# INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

**The quality of this reproduction is dependent upon the quality of the copy submitted.** Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

# Frequency-Dependent Actions of GABA Enhancing Anticonvulsant Drugs

**Michael F. Jackson**

Department of Pharmacology and Therapeutics
McGill University, Montreal

August, 1998

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of

**DOCTOR of PHILOSOPHY**

**Abstract**

The contribution of use-dependence to the actions of two novel antiepileptic drugs acting upon the GABA neurotransmitter system was investigated using the rat hippocampal slices preparation. The first study of this thesis demonstrated that tiagabine, a GABA uptake blocker, causes a more pronounced increase of GABAergic responses evoked by high-frequency stimulation (HFS) than of those elicited by a single stimulus. The predominant effect of tiagabine on HFS-evoked responses was to facilitate the generation of $GABA_A$ receptor-mediated depolarizations (DRs) which were capable of triggering bursts of action potentials when evoked from the stratum radiatum.

Since these results suggested that tiagabine may paradoxically promote rather than inhibit epileptiform discharges, the second study investigated the functional consequences of tiagabine-augmented DRs. Consistent with our observation of enhanced inhibition following HFS, the amplitude of evoked EPSPs superimposed upon tiagabine-augmented DRs were markedly reduced and could no longer trigger action potentials. A similar inhibitory effect on evoked EPSCs was also observed. The large conductance increase associated with the occurrence of DRs suggests that tiagabine can reduce the depolarizing influence of excitatory transmission by increasing the effectiveness of GABA-mediated shunting inhibition.

In the third study of this thesis, the effects of γ-vinyl GABA (GVG, vigabatrin) were investigated in slices prepared from animals pretreated with either an anticonvulsant dose of GVG or saline. GVG pretreatment produced a frequency-dependent strengthening of GABA-mediated transmission such that repetitive stimulation at low-frequencies (2.5-10 Hz) was no longer associated with a depression of inhibition. A reduced sensitivity of evoked IPSCs to the actions of baclofen, a $GABA_B$ receptor agonist, suggests that GVG may have produced its frequency-dependent effects by depressing the efficacy of the GABA release regulating negative feedback mechanisms through the presynaptic $GABA_B$ autoreceptors.

In the final study of this thesis, the acute effects of bath applied GVG on GABAergic inhibition were examined. GVG (100-500 μM) caused a concentration-dependent disinhibitory effect which did not appear to be mediated through an antagonism of postsynaptic $GABA_A$ receptors. These results suggest that the acute actions of GVG, distinct from those mediated through its inactivation of GABA-T, do not contribute to the anticonvulsant properties of this drug.

In conclusion, the results presented suggest that both tiagabine and GVG produce use-dependent increases in inhibition, an effect which may importantly contribute to their anticonvulsant properties.

**Résumé**

Nous avons utilisé la préparation de tranche d'hippocampe chez le rat pour évaluer la contribution de propriétés dites *usage-dépendantes* aux mécanismes d'action de deux nouveaux anticonvulsants agissant sur la transmission impliquant le neurotransmetteur GABA. La première étude de cette thèse démontre que l'augmentation des réponses GABAergiques induite par la tiagabine, un inhibiteur de la capture du GABA, est plus prononcée suite à une stimulation à haute fréquence (SHF) que par une stimulation unitaire. L'effet prédominant de la tiagabine sur les réponses évoquées par SHF fût de faciliter l'induction de dépolarisations résultant de l'activation des récepteurs de type $GABA_A$. De telles réponses peuvent provoquer une bouffée de potentiels d'action lorsqu'elles sont induites à partir du stratum radiatum.

Ces résultats suggèrent que la tiagabine pourrait paradoxalement promouvoir plutôt qu'inhiber les décharges épileptiformes. Nous avons donc décidé d'évaluer les conséquences fonctionnelles de la facilitation des réponses dépolarisantes (RD) par la tiagabine. Nous avons observé une réduction marquée de l'amplitude des potentiels postsynaptique excitateurs (PPSEs) engendrés par stimulation électrique au cours de RDs dont l'amplitude avait été augmentée par la présence de tiagabine. La réduction de l'amplitude des PPSEs fût telle qu'ils ne pouvaient plus engendrer de potentiels d'action. Un effet semblable aux RDs fût observé sur les courants postsynaptiques excitateurs (CPSEs). Ces résultats sont entièrement en accord avec l'augmentation de l'efficacité de l'inhibition produite suite à la SHF en présence de tiagabine. La présence d'une forte augmentation de la conductance transmembranaire lors des RDs suggère que la tiagabine peut réduire l'influence dépolarisante des PPSEs en augmentant l'efficacité de l'inhibition GABAergique via un mécanisme de court-circuit membranaire.

Lors de la troisième étude de cette thèse, nous avons examiné les effets du γ-vinyl-GABA (GVG) sur des tranches d'hippocampe préparées à partir d'animaux ayant reçu préalablement, par injection, une dose anticonvulsante de

GVG ou de salin. Le traitement au GVG causa une augmentation de l'inhibition GABAergique en fonction de la fréquence de stimulation. Cela fut observé lors de stimulations répétitives à basse fréquence (2.5-10 Hz), qui ont normalement pour effet de réduire l'efficacité de l'inhibition synaptique, mais qui, suite à l'injection de GVG, produisirent l'effet contraire. Etant donné que nous avons observé une réduction de l'action inhibitrice de la baclofen, un agoniste des récepteurs de type $GABA_B$, sur l'amplitude des courants postsynaptiques inhibiteurs (CPSIs), nos résultats suggèrent que l'habileté du GVG à produire une facilitation de l'inhibition en fonction de la fréquence résulte d'une réduction de la fonction régulatrice des autorécepteurs présynaptiques $GABA_B$ sur le relâchement synaptique du GABA.

La dernière étude de cette thèse traite des effets du GVG sur l'inhibition GABAergique suite à son administration par superfusion. Le GVG (100-500 µM) causa une réduction, en fonction de sa concentration, de l'inhibition synaptique. Le mécanisme par lequel cet effet du GVG fût produit ne semble pas être dû à un antagonisme des récepteurs $GABA_A$. Ces résultats indiquent qu'un mécanisme d'action aiguë du GVG, distinct de ceux impliquant l'inhibition de la GABA-T, pourrait provoquer, plutôt que de prévenir, les crises d'épilepsie.

En conclusion, nos résultats suggèrent que le GVG et la tiagabine produisent une augmentation de l'inhibition GABAergique via un mécanisme qui est fonction de l'usage, ce qui pourrait représenter le substrat principal de l'action anticonvulsante de ces composés.

**Acknowledgments**

I would most importantly like to thank my parents, as well as my brother and sister, for their continued love, encouragement and support. It is to them that I dedicate this thesis.

I wish to sincerely thank Dr. Radan Čapek for his guidance throughout my graduate studies. I am grateful to him for his patience and continued interest in all my undertakings. He greatly contributed towards making my stay in the Department an enjoyable one and from him I have learnt a great deal over the years. A special thank you to Dr. Esplin for her enthusiastic support over the years and especially for her words of encouragement which were always greatly appreciated.

Annie Constantin, pour des raisons que j'ignore encore, je ne me suis jamais senti à l'aise de te parler en français (go figure!). Je me suis dis que je pouvais alors au moins te remercier en français pour avoir contribuer à rendre la vie dan'lab amusante. J'ai également beaucoup apprecié notre visite au Lac Labelle (Merci également à Michel...I enjoyed staying at your chalet, driving your boat and especially drinking your beer!) et aussi le voyage de ski que nous avons passé ensemble (I'd never driven into a snow bank at 100 kph before...I won't soon forget that one!). Je regrette que nous n'avions pas été capable de profiter plus souvent de tes nombreuses invitations pour aller au chalet mais peut être que lors d'un de nos voyage à Val D'Or on aura la chance d'arrêter vous voir.

Thanks also to Caroline Saucier, Shyam Ramchandani, Martin Gagnon, Naomi Belinski and Sylvain "Costanza" Côté for good times spent during lunchtimes, water cooler breaks, late night poker games and ski trips (why didn't Costanza ever come to one of these?). Special thanks to Shyam and Sylvain for organizing those great hockey pools (Sylvain, the tent we bought still looks great and Shyam, you'll never win one these things if you keep going for the Maple Leafs!).

I would also like to thank Dr. Yves De Koninck for his interest in my work and much helpful advice. I will miss our lengthy stair-well discussions after Dr. Krnjević's Journal Clubs.

Thanks as well to the many members of the faculty for their encouragement over the years and in particular to Dr. Alfredo Ribeiro da Silva, Dr. Donald Ecobichon, Dr. Ante Padjen, Dr. Brian Collier, Dr. Paul Clarke and Dr. Claudio Cuello.

I wish to also thank Tony Assaf, Nada Awad, Yves Tremblay, Catherine Leclerc, Marc Beasse, Leslie Glass, Martin Guarda, Debbie Guarda, Jean-Paul Sarkis and Paul Couturier. Your continued friendship has and will continue to mean a great deal to me.

Finally, and most importantly, I would like to thank Natalie Lavine, the one person who more than any other has helped make these many years seemingly fly by. I thank her especially for helping me keep some measure of sanity over the long months during which I incessantly complained and procrastinated about completing this thesis. I assure you Nat that our life together can now only get much better.

**Contribution of Authors**

In accordance with McGill University's "Guidelines for Thesis Preparation" this thesis is written in manuscript format. Thus, the results sections (Sections 3,4,5 and 6) are composed of text and figures which are duplicated from manuscripts previously published, under revision or about to be submitted for publication. The following statement lists the contributions made by each author:

**Section 3.** Frequency-dependent enhancement of hyperpolarizing and depolarizing GABAergic synaptic responses. Jackson, M.F., Esplin, B. and Čapek, R. *Epilepsy Research* (under revision).

I performed all the work and wrote the manuscript on which this section is based under the supervision of Drs. Čapek and Esplin.

**Section 4.** The inhibitory nature of tiagabine-augmented $GABA_A$ receptor-mediated depolarizing responses (DRs) in hippocampal pyramidal cells. Jackson, M.F., Esplin, B. and Čapek, R. *J.Neurophysiology* (under revision).

I performed most of the work described in this section. Ms. Annie Constantin assisted with or performed some of the extracellular recordings described. I wrote the manuscript on which this section is based with revisions to the text and figures provided by Drs. Čapek and Esplin.

**Section 5.** Reversal of the activity-dependent suppression of GABA-mediated inhibition in hippocampal slices from γ-vinyl GABA (vigabatrin)-pretreated rats. Jackson, M.F., Esplin, B. and Čapek, R. (to be submitted).

I performed all the work and wrote the manuscript on which this section is based under the supervision of Drs. Čapek and Esplin.

**Section 6.** Acute effects of γ-vinyl GABA (vigabatrin) on hippocampal GABAergic inhibition *in vitro*. Jackson, M.F., Dennis, T., Esplin, B. and Čapek, R. *Brain Res.* (1994) 651: 85-91.

I performed all the work which is described in this section. Binding experiments were undertaken in the laboratory of Dr. Dennis. I wrote the manuscript on which this section is based with revisions to the text and figures provided by Drs. Čapek, Esplin and Dennis.

**TABLE OF CONTENTS**

## List of Abbreviations

| | |
|---|---|
| aCSF | artificial cerebrospinal fluid |
| 4-AP | 4-aminopyridine |
| CNQX | 6-cyano-7-nitroquinoxaline-2,3-dione |
| CPP | 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid |
| CSF | cerebrospinal fluid |
| DAP | depolarizing after-potential |
| DR | depolarizing response |
| EPSC | excitatory postsynaptic current |
| EPSP | excitatory postsynaptic potential |
| GABA | γ-aminobutyric acid |
| GABA-T | GABA-transminase |
| GAD | glutamic acid decarboxylase |
| GAG | γ-acetylenic GABA |
| GVG | γ-vinyl GABA |
| HFS | high-frequency stimulation |
| IPSC | inhibitory postsynaptic current |
| IPSP | inhibitory postsynaptic potential |
| ISI | interstimulus interval |
| NA | nipecotic acid |
| PDS | paroxysmal depolarizing shift |
| PLP | pyridoxal 5'-phosphate |
| PS | population spike |
| RMP | resting membrane potential |
| SSADH | succinic semialdehyde |
| SWD | spike wave discharge |
| TBOB | *butylbicycloorthobenzoate* |
| TGB | tiagabine |

# General Introduction

## 1. Research goals and literature review

The decline of society's long held superstitious beliefs attributing epilepsy to supernatural forces paralleled scientific discoveries which increased our understanding of how the brain functions. The first suggestion that there exists a physiological basis for epilepsy is attributed to Hippocrates who suggested that the underlying cause of epilepsy was an abnormal brain consistency due to an accumulation of phlegm. Since then, basic epilepsy research has helped to identify the intrinsic (voltage-gated ion channels) and extrinsic (neurotransmitters and neuromodulators acting at ligand-gated ion channels) elements involved in the electrogenesis of the abnormal brain activity which underlies convulsive disorders. Given the fact that these same elements are involved in the normal functioning of the brain it is therefore not surprising that anticonvulsant therapy, which targets these systems, can be associated with numerous side-effects.

Established and novel anticonvulsants are predominantly thought to act on one or more of the following targets: (1) *voltage-dependent ion channels involved in action potential propagation or burst generation* (2) *glutamatergic excitatory neurotransmission* and (3) *GABAergic inhibitory neurotransmission* (see Appendix, Tables 1 and 2). A common property of therapeutically effective ion channel modulators, such as phenytoin and carbamazepine, is their ability to produce a voltage and frequency-dependent block of voltage-gated $Na^+$ channels. The greater degree of channel block produced by these drugs under strong depolarizing conditions, as is presumed to occur during seizures, is believed to allow these drugs to selectively target the neurons involved in the initiation and spread of seizure activity while minimizing the occurrence of side effects. Although numerous *in vitro* electrophysiological studies have investigated the actions of antiepileptic drugs believed to target the GABAergic neurotransmitter system, few have used stimulation patterns designed to more accurately reflect the hypersynchronous activation of neurons occurring during seizures. The contribution of frequency- or activity-dependent properties to the

mechanisms of action of GABA-enhancing antiepileptic drugs has therefore not been investigated in any systematic way.

The main goals of my thesis research have been to characterize the effects of stimulation patterns intended to mimic the activation of neurons during a seizure on 1) the ability of novel antiepileptic drugs to produce an enhancement of GABA-mediated neurotransmission and 2) $GABA_A$ and $GABA_B$ receptor-mediated post-synaptic responses. More specifically, the focus of these studies has been to elucidate the actions of the anticonvulsants vigabatrin, an irreversible inhibitor of GABA-transaminase, and tiagabine, a selective blocker of GABA re-uptake, on GABA-mediated synaptic responses recorded from pyramidal neurons of the CA1 region of rat hippocampal slices.

The following introductory chapter is intended to provide relevant background information on: epileptic syndromes, the involvement of various neurotransmitter systems and voltage-gated ion channels in the electrogenesis of epileptic seizures, as well as to provide a description of the effects of vigabatrin and tiagabine on the GABAergic neurotransmitter system.

## 1.1 Epilepsy

Current epidemiological data suggests that the prevalence of active epilepsy ranges from 4-10 in 1000 (Sander and Shorvon, 1996) and it is estimated that as many as 1 in 10 will suffer a seizure in their lifetime, making epilepsy one of the most common neurological disorders in man. Over 100 years ago John Hughlings Jackson described epilepsy as "...an occasional, an excessive, and a disorderly discharge of nerve tissue on muscles". He furthermore introduced a classification scheme which divided seizures into two broad categories: 1) *generalized seizures* "...in which the spasm affects both sides of the body almost contemporaneously...", and 2) *partial seizures* "...in which the fit begins on one side of the body, and in which parts of the body are affected one after another" (Jackson, 1870). Although greatly expanded upon, such a scheme continues to be in use today as part of the International League Against Epilepsy's (ILAE) standardized classification of epileptic seizures (Commission on Classification and Terminology of the ILAE, 1981).

## 1.2 Epilepsy and seizure classification

The term epilepsy encompasses a large number of syndromes and since the seizures associated with each of these may respond very differently (or even selectively) to the various treatment options which exist, the proper identification of the disease is of paramount importance in selecting the most appropriate treatment regimen. This is one of the primary reasons for which, in addition to a classification of individual seizure types, the ILAE has introduced a Classification of Epilepsies and Epileptic Syndromes (Commission on Classification and Terminology of the ILAE, 1989). Over 30 epilepsies and epileptic syndromes are identified according to a number of factors which include: the type of seizure (often based on EEG findings), etiology, anatomy, precipitating factors, age of onset, severity, chronicity, diurnal and circadian cycling, and sometimes prognosis. The ILAE proposal divides the epilepsies into four main groups: 1)

*localization-related syndromes (commonly referred to as focal or partial epilepsy)*, 2) *generalized syndromes*, 3) *syndromes undetermined whether focal or generalized* and 4) *special syndromes*.

Based on this classification, epidemiological studies have found that the most common forms of epilepsy in adults are the focal or partial epilepsies which account for as many as two thirds of all cases (Gastaut et al., 1975; Hauser et al., 1993; Bauer, 1994). The partial epilepsies can be further subdivided based on whether or not consciousness is impaired during the attack. When consciousness is not impaired the seizures are classified as simple partial seizures whereas when consciousness is affected the seizures are termed complex partial seizures. Complex partial seizures are the single most common type of seizures in humans. Although the overall rate of occurrence of drug resistant epilepsy is estimated at between 20-40%, resistance to anticonvulsant therapy is reported to occur in about 70% of patients suffering from complex partial seizures (Löscher and Schmidt, 1994b).

## 1.3 Focal epilepsy

Tonic-clonic seizures, often referred to as grand-mal, as well as absence seizures, also known as petit mal, are examples of generalized epilepsy. Electroencephalographic (EEG) findings have demonstrated that such seizures are bilaterally synchronous and involve widespread areas of the cortex almost simultaneously from the onset, suggesting the involvement of deep subcortical gray matter. In contrast, focal (partial) epilepsies are the result of discrete cortical abnormalities from which the epileptic activity may or may not spread to the rest of the brain. The EEG hallmarks of focal epilepsy in both animal models and in humans are the ictal, or seizure discharge, and the interictal discharge. The term ictal, from the Latin ictus meaning to strike, appropriately reflects the sudden and unpredictable way with which seizures commence. Interictal discharges, seen as a sharp spike on the EEG, are typically observed between seizure events. They are not generally associated with any behavioral

manifestations and are believed to reflect the neuronal activity of the hyperexcitable focus.

### 1.3.1 Focal epilepsy: Animal models

The vast majority of studies investigating the mechanisms involved in the generation of seizures have been undertaken in acute and chronic models which more closely approximate the focal epilepsies. One of the primary reasons why research efforts were concentrated in this way was that such models provided restricted targets in which electrophysiologists and anatomists could search for alterations in neuronal function which would underlie the epileptiform activity.

Early studies in acute models often involved the topical application of convulsant drugs to the exposed cortex of animals. These drugs typically interfered with inhibitory neurotransmission mediated by either GABA (penicillin, bicuculline and picrotoxin) or glycine (strychnine). The great ease with which seizures could be generated following the application of agents which interfered with GABA-mediated transmission contributed to an early acceptance of the importance of GABA in the control of central nervous system (CNS) excitability. Discovered during neurosurgical procedures in which the antibiotic was applied to the exposed brain to prevent infection (Walker and Johnson, 1945), the penicillin model in particular has been extensively used to study the neuronal basis of focal epilepsy and a wealth of information, including the initial observation and characterization of the paroxysmal depolarizing shift (described in the following section), was derived from the use of this model. Although *in vivo* recordings from acute epileptic foci following the application of convulsants contributed enormously to our understanding of factors involved in the generation of epileptiform activity, electrophysiological recordings in these models were technically laborious due to the mechanical instability resulting from cardiorespiratory pulsations, the presence of dura, subarachnoid membranes and the blood-brain-barrier, and the confounding effects of the anesthetic agents used in these studies made the interpretation of results more difficult. Increasing

use has thus been made of neocortical brain slices since their introduction over 30 years ago (Yamamoto and McIlwain, 1966). The ability to elicit evoked and spontaneous epileptiform activity in thin slices of the hippocampus and the involvement of this structure in complex partial seizures has contributed to the popularity of the hippocampal slice preparation as a model system for the study of mechanisms contributing to the generation of focal seizures. In fact, many of the results to be discussed in the following sections were derived from studies making use of this very model. Other acute models of focal seizures include the direct electrical stimulation of cortical tissue, topical application of cholinergics and anticholinergics and withdrawal of GABA following its continued infusion for days or weeks.

Chronic models can be produced by brain injury following the implantation of metals (alumina hydroxide gel model, iron salts and other metals)(Kopeloff et al., 1955; Blum and Liban, 1960; Dow et al., 1962; Reid et al., 1979; Feria-Velasco et al., 1980; Lange et al., 1980; Pei et al., 1983), freeze lesions (Hanna and Stalmaster, 1973; Loiseau et al., 1987), injection of tetanus toxin (Mellanby et al., 1984) or pilocarpine (Turski et al., 1983; Turski et al., 1984), and kindling (Alonso-DeFlorida and Delgado, 1958; Goddard, 1967).

### 1.3.2 Focal epilepsy: Basic mechanisms

One of the key findings in early electrophysiological investigations of focal epilepsy, which launched the modern era of studies into the cellular mechanisms of the disease, was the observation that the electroencephalographically recorded interictal discharge coincided with a large (25-40 mV), prolonged (100-300 msec) intracellularly recorded depolarization which was termed the paroxysmal depolarizing shift (PDS) (Matsumoto and Ajmone Marsan, 1964b). The PDS triggers a burst of action potentials and is typically followed by an afterhyperpolarizing potential (Dichter and Spencer, 1969a; Dichter and Spencer, 1969b; Ayala et al., 1973). Although such a depolarizing-hyperpolarizing sequence was characteristically recorded from neurons within the seizure focus,

regardless of the pharmacological or physical agent used to produce the epileptic foci, a peripheral zone was identified where neurons experienced only strong hyperpolarization coinciding with the interictal paroxysm (Prince and Wilder, 1967; Dichter and Spencer, 1969a; Dichter and Spencer, 1969b). Thus, the hyperexcitable seizure focus discharging at high rates is surrounded by a region of strong inhibition which serves to limit the spatial extent over which the epileptiform activity may spread.

In acute models of focal epilepsy, a characteristic sequence of events has been observed to occur during the transition from interictal to ictal (seizure) discharge: the afterhyperpolarization, which normally follows the PDS, gradually fades and is replaced by a depolarization capable of triggering afterdischarges (Matsumoto and Ajmone Marsan, 1964a; Dichter and Spencer, 1969a; Ayala et al., 1970). As the afterdischarges become progressively longer with each successive interictal discharge, the surround inhibition is reduced allowing the activity of more and more distant areas of the brain to become synchronized. Eventually, a full blown seizure develops which is characterized by the maintenance of the membrane potential at a relatively sustained level of excessive depolarization and the prolonged synchronous firing of afterdischarges. This activity eventually subsides and is followed by a post-ictal period of strong membrane hyperpolarization.

It is important to note that although a number of anatomical and/or functional abnormalities are likely to be found within the epileptic focus, the majority of neurons which are recruited during the spread of the seizure activity from the focal area are "normal". That is to say that they have not been directly exposed to either the convulsant agent or pathological process which underlies the focal abnormality. In the following sections, the different mechanisms involved in the generation and spread of epileptiform activity will be discussed.

### *A) Paroxysmal depolarizing shift: Giant EPSP or endogenous burst?*

Two hypotheses were initially proposed to explain the origin of the paroxysmal depolarizing shift: the *giant excitatory postsynaptic potential (EPSP) hypothesis* and the *endogenous burst hypothesis*. The former stated that the PDS was a network-driven event resulting from an increase in the strength of excitatory inputs (Prince, 1968a; Dichter and Spencer, 1969a; Dichter and Spencer, 1969b; Ayala et al., 1970; Ayala et al., 1973), whereas the latter suggested that the PDS was the result of an abnormally large response of the neuronal membrane to an excitatory input of normal strength (Wong and Prince, 1979; Schwartzkroin and Prince, 1980; Alger, 1984).

Initial observations from experiments aimed at characterizing the properties of the PDS were most consistent with the *giant EPSP hypothesis*. Comparison of membrane properties after the establishment of an experimental foci with those determined in recordings from normal cortex revealed no difference in either the neuronal resting membrane potential or input resistance. Action potential generating mechanisms also appeared normal within the epileptic focus and although high frequency spiking could be achieved, no amount of depolarizing current injection could reproduce the PDS (Prince, 1968a). Arguments in favor of the PDS being synaptic in nature were based on a number of initial observations: 1) the PDS was graded in amplitude when repeatedly elicited, 2) it was associated with a conductance increase, 3) its amplitude varied with changes in membrane potentials and its polarity could be reversed with strong depolarization (which would be impossible if the PDS was entirely a voltage-activated event) and 4) its frequency and probability of occurrence was independent of membrane potential. Activation of recurrent excitatory pathways were thought to be particularly important for the generation of the PDS by providing a positive feedback mechanism which would allow the recruitment and synchronization of large populations of neurons. Furthermore, the lack of such recurrent excitatory connections was suggested to underlie the

inability of the cerebellar cortex to develop spontaneous PDSs following the application of convulsive agents (Ayala et al., 1973).

Acceptance of the *endogenous burst hypothesis* grew with the advent of brain slice preparations which provided the greater mechanical stability necessary for high quality recordings and allowed accurate and convenient control over the extracellular environment. These preparations became popular model systems for the study of epileptic phenomena following the demonstration that similar paroxysmal discharges could be recorded in the presence of convulsant agents such as penicillin or picrotoxin (Schwartzkroin and Prince, 1980). The hippocampal slice preparation became particularly popular and its use permitted the identification of a number of intrinsic membrane properties which could participate in the generation of epileptiform discharges. Hippocampal pyramidal cells were shown to have an innate ability to fire asynchronous bursts in response to depolarizing current injection. These bursts resembled PDSs in that they were composed of a slow depolarizing component with superimposed repetitive action potentials. The slow depolarizing component appeared to be due to the summation of voltage-gated calcium ($Ca^{2+}$) channel-dependent depolarizing after-potentials (DAPs) (Schwartzkroin and Prince, 1980) which are triggered by membrane depolarization and can conversely be prevented by hyperpolarization . The demonstration of the ability of hippocampal pyramidal cell dendrites to fire $Ca^{2+}$-dependent action potentials suggested a further role for voltage-gated $Ca^{2+}$ channels in the intrinsic capabilities of these cells to burst. The much smaller capacity of granule cell dendrites to fire $Ca^{2+}$ potentials was suggested to underlie the relatively high seizure threshold of the dentate gyrus. The demonstration that the amplitudes of evoked EPSPs were unchanged following the application of either penicillin or bicuculline to hippocampal slices (Wong and Prince, 1979; Schwartzkroin and Prince, 1980) added to arguments in favor of the *endogenous burst hypothesis*.

A more unified view of cortical epileptogenesis (Prince and Connors, 1986), supported by evidence from computer simulations of epileptiform activity (Traub and Wong, 1983), is now generally accepted. The interaction of three

general factors are believed to contribute to the propensity of a given CNS region to develop seizures: 1) the presence of neurons with an intrinsic ability to fire bursts of action potentials. Subpopulations of such neurons would serve to initiate synchronous discharges and therefore would act as pacemakers. 2) Reduction in the efficacy of inhibitory synaptic mechanisms. GABA-mediated inhibition has been shown to play an important role in suppressing intrinsically generated bursts and block of inhibition by convulsive agents is one of the most reliable and extensively used methods of inducing epileptiform activity. 3) Presence of excitatory interconnections allowing the synchronization of large populations of neurons.

### *B) Paroxysmal hyperpolarization and surround inhibition*

Whereas the hyperpolarization which follows the PDS (post-PDS HP) plays a critical role in controlling the duration and determining the frequency of the interictal discharge, both the post-PDS HP and the presence of a strong inhibitory surround are thought to limit the spread of the paroxysmal activity. Although a number of mechanisms could potentially contribute to the generation of the post-PDS HP including the activation of voltage-gated potassium ($K^+$) conductances (Rudy, 1988; Storm, 1990), $Ca^{2+}$-dependent $Cl^-$ conductances (Owen et al., 1984; Mayer, 1985) and the electrogenic $Na^+$ pump (Heinemann and Gutnick, 1979; Haglund and Schwartzkroin, 1990), the hyperpolarization seems to be predominantly due to GABA-mediated IPSPs and the activation of $Ca^{2+}$-activated $K^+$ conductances.

Early evidence favoring a role for GABA-mediated IPSPs came from studies which showed that the post-PDS HP was graded in amplitude when repeatedly evoked, was associated with a drop in membrane resistivity and that its amplitude varied with changes in membrane potential eventually reversing at negative membrane potential values. Furthermore, the observation of paroxysmal hyperpolarizations occurring in the absence of a preceding depolarization (inhibitory surround) suggested that mechanisms other than the

activation of voltage-dependent afterhyperpolarizing currents were responsible for the generation of the post-PDS HP. However, the strongest evidence in support of a role for IPSPs came with the demonstration that, at least in the penicillin model of focal epilepsy, the post-PDS HP could be reversed following the intracellular injection of $Cl^-$ anions (Prince, 1968b; Hablitz, 1981).

The continued presence of the post-PDS HP following the blockade of $GABA_A$ receptors by the specific antagonists bicuculline and picrotoxin (Schwartzkroin and Prince, 1980; Alger and Nicoll, 1980; Hablitz, 1981) suggested additional mechanisms contributed to the electrogenesis of this event. The post-PDS HP recorded under these conditions was unaffected by intracellular loading of $Cl^-$ ions (Alger and Nicoll, 1980; Hablitz, 1981) and could be blocked by bath application of $Ba^{2+}$ (Alger and Nicoll, 1980) a divalent cation which reduces $K^+$ conductance, as well as by the intracellular application of the $Ca^{2+}$ chelator EGTA (Schwartzkroin and Prince, 1980; Hablitz, 1981). These properties were similar to those of the long-lasting afterhyperpolarization which had recently been described to follow the current-induced repetitive firing of hippocampal pyramidal cells (Hotson and Prince, 1980) and suggested that $Ca^{2+}$-activated $K^+$ conductances could contribute to the post-PDS HP.

Although the relative contribution of each of these potential mechanisms to the generation of the long-lasting hyperpolarizations which follow paroxysmal discharges *in vivo* remains to be determined, *in vitro* studies have demonstrated that the underlying mechanisms may depend on the means by which the epileptiform activity is elicited within brain slices. Thus, GABA-mediated IPSPs appear to be predominantly responsible for the post-PDS HP generated in the presence of penicillin (Schwartzkroin and Stafstrom, 1980; Hablitz, 1981) whereas the activation of $Ca^{2+}$-dependent $K^+$ potentials appears to underlie afterhyperpolarizations generated in the presence of bicuculline or picrotoxin (Alger and Nicoll, 1980; Hablitz, 1981). Although each of these convulsant agents are believed to act through identical mechanisms, namely by antagonizing $GABA_A$ receptor-mediated inhibition, differences in mechanisms underlying the post-PDS HP may be related to observed inability of penicillin to

completely abolish postsynaptic inhibition (Davenport et al., 1978; Davenport et al., 1979).

### *C) Spread of seizure activity into "normal" brain areas*

Given the fact that overt behavioral signs of underlying focal abnormalities are only observed during ictal discharges which involve large portions of the CNS, a great deal of research has focused on gaining a better understanding of the mechanisms by which epileptiform activity spreads in anatomical space. Several contributing factors have been proposed by which the activity of progressively larger populations of neurons become synchronized. These include: (1) positive-feedback mechanisms mediated through recurrent synaptic excitation, (2) nonsynaptic propagation of excitation, (3) diffuse actions of neuromodulators causing a reduction in the effectiveness of inhibitory mechanisms and (4) reductions in the strength of synaptic inhibition during the repeated activation of inhibitory pathways.

Anatomical differences between nonpyramidal circuit neurons, the activity of which provides the basis for synaptic inhibition, and pyramidal neurons, which are the principal cells of the cortex and whose activation results in synaptic excitation, have important implications for the propagation of epileptiform activity. Whereas the axons of nonpyramidal cells generally terminate within restricted cortical regions, those of pyramidal cells may project to more distant locations thereby providing an anatomical substrate by which excitation in one focal area of the brain can influence the activity in another more distant region. Furthermore, anatomical and physiological evidence has demonstrated the existence of extensive recurrent excitatory collaterals which provide powerful positive feedback which can result in a pronounced amplification of excitation. The presence of such highly divergent positive feedback mechanisms have been identified in the CA3 region of the hippocampus (Miles and Wong, 1986) as well as in the neocortex (Mason et al., 1991) and are thought to underlie the ability of these regions to support widespread synchronous activity under conditions of

partial disinhibition. The strength of these mechanisms is amply illustrated by the demonstration that the activation of a single pyramidal cell from the CA3 region of disinhibited hippocampal slices can initiate burst discharges (Miles and Wong, 1983; Miles and Wong, 1987).

Although the relative contribution of nonsynaptic interactions to inter-neuronal communication is unclear, their dependence on the level of neuronal activity suggests that they are more likely to contribute to excitation during synchronized high-frequency neuronal discharges. The ability of these nonsynaptic mechanisms to synchronize neuronal discharges is amply illustrated by the occurrence of spontaneous burst discharges following the superfusion of hippocampal slices with calcium-free solutions which abolish synaptic transmission (Taylor and Dudek, 1982; Haas and Jefferys, 1984; Konnerth et al., 1986). Nonsynaptic interactions between populations of neurons can occur through gap (or electrotonic) junctions, extracellular electrical field effects and activity-induced changes in the extracellular concentrations of various ionic species. Gap junctions, which provide electrical continuity between interconnected neurons, are formed through the interaction of specific membrane "connexin" proteins (Kumar and Gilula, 1996). Although electrophysiological and anatomical evidence supports the existence of gap junctions in the mature mammalian nervous system (Gutnick and Prince, 1981; MacVicar and Dudek, 1982; Kosaka and Hama, 1985; Shiosaka et al., 1989), their rate of occurrence is fairly low (Llinás and Yarom, 1981; O'Beirne et al., 1987). The involvement of electrotonic interactions in epileptiform phenomena is however strengthened by the observation that the gap junctional conductance is increased during episodes of hyperexcitability as well as by alkaline shifts of the intracellular pH (Perez-Velazquez et al., 1994). Such shifts have been shown to occur during the repetitive activation of pyramidal neurons (Somjen, 1984; Walz, 1989; Chen and Chesler, 1992; Taira et al., 1995), suggesting that transient changes in pH during interictal discharges may favor gap junctional communication between neurons and thus facilitate the propagation of epileptiform activity.

Changes in the extracellular pH are believed to be produced as a result of the flow of $H^+$ and $HCO_3^-$ ions resulting from the activation of receptor-gated ion channels or of $Ca^{2+}/H^+$ exchangers following an influx of $Ca^{2+}$ through voltage-gated channels (Chen and Chesler, 1992; Paalasmaa and Kaila, 1996). In addition, during epileptiform activity the extracellular concentration of $K^+$ has been shown to rise while that of $Ca^{2+}$ decreases (Heinemann et al., 1977; Somjen and Giacchino, 1985; Hablitz and Heinemann, 1987). Both of these changes will further facilitate the progressive recruitment of surrounding neurons. Increases in extracellular $K^+$ will favor membrane depolarization. In contrast, a drop in extracellular $Ca^{2+}$ may increase excitability through one of several mechanisms including: 1) a reduction in the screening of negative charges at the surface of neuronal membranes thereby altering the surrounding electrical field which is sensed by voltage-dependent channels, 2) an augmentation of the electrotonic coupling of neurons (Perez-Velazquez et al., 1994) and 3) the activation of a recently described non-selective cation channel (Xiong et al., 1997).

Release of neurotransmitters and neuromodulators, such as acetylcholine, norepinephrine, dopamine, serotonin as well as endogenous opiates, during interictal activity may provide an additional means of synchronizing the firing of progressively larger populations of neurons. Such an effect could be mediated via second messenger cascades following the activation of their respective ligand-gated G-protein coupled receptors, resulting in a decrease in either or both $Ca^{2+}$-dependent $K^+$ conductances, which contributes to the generation of the post-PDS HP, and GABA-mediated IPSPs. In particular, an increase in the activation of the cholinergic receptor system has been shown to produce a proconvulsant effect.

Finally, numerous studies have demonstrated that the effectiveness of GABA-mediated inhibition is decreased during the repeated synchronous activation of inhibitory pathways at low (0.2-20 Hz) frequencies. Given the importance of GABA-mediated mechanisms in controlling CNS excitability and the significant role of such mechanisms in the generation of the post-PDS HP, which plays an important role in limiting the spread of epileptiform discharges,

such activity-dependent depression of inhibition is thought to importantly contribute to the onset of seizure activity (Ben-Ari et al., 1979; Deisz and Prince, 1989).

## 2. The GABA neurotransmitter system: A target for anticonvulsant drugs

Given the importance of GABA-mediated inhibition in the control of CNS excitability and the demonstration that a number of established conventional antiepileptic drugs produce their therapeutic actions through an enhancement of GABA-mediated transmission, the design of compounds capable of enhancing synaptic inhibition has become an important strategy in the search for novel antiepileptic drugs. Such a strategy is used in the hope that it may yield new drugs with a greater therapeutic index allowing for a greater separation between anticonvulsant efficacy and general CNS toxicity.

The much greater understanding of the GABAergic neurotransmitter system has helped to identify a number of mechanisms through which a pharmacological intervention could produce an enhancement of GABA-mediated inhibition. These include: (1) enhancement of the presynaptic stores of GABA via an increase in GABA synthesis or (2) a decrease in its degradation, (3) direct activation of GABA receptors which mediate pre and postsynaptic inhibition, (4) allosteric enhancement of the actions of GABA at the $GABA_A$ receptor subtype, and (5) reduction of neurotransmitter uptake.

In the following sections, the GABA system will be described with regards to the targets involved in the above mentioned mechanisms namely (1) glutamic acid decarboxylase (GAD, E.C. 4.1.1.15), (2) GABA transaminase (GABA-T), (3) the pre and postsynaptically located $GABA_A$ and $GABA_B$ receptor subtypes through which GABA-mediated signaling occurs, and (4) the GABA transporter. Whenever appropriate, sites through which established and novel anticonvulsants are thought to produce their anticonvulsant effects will be identified. Although GABA is detected in the peripheral nervous system (PNS), endocrine, as well as in other non-neuronal tissues, only its actions in the CNS

are relevant to the control of neuronal excitability. For this reason, the following sections will be limited to a description of GABA in the CNS.

## 2.1 Synthesis of GABA

CNS stores of GABA are predominantly derived through the α-decarboxylation of L-glutamate, a reaction which is catalyzed by the enzyme glutamic acid decarboxylase (GAD, E.C. 4.1.1.15), which uses pyridoxal 5'-phosphate (PLP), a form of vitamin $B_6$, as a cofactor. Two different forms of GAD have been identified in the brain and have been designated $GAD_{65}$ and $GAD_{67}$ based on their respective apparent molecular weights of 65 and 67 kDa. These two forms, which are the product of two separate genes (Erlander et al., 1991), differ in their amino acid sequence, pattern of expression in different brain regions and during development, subcellular localization, as well as in their interaction with the cofactor, PLP. While $GAD_{67}$ is widely distributed throughout the neuron, $GAD_{65}$ is the predominant form found in nerve terminals (Kaufman et al., 1991; Rimvall and Martin, 1994) and is therefore believed to play an important role in the regulation of the transmitter pool of GABA (Martin and Rimvall, 1993). Furthermore, the recent demonstration of a lower threshold for chemically-induced seizures in genetically-altered $GAD_{65}$ (-/-) mice (Asada et al., 1996) suggests that the synthesis of GABA by the lower molecular weight form of GAD is more directly involved in mediating synaptic inhibition.

Unlike $GAD_{67}$, the activity and protein levels of $GAD_{65}$ are not regulated by the intracellular levels of GABA (Rimvall et al., 1993; Rimvall and Martin, 1994). However, 50% of $GAD_{65}$ is found as apoenzyme, that is as inactive GAD without covalently bound PLP (apoGAD) (Kaufman et al., 1991). The conversion from the inactive to the active form of the enzyme appears to be regulated by the levels of adenosine 5'-triphosphate (ATP) and inorganic phosphates ($P_i$), with increases in the former favoring the inactivation of the enzyme while increases in the latter favors its activation. Therefore, it has been suggested that alterations in the levels of ATP and $P_i$, which are likely to occur during an increase in

neuronal activity, may allow $GAD_{65}$ to respond to an increased or decreased transmitter demand during changes in neuronal activity (Martin and Rimvall, 1993).

Convulsions in experimental animals as well as in humans can be induced by compounds which interfere with the synthesis of GABA. Agents such as isoniazid, mercaptopropionic acid and aminooxyacetic acid cause a reduction in the synthesis of GABA by interfering with either the availability of the required GAD cofactor, PLP, or with the substrate, glutamate. In addition, infants fed a diet deficient in vitamin $B_6$ have an increased susceptibility to seizures that can be almost immediately reversed following the addition of pyridoxine to the diet. Given the importance of the synthesis of the inhibitory transmitter GABA for the control of CNS excitability, the design of compounds which would potentiate the enzymatic activity of GAD would be expected to produce an anticonvulsant effect. Such an effect has in fact recently been described for 3-alkyl GABA and 3-alkylglutamic acid analogues, which were demonstrated to cause an activation of GAD (Silverman et al., 1991; Taylor et al., 1992). In these same studies, the antiepileptics sodium valproate, milacemide and gabapentin were similarly shown to produce an increase in the activity of GAD suggesting that such a mechanism may contribute to the clinical actions of these drugs.

## 2.2 Degradation of GABA

GABA which has been taken back up into neuronal and glial cells, following its release from presynaptic terminals, is destined to be catabolized. The first step in this process is a transamination reaction catalyzed by the enzyme γ-aminobutyrate-α-oxoglutarate aminotransferase (GABA transaminase, GABA-T, E.C. 2.6.1.19) which proceeds as follows: the α-amino group of GABA is transferred to the α-carbon of α-oxoglutarate thus transforming GABA into succinic semialdehyde (SSADH), and 2-oxoglutarate into glutamate. Given the presence of a large metabolic pool of SSADH, GABA could theoretically be

generated by the reversal of the transamination reaction. However, this does not seem to occur *in vivo* since succinic semialdehyde is rapidly converted, by the enzymatic activity of succinic semialdehyde dehydrogenase, to succinate, which then enters the tricarboxylic acid pathway (TCA, Krebs cycle). Since the precursor for GABA, glutamate, can be formed from α-oxoglutarate through a transamination reaction with glutamine, the portion of the Krebs cycle which normally converts α-oxoglutarate to succinate can be bypassed through the GABA metabolic pathway. This route, referred to as the "GABA shunt", can therefore allow GABA or glutamate to serve as an alternate energy source at, however, a cost since this pathway yields 25% less energy than the Krebs cycle (3 ATP equivalents versus 3 ATP + 1 GTP for the Krebs cycle).

GABA-T has been purified to homogeneity from several species (Schousboe et al., 1973; John and Fowler, 1976), including man (Cash et al., 1974). Biochemical studies of its properties have revealed that the brain enzyme consists of two identical subunits with a total molecular weight of ~109 kDa. As with GAD, the activity of GABA-T requires PLP as a cofactor. The existence of multiple forms of the enzyme, including a "cytoplasmic" and "synaptosomal" form, has been proposed based on subcellular fractionation techniques. Each of these forms differed from the other with respect to their affinity for GABA, optimal pH and inhibition by GABA analogs (Buu and van Gelder, 1974; Tunnicliff et al., 1977). Although other groups have failed to isolate more than a single form of GABA-T from highly purified preparations (Schousboe et al., 1973; Maître et al., 1975; John and Fowler, 1976) the existence of multiple forms of the enzyme is supported by the isolation, from a rat hippocampal cDNA expression library, of two mRNAs of different lengths (~2.1 and 6.4 kb long). Both of these mRNA species are proposed to originate from a single gene (Medina-Kauwe et al., 1994). Examination of the deduced amino acid sequence for GABA-T reveals the presence of a putative mitochondrial targeting sequence within the first 27 amino acids of the N-terminal domain (Kwon et al., 1992; Medina-Kauwe et al., 1994). The presence of such a targeting sequence is consistent with studies

which have localized GABA-T to the inner mitochondrial membrane (Schousboe et al., 1977).

### 2.2.1 GABA-T as a target for antiepileptic drug therapy

The elucidation of the chemical reactions catalyzed by GABA-T during the catabolism of GABA allowed for the initial design of compounds capable of inhibiting this process. As stated previously, the enzymatic activity of GABA-T has an absolute requirement for PLP. Such a dependence is due to the presence, within the structure of PLP, of a reactive aldehyde group which is capable of forming a covalent intermediate (Schiff-base) with the α-amino group of GABA. This allows PLP to act as a carrier of the amino group from one reactant (GABA) to the other (α-oxoglutarate) during transamination. The key to the blocking action of the first compounds designed to inhibit GABA-T therefore resided in their ability to form a more stable Schiff-base with PLP than could GABA itself. As such, these compounds were termed aldehyde antagonists or carbonyl-trapping agents. Numerous examples of these reagents were introduced including hydroxylamine (Baxter and Roberts, 1961), hydrazine (Medina, 1963), as well as the more widely studied aminooxyacetic acid (AOAA) (Kuriyama et al., 1966).

Although an anticonvulsant effect, which coincided with elevated GABA levels, could be demonstrated following the *in vivo* administration of low doses of either AOAA or hydrazine, higher doses were observed to result in a proconvulsant effect. The opposite effects of low and high doses of carbonyl-trapping agents have been attributed to their lack of specificity. Indeed, in addition to serving as a prosthetic group for numerous transaminases involved in the catabolism of several amino acids (alanine, arginine, asparagine, aspartate, cysteine, isoleucine, leucine, phenylalanine, tryptophan, tyrosine, and valine), PLP has been implicated in decarboxylation reactions as well, and in this respect is most notably, as mentioned above, essential for the activity of GAD. It

therefore becomes apparent that carbonyl-trapping agents, which react with PLP, will display very little selectivity in their inhibition of enzymatic activity. Nevertheless, the ability of PLP-inactivating agents to produce an increase in brain GABA content despite causing an inhibition of GAD, was thought to reflect a higher sensitivity of GABA-T to these agents compared to GAD (Metcalf, 1979). Such a relatively selective inhibitory action therefore allowed low concentrations of these agents, which caused minimal inhibition of GABA synthesis, to produce an anticonvulsant effect and gave hope that the discovery of more selective GABA-T inhibitors could lead to the development of novel antiepileptics.

### 2.2.2 Enzyme-activated irreversible inhibitors of GABA-T: Development of GVG

Variably referred to as "$K_{cat}$" inhibitors or "suicide enzyme inactivators", compounds which produce an irreversible inhibition of GABA-T following the activation of a latent reactive group were first described over 25 years ago by Fowler and John with their introduction of ethanolamine-*o*-sulfate (EOS) (Fowler and John, 1972). In contrast to the carbonyl-trapping agents, which cause an irreversible inactivation of the GABA-T coenzyme PLP, $K_{cat}$ inhibitors are transformed by the target enzyme's own mechanism of action into reactive species (alkylating agent) which irreversibly inactivate the enzyme by forming a covalent bond with a nucleophile present within the active site. Since the inactivator must be accepted by GABA-T as a substrate, in a manner comparable to GABA itself, the inhibitory actions of compounds possessing a mechanism similar to that of EOS are expected to be highly specific. Consistent with this expectation, EOS was demonstrated to possess no *in vitro* inhibitory effects on the activity of GAD, alanine aminotransferase or aspartate aminotransferase (Fowler and John, 1972; Fowler, 1973). However, the relatively poor ability of EOS to penetrate the blood-brain-barrier following its systemic administration, as evidenced by the necessity of using large doses in order to influence the activity of the CNS enzyme (Löscher, 1981), as well as the demonstration that this

compound is a rather weak inhibitor of GABA-T ($IC_{50}$ = 3.6 mM) (Löscher, 1980b), may have precluded its further development as an antiepileptic.

The successful demonstration that rationally designed enzyme-activated irreversible inhibitors of GABA-T could achieve a high degree of specificity encouraged the further development of mechanistically similar compounds and led to the introduction in the mid to late 70s of γ-acetylenic GABA (4-amino-5-ynoic acid, GAG)(Jung and Metcalf, 1975) and γ-vinyl GABA (4-amino-hex-5-enoic acid, vigabatrin, GVG) (Lippert et al., 1977). Although both of these compounds were demonstrated to selectively inhibit the transamination of GABA, GAG, but not GVG, was also shown to inhibit the decarboxylation of glutamate by GAD (Jung et al., 1977b). Such inhibition, although unexpected at the time, is thought to occur due to the acceptance of GAG as a substrate for the reversed reaction of GAD, that is, the potential carboxylation of GABA leading to the formation of glutamate (Metcalf, 1979). Although both GAG and GVG have been demonstrated to produce elevated GABA levels, which are believed to protect animals against experimentally-induced convulsions, the inhibition of the synthesis of GABA by GAG is thought to explain the more frequent proconvulsant effects observed in animals at higher doses of this drug. Thus, as a result of its greater selectivity, the neurochemical and anticonvulsant properties of GVG have been much more extensively studied, in both animals and humans, than GAG.

### 2.2.3 Preclinical pharmacology of GVG

Although GVG is typically synthesized and administered as a racemic *R, S* mixture, only the *S*(+) enantiomer possesses pharmacological activity. Such selectivity in the activity of GVG's enantiomers has been demonstrated with respect to both the inhibition of GABA-T (Larsson et al., 1986; Gram et al., 1989) as well as the high affinity uptake of the enantiomers into neurons and astrocytes (Schousboe et al., 1986). The uptake of GVG into neurons and astrocytes is an

obvious necessary step for inhibition of the intracellularly located GABA-T to occur. In this respect, it is interesting to note that the affinity of the transport system for GVG is much greater in neurons than in astrocytes (Schousboe et al., 1986), a finding which may explain the previously observed preferential inhibition of the neuronal GABA-T over that found in astrocytes (Larsson et al., 1986).

Due to its relatively poor ability to penetrate the CNS following systemic administration, large doses of GVG (1500 mg/kg compared to only 100 mg/kg for GAG) are needed to produce a substantial (> 500%) increase in whole brain GABA levels by a single intraperitoneal (i.p.) injection in mice. The time course of the rise in GABA levels following such an acute single injection of GVG has been characterized (Schechter et al., 1977). The maximum rise in whole brain GABA levels (> 500% over control) occurs approximately 4 hours following injection. These elevated levels are well maintained for over 24 hours and up to 5 days are needed for the concentration of GABA to return to its control level. Given that the brain levels of GVG peak within 1 hour of its administration and then decline rapidly with an elimination half life of 5-8 hours (Bernasconi et al., 1988), the prolonged duration of GVG's neurochemical actions are therefore due to the irreversible nature of its block of GABA-T. Therefore, the recovery of GABA-T activity most likely necessitates the synthesis of new enzyme and thus occurs with a time course of several days (Larsson et al., 1986).

Testing of the anticonvulsant effectiveness of GVG in mice susceptible to audiogenic seizures (DBA/2) demonstrated that the seizure protection afforded by a single i.p. injection of GVG was well correlated with the maximum percent increase in GABA levels. Accordingly, complete protection from seizures in 50% of animals ($ED_{50}$) was observed at a dose of 990 mg/kg of GVG which raised the levels of GABA by 324% (Schechter et al., 1977). In addition to evaluating its anticonvulsant potential in animal models of spontaneously occurring seizures, the potency of GVG has been measured in numerous animal tests of anticonvulsant effectiveness. These experiments demonstrated that when given as a single i.p. injection, large doses of GVG (750-2000mg/kg) were necessary to protect animals from experimentally-induced seizures (Sarhan and Seiler, 1979;

Löscher, 1980a; Shin et al., 1986; Bernasconi et al., 1988). In contrast, when GVG was administered repeatedly, a marked reduction in the dose necessary for seizure protection was observed (20-400mg/kg)(Schechter et al., 1977; Löscher, 1982). The increased anticonvulsant effectiveness most likely occurred as a result of the much greater inhibition of GABA-T, with correspondingly higher concentrations of brain GABA, achieved following the repeated administration of GVG in rats (Valdizán and Armijo, 1991; Valdizán and Armijo, 1992). Since GVG penetrates the blood-brain-barrier only poorly following a single dose, the possibility exists that the drug may accumulate in the brain during repeated dosing thereby allowing a given dose of GVG to exert a more pronounced effect. Consistent with this hypothesis, the cerebrospinal fluid (CSF) concentration of GVG reaches only 10-15% of blood levels following the first dose and increases further during daily dosing of epileptic patients (Ben-Menachem, 1989). Finally, another possible explanation for the increased actions of GVG is that due to the irreversible nature of its effect on GABA-T, the enzyme may accumulate in an inactivated state during repeated dosing.

### 2.2.4 Human studies with GVG

#### *A) Pharmacokinetics of GVG*

The pharmacokinetics of GVG have been studied in detail (Grove et al., 1984; Haegele and Schechter, 1986; Saletu et al., 1986; Mumford, 1988; Frisk-Holmberg et al., 1989; Schechter, 1989; Hoke et al., 1993). The absorption of GVG from the gastrointestinal tract is unaffected by food and proceeds rapidly and almost completely. Peak plasma level occurs within the first two hours following oral administration of 0.5-3 g of GVG and the absorption half-life has been estimated to range from 0.18 to 0.59 hours. Most likely as a result of its inability to bind with GABA-T, the inactive (R(-)) enantiomer of GVG has been observed to reach higher peak plasma levels than the drug's active (S(+)) form. Given the fact that GVG is administered as a racemic mixture, it is important to

point out that studies of the pure S(+) form of GVG have indicated that the pharmacokinetics of the active form are not influenced by R(-) GVG. In addition, no R(-) GVG is detected following the administration of pure S(+) GVG indicating that chiral inversion to the inactive form does not occur *in vivo*. Once absorbed, the apparent volume of distribution of GVG is 0.8 L/kg and its bioavailability is at least 60-80%. GVG does not bind to plasma proteins, nor does it cause hepatic induction through the cytochrome P450-dependent enzymes. As a result, GVG does not appear to significantly interact with other simultaneously administered antiepileptic drugs. The only exception being a 20-30% reduction in the plasma concentrations of phenytoin following addition of GVG to the therapy (Browne et al., 1987; Rimmer and Richens, 1989). The mechanism underlying this reduction is not currently known. The elimination of GVG from plasma follows a biexponential decay with a reported half-life of approximately 7 hours. Elimination occurs primarily through renal excretion and up to 80% of GVG can be recovered unchanged within 24 hours in a patient's urine with no detectable metabolites.

***B) Effects of GVG on CSF amino acid levels***

Due to both the irreversible nature of GVG's actions and its short elimination half-life, the duration of the resulting inhibition of GABA-T far outlasts the presence of GVG in plasma. As a result, no correlation is found between the plasma concentrations of GVG and its clinical effect (Gram et al., 1985; Browne et al., 1987; Cocito et al., 1989). Animal studies have previously demonstrated a linear correlation between the increased levels of GABA in the brain and CSF following GVG treatment. Thus, given the impossibility in humans of directly measuring the concentration of GABA in the brain, the CSF levels have been monitored in an attempt to correlate therapeutic effectiveness with a biochemical marker of GVG's activity. These studies typically measured the levels of free GABA, total GABA (i.e. GABA detected following hydrolysis of CSF), homocarnosine (conjugate of GABA and histidine) and β-alanine, which can

serve as an alternate substrate for GABA-T. Dose-related increases in each of these parameters have been demonstrated in patients treated with 1 to 6 g/day of GVG. The CFS levels of acetylcholine, somatostatin, β-endorphins, prolactin, cAMP or cGMP were unaltered (Pitkänen et al., 1987; Sivenius et al., 1987) and, in addition, no changes were detected in the levels of either homovanillic acid (HVA, the metabolite of dopamine), 5-hydroxyindoleacetic acid (5-HIAA, the metabolite of serotonin) or of the excitatory amino acids (Schechter et al., 1984; Riekkinen et al., 1989a; Sivenius et al., 1987; Ben-Menachem et al., 1991), indicating a fairly specific interaction of GVG with the GABAergic system. More recently, the brain levels of GABA have been estimated more directly using nuclear magnetic resonance spectroscopy. Using this technique, a close correlation was demonstrated between GVG dosage and brain GABA levels (Mattson et al., 1994; Petroff et al., 1996; Petroff and Rothman, 1998). As a general rule, these studies have demonstrated that GVG causes an increase in the levels of GABA in the CNS of epileptic patients which is associated with a decrease in seizure frequency (Riekkinen et al., 1989b; Ben-Menachem, 1989).

### *C) Anticonvulsant efficacy of GVG in humans*

The international clinical experience with GVG has grown tremendously since the initial single-blind, placebo controlled studies were performed (Gram et al., 1983; Schechter et al., 1984). Results from over 22 North American and European single or double-blinded as well as several open long-term studies have now been described. These studies have typically been carried out in previously drug-resistant patients suffering from complex partial seizures, with or without secondary generalization. In these patients, GVG is added to the existing therapy, which can include anywhere from 1 to 3 of the established antiepileptic drugs (carmabazepine, phenytoin, valproic acid, benzodiazepines and barbiturates). Therefore, a demonstration of anticonvulsant effectiveness in these difficult to treat patients is all the more impressive.

The therapeutic response of patients treated with GVG is remarkably similar across the numerous trials that have been carried out. A reduction in seizure frequency of at least 50% is typically observed in approximately 50% of patients (Gram et al., 1983; Tartara et al., 1986; Tassinari et al., 1987; Browne et al., 1991; Ylinen et al., 1995). Furthermore, these studies showed that GVG is much more effective in reducing the frequency of partial seizures than of those of other types (Tassinari et al., 1987; Michelucci and Tassinari, 1989). Similarly, in 135 children, response rates during treatment with GVG were better in partial seizures compared to those obtained when treating generalized seizures (Livingston et al., 1989). In addition to being effective in the treatment of partial seizures, GVG has proven itself to be highly efficacious in treating a number of specific childhood syndromes and seizure types. In particular, high response rates have been observed in pediatric patients suffering from infantile spasms (West syndrome). In these patients, treatment with GVG results in a greater than 50% reduction of spasms in 50-100% of patients (Chiron et al., 1991; Aicardi et al., 1996). These results are all the more impressive given the fact that West syndrome is one of the most difficult types of epilepsy to treat. Results have been especially impressive in patients suffering from symptomatic infantile spasms due to tuberous sclerosis. In fact, a recent study reported complete resolution of spasms in 100% (11/11) of patients treated with GVG compared to only 45% (5/11) of patients receiving the reference drug, hydrocortisone (Chiron et al., 1997). These authors went on to suggest that GVG should be considered as the first choice treatment for infantile spasms due to tuberous sclerosis.

Although animal studies have suggested that tolerance to the anticonvulsant effects of GVG may develop during repeated dosage (Löscher and Frey, 1987; Rundfeldt and Löscher, 1992), long-term follow-up studies in humans have, in contrast, demonstrated that the therapeutic response is well maintained in patients receiving GVG for up to 7 years (Pedersen et al., 1985; Dam, 1989; Tartara et al., 1989; Tartara et al., 1992; Cocito et al., 1993; Ylinen et al., 1995). Furthermore, these studies demonstrated that GVG is generally well tolerated, with the most commonly reported side effects being mild

drowsiness, fatigue, dizziness and weight gain. Interestingly, the side effects associated with GVG therapy are frequently transient and disappear after the first few weeks of treatment. Of graver concern however are the reports of increased confusion and psychotic reactions which have been described in 3-6% of patients receiving GVG (Browne et al., 1987; Ben-Menachem et al., 1990; Sander et al., 1990). In all cases, the psychiatric symptoms disappeared upon discontinuation of the drug. More recently, vigabatrin-associated visual disturbances, such as constricted visual fields or blurred vision have been reported (Krauss et al., 1998). The cause of such effects are not yet known and their prevalence in a larger group of patients remains to be determined.

### 2.2.5 Mechanism of GVG's anticonvulsant action

GVG is one of the first drugs introduced to market that was developed through a rational drug design approach and has repeatedly been said to be one of the few antiepileptic drugs whose mechanism of action, namely inhibition of GABA-T, has been clearly defined (Richens, 1989; Rogawski and Porter, 1990; Guberman, 1996). Although the results presented in the previous sections are consistent with a mechanism by which GVG produces its anticonvulsant effect by raising CNS levels of GABA following the irreversible inhibition of GABA-T, the possibility that GVG may act at additional sites within the GABAergic neurotransmitter system must not be underestimated given the fact that GVG is a close structural analog of GABA. In fact, additional effects on the GABAergic system have been reported in a number of studies of the neurochemical actions of GVG. As stated previously, GVG is a substrate for the GABA-transporter. It is therefore not surprising that GVG has been reported to act as an inhibitor of the uptake of GABA (Löscher, 1980b; Schousboe et al., 1986; Jolkkonen et al., 1992). Furthermore, as with other compounds which act as substrates for the GABA transporter, GVG possesses GABA-releasing properties (Abdul-Ghani et al., 1981) which, in addition to its GABA uptake blocking properties, may contribute to the reported rapid rise in the extracellular levels of GABA during

GVG administration as studied by microdialysis (Jolkkonen et al., 1992). Recently, a study of the effects of GVG on the GABA stimulated net uptake of $^{36}Cl^-$ into cortical membrane vesicles suggested that, over a concentration range of 100-1000 µM, GVG may act as a non-competitive antagonist of the $GABA_A$ receptor complex (Suzuki et al., 1991), an action expected to exacerbate, rather than prevent seizures. Although the use of receptor binding methods showed very little inhibition of the binding of GABA to its receptors ($IC_{50}$ > 1mM)(Löscher, 1980b), the possibility that GVG produces an antagonism of the $GABA_A$ receptor through one of its associated modulatory binding sites remained to be investigated.

Together, these results amply demonstrate that other mechanisms, in addition to inhibition of GABA-T, may contribute to the reported effects of GVG following its administration in animal as well as in human studies. The elucidation of the functional consequences of these neurochemical actions was therefore an additional objective of this thesis.

## 2.3 GABA receptors

### 2.3.1 $GABA_A$ receptors: Structure and function

The $GABA_A$ receptor is a macromolecular protein which, in addition to forming an intrinsic chloride ion-selective channel, possesses several binding sites for clinically useful pharmacological agents (barbiturates, benzodiazepines and anesthetic steroids) and experimental convulsants (bicuculline, penicillin and picrotoxin to name only a few). It belongs, along with the nicotinic acetylcholine receptor (nAchR), the inhibitory glycine receptor and the $5\text{-}HT_3$ receptor, to a superfamily of neurotransmitter-gated ion channels which mediate fast synaptic transmission in the CNS. The deduced amino acid sequence of the subunits from which these receptors are formed share significant sequence identity (20-30%) and thus appear to be evolutionarily related. Structural motifs, first proposed for the nAchR (for review see Devillers-Thiéry et al., 1993) and by

analogy believed to be common to all members of this superfamily, have been identified, including: (1) a large N-terminal extracellular domain which is thought to be the site of agonist binding and which contains consensus *N*-glycosylation sites and two disulfide-linked conserved cysteine residues, (2) four putative membrane-spanning domains (TM1-TM4), with TM2 being involved in the formation of the channel wall, (3) a large intracellular loop between the third and fourth membrane spanning domains possessing consensus phosphorylation sites, and (4) an extracellularly located C-terminal domain. Furthermore, again by analogy with nAchR, these receptors are generally thought to be generated following the assembly of various combinations of subunits into heteropentameric isoforms.

Support for the pentameric structure of the $GABA_A$ receptor has been obtained from electrophysiological estimates of the channel's pore diameter. By comparing the relative permeabilities of poorly hydrated anions of varying spherical diameter, the $GABA_A$ receptor channel pore diameter was estimated to be about 6 Å. Assuming the channel forming transmembrane domains (TM2) of each subunit contributing to the formation of a receptor are made up of α-helices with an assumed diameter of 8.4 Å, then five such helices in close apposition would be needed in order to form a channel with a pore diameter of ~6 Å (Bormann et al., 1987). The pentameric structure of the $GABA_A$ receptor has recently been confirmed more directly using electron microscopic image analysis (Nayeem et al., 1994).

### *A) Subunit composition of $GABA_A$ receptors*

The $GABA_A$ receptor family has grown steadily since the cloning of its first two members (Schofield et al., 1987) and is now composed of 18 polypeptide subunits, including: six $\alpha$, four $\beta$, three $\gamma$, one $\delta$, one $\varepsilon$, and three $\rho$ (Macdonald and Olsen, 1994; Ogurusu and Shingai, 1996; Davies et al., 1997). Comparison of subunit amino acid sequences reveal 30-40% identity between the subunit

families and up to 70-80% identity amongst the members of each family (Olsen and Tobin, 1990; Cutting et al., 1991; Wang et al., 1994; Davies et al., 1997). Further diversity can be generated by alternative RNA splicing which has been identified for several of the GABA receptor subunits, the most ubiquitous being that of the $\gamma_2$ subunit ($\gamma_{2S}$ and $\gamma_{2L}$), the variants of which have intracellular loops differing in length by 8 amino acids. Thus, a bewildering degree of heterogeneity in the subunit composition of $GABA_A$ receptors has been uncovered by molecular cloning studies.

Given the random assembly of five subunits, selected from a repertoire of 18, into an intact receptor, the number of possible combinations of subunits would theoretically be well over a million ($18^5$). Fortunately, if any sense is ever to be made of the physiological role of $GABA_A$ receptor heterogeneity in the CNS, recent studies of $GABA_A$ receptors expressed in *Xenopus* oocytes and mammalian cell lines as well as studies of $GABA_A$ receptor subunit distribution using in situ hybridization and immunohistochemistry suggest that constraints in the manner in which subunits may assemble exist and that the actual number of $GABA_A$ receptors in the CNS is likely to be limited to approximately one or two dozen distinct isoforms.

As previously mentioned, endogenously expressed $GABA_A$ receptors have for a long time been known to be modulated by benzodiazepines. Although initial studies of recombinant receptors demonstrated that the coexpression of $\alpha$ and $\beta$ subunits alone was sufficient to reconstitute GABA receptors whose function could be potentiated by barbiturate, little if any modulation by benzodiazepines could be demonstrated (Schofield et al., 1987). The subsequent cloning, identification and coexpression with $\alpha$ and $\beta$ subunit subtypes of the $\gamma_2$ subunit demonstrated that the resulting receptors could now display the full pharmacological profile of endogenously expressed $GABA_A$ receptors, including modulation by benzodiazepines (Pritchett et al., 1989), suggesting that native receptors were composed of a minimum assembly of at least one $\alpha$, one $\beta$ and one $\gamma$ subunit. These studies could not however give any indication of the

stoichiometry of the expressed receptors. Therefore, given such a constraint on the formation of $GABA_A$ receptors, the possible number of distinct isoforms is reduced to approximately 20000 (6 $\alpha$s x 4 $\beta$s x 3 $\gamma$s x 18 x 18)

Studies of the distribution of the various subunits have demonstrated further restrictions in the number of possible $GABA_A$ receptor isoforms. The $\rho$ subunit subtypes have been shown to have a very limited distribution in the CNS and have most commonly been found in the retina (Cutting et al., 1991; Enz et al., 1995; Ogurusu et al., 1997). Although retinal cells have additionally been shown to express $\alpha$ and $\beta$ subunits, recent evidence suggests that $\rho$ subunits assemble exclusively with either other identical subunit subtypes (homooligomers) or with other members of the $\rho$ family (heterooligomers) (Hackam et al., 1997; Hackam et al., 1998). Expression of $\rho$ subunits in *Xenopus* oocytes revealed that the resulting receptors display a pharmacological profile (Wang et al., 1994; Shimada et al., 1992) which resembles that reported for the previously described retinal $GABA_C$ receptor (Qian and Dowling, 1993; Feigenspan and Bormann, 1994; Johnston, 1996). Thus, $\rho$ subunits are thought to be the molecular components of $GABA_C$ receptors. A similarly restricted distribution has been observed for the most recently cloned member of the $GABA_A$ receptor, the $\varepsilon$ subunit, which reportedly imparts an insensitivity to the potentiating effects of intravenous anesthetic agents when assembled with $\alpha$ and $\beta$ subunit subtypes (Davies et al., 1997). Indeed, although the expression of $\varepsilon$ was found to be low in whole brain samples from rat, this subunit was relatively enriched in the amygdala and thalamus, being particularly abundant in the subthalamic nucleus. Among the members of the $\alpha$ subunit family, the restricted distribution of $\alpha_4$ and $\alpha_6$ suggests that these subunits may contribute to a lesser extent to the formation of native receptors. The $\alpha_6$ subunit is found almost exclusively in the granule cells of the cerebellum (Persohn et al., 1992; Wisden et al., 1992), while expression of the $\alpha_4$ is largely limited to some regions of the thalamus and hippocampus (Wisden et al., 1992; Laurie et al., 1992).

Immunoprecipitation studies using subunit-specific antibodies have revealed further restrictions for the assembly of $GABA_A$ receptors. The δ subunit, which, when assembled with α and β subunits, forms a receptor having a reduced sensitivity to benzodiazepines while displaying higher affinity binding for muscimol when compared to γ containing receptors (Quirk et al., 1995), appears to be relatively enriched in the cerebellum (Quirk et al., 1995). Furthermore, comparison of the distribution of δ and γ mRNA indicates that these subunits are present in distinct neuronal populations (Shivers et al., 1989). This suggests that the expression of these two subunits is largely mutually exclusive and that δ subunits may substitute for γ in the assembly of $GABA_A$ receptors.

Finally, although the $\beta_4$ subunit subtype has been identified in chick (Bateson et al., 1991), a mammalian homologue has yet to be identified. Thus, we are left with four α, three β and four γ/δ subunits that are likely to make up a majority of $GABA_A$ receptors in the mammalian CNS. If, as has recently been suggested (Chang et al., 1996; Tretter et al., 1997), the stoichiometry of the pentameric receptor is 2α2β1γ/δ then, assuming no further restrictions in the manner in which the subunits may combine, a total of 576 (4 αs x 4 αs x 3 βs x 3βs x 4 γ/δs) receptor combinations would be possible. However, a consideration of the evidence gathered from experiments analyzing the subunit composition of native receptors has lead to the recent suggestion that there are probably less than ten major subtypes of $GABA_A$ receptors (McKernan and Whiting, 1996), the most ubiquitous of which appears to be the $\alpha_1\beta_2\gamma_2$, proposed to account for up to 43% of $GABA_A$ receptors in the rat brain.

### *B) Properties of GABA-gated chloride channels*

Concentration-response curves of whole-cell currents induced following the application of GABA have revealed that the $EC_{50}$ (effective concentration causing a response which is 50% of the maximal) values for GABA are highly

variable from one region of the CNS to the next as well as, within any given region, from one neuronal population to the next (White, 1992; Schönrock and Bormann, 1993; Feigenspan and Bormann, 1994). Such variability is likely a reflection of the heterogeneity of $GABA_A$ receptors. In these studies, the GABA concentration-response curves were also shown to generally exhibit a Hill coefficient of approximately 2. A Hill coefficient having a value which is greater than unity indicates the existence of positive cooperativity, whereby the binding of one agonist molecule facilitates the subsequent binding of a second agonist molecule, between multiple binding sites. Its maximum value can never exceed the number of binding sites within a receptor complex and thus a Hill coefficient of 2 suggests that the binding of two GABA molecules are required in order to fully activate the $GABA_A$ receptor (Sakmann et al., 1983; Bormann and Clapham, 1985).

Ion substitution experiments which, as alluded to earlier, permitted an estimate of the $GABA_A$ receptor channel pore diameter have also allowed a determination of the ionic selectivity of the channels. Measurements of single-channel conductances recorded in the presence of equal transmembrane concentrations of small anions demonstrated a conductance sequence of $Cl^- > Br^- > I^- > SCN^- > F^-$ which was almost exactly the reverse order of the permeability sequence ($SCN^- > I^- > Br^- > Cl^- > F^-$). The inversion of these sequences was interpreted as being indicative of the existence of permeant ion binding to sites located within the channel pore (Bormann et al., 1987) and suggested that the channel is not simply a water-filled pore which acts as a molecular sieve limiting access through the channel to anions displaying a molecular diameter less than that of the channel pore. Of the small anions, it is clear that, under physiological conditions, $GABA_A$ receptor-mediated currents will be predominantly carried by the translocation of $Cl^-$ ions. As discussed above, the estimates of $GABA_A$ receptor channel pore diameter were based on measurements of the relative permeability of large, poorly hydrated, polyatomic anions which demonstrated the following permeability sequence: formate > bicarbonate ($HCO_3^-$) > acetate > phosphate > propionate. Again, of these, the only anion likely to play a

physiological role is $HCO_3^-$, which as shown by Bormann et al. and others (Akaike et al., 1989; Kaila et al., 1989; Mason et al., 1990) has a permeability of ~0.2 relative to that of $Cl^-$.

$HCO_3^-$ ions are present both intracellularly and extracellularly where their concentrations are regulated by the enzymatic activity of carbonic anhydrase, which catalyzes the following reaction:

$$CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^-$$

The enzymatic regulation of the intracellular and extracellular concentrations of $HCO_3^-$ generates a reversal potential which is much more positive than the resting membrane potential of neurons, indicating that under physiological conditions flux of $HCO_3^-$ through $GABA_A$ receptors would be outwardly directed resulting in an inward current. The first indication that $HCO_3^-$ could contribute to the physiological actions of GABA came from experiments performed on crayfish muscle fibers where GABA-mediated depolarizations were shown to be both blocked by the specific $GABA_A$ receptor blocker, picrotoxin, and carried by $HCO_3^-$ ions (Kaila et al., 1989). Similarly, recent experiments in rat neocortical neurons showed that alterations of the intracellular pH (and thus of the intracellular concentration of $HCO_3^-$) caused shifts in the reversal potential of $GABA_A$ receptor-mediated IPSPs indicating that $HCO_3^-$ may contribute to GABAergic neurotransmission in the mammalian CNS (Kaila et al., 1993). Interestingly, application of the convulsant compound, 4-aminopyridine (4-AP), has been shown to induce spontaneously occurring, synchronous activity which corresponded to an intracellularly recorded long-lasting, $GABA_A$ receptor-mediated depolarization (Perreault and Avoli, 1992). Consistent with a mechanism involving the extrusion of $HCO_3^-$ through $GABA_A$ receptors, such GABA-mediated spontaneous activity was demonstrated to be associated with alkaline transients in extracellular pH (Lamsa and Kaila, 1997). Together with experimental evidence demonstrating the anticonvulsant properties of the carbonic anhydrase inhibitor, acetazolamide, these results were interpreted as suggesting that GABA-mediated mechanisms may, under some circumstances, contribute to epileptogenesis.

The kinetics of the gating of $GABA_A$ receptors have been studied in great detail using fluctuation (or noise) analysis (Barker and Mathers, 1981; Barker et al., 1982; Cull-Candy and Usowicz, 1989; De Koninck and Mody, 1994) as well as, more directly, using the patch clamp technique under conditions which permit the recording of currents generated by single channels (Hamill et al., 1983; Bormann et al., 1987; Macdonald et al., 1989; Twyman et al., 1990; Newland et al., 1991; Kaneda et al., 1995). In both recombinant as well as native $GABA_A$ receptors numerous single-channel current amplitudes have been observed, indicating that the receptor-gated channels can open to multiple (2-4) conductance levels. Nevertheless, a main conductance level, defined from the average amplitude of the most frequently occurring current step, can typically be identified that accounts for over 90% of the current through the channel. The reported main state conductance from single-channel recordings is typically 16-30 pS, almost indistinguishable from the values of 12-28 pS estimated from fluctuation analysis. The molecular basis and physiological function of the additional conductance states remain unknown.

Since the transition of the $GABA_A$ receptor-gated channel from an opened to a closed state occurs rapidly, the onset and offset of single-channel currents appear instantaneous such that square current pulses are recorded. Furthermore, since the duration of channel openings is variable and separated by equally variable closing events, the recorded currents have a burst-like appearance. Plots of the open and closed time distributions are typically best fit by the sum of multiple exponentials, suggesting that $GABA_A$ receptors enter into multiple open and closed states. Based on these observations of the main state conductance gating behavior, a model has been proposed which can account for the complex behavior of $GABA_A$ receptor-gated channels (Twyman et al., 1990). The model incorporates two binding sites to which GABA binds in a sequential manner, three open states ($O_{1-3}$), the first of which ($O_1$) corresponds to openings of the channel from a singly liganded closed state, and ten closed states ($C_{1-10}$). In keeping with the consistent observation of a progressive decline in the amplitude of $GABA_A$ receptor-mediated currents in the continued presence of

agonist, the model also includes a desensitized state. Such a model has contributed to a greater understanding of the manner in which the anticonvulsant benzodiazepines and barbiturates produce an enhancement of $GABA_A$ receptor function.

### *C) Anticonvulsant therapies aimed at enhancing $GABA_A$ receptor function*

Drugs acting at the $GABA_A$ receptor complex can be divided into two broad categories based on their mechanisms of action: (1) those which produce a direct activation of the receptor through the agonist binding site, and (2) those which allosterically enhance neurotransmitter-mediated actions at the receptor binding site. Allosteric modulation of $GABA_A$ receptor function, as exemplified by the actions of the barbiturates and benzodiazepines, has proven to be the most successful mechanistic approach to producing an enhancement of $GABA_A$ receptor-mediated inhibition. The barbiturate phenobarbital was first introduced into clinical practice in 1912 (Hauptmann, 1912) as an effective treatment against seizures, while the first benzodiazepine, diazepam, was approved for adjunctive therapy in 1968.

Both the barbiturates and benzodiazepines have been shown to produce an increase in the amplitude as well as the duration of $GABA_A$ receptor-mediated synaptic responses (Barker and McBurney, 1979; Segal and Barker, 1984; Weiss and Hablitz, 1984; De Koninck and Mody, 1994). Although their effects on GABAergic transmission are qualitatively similar, the mechanisms by which they enhance GABA function are distinct and characteristic of each group of compounds. The barbiturates have been shown to increase the mean duration of channel openings without altering their frequency of occurrence. In addition, they are known to cause, in higher concentration, a direct activation of the receptor complex, even in the absence of GABA. In contrast, the benzodiazepines produce an increase in the frequency of channel openings while having no effect on their duration. Both the barbiturates and benzodiazepines have generally been reported to have no effect on single

channel conductance. Recently, however, a study of $GABA_A$ receptors in cultured rat hippocampal neurons demonstrated that diazepam could produce a several-fold increase in the single channel conductance (Eghbali et al., 1997). The actions of both groups of compounds have been explained in relation to the proposed model of $GABA_A$ receptor gating. The barbiturates have been proposed to increase mean open time by favoring the transition of the channels to the open state having the longest duration ($O_3$) over those having shorter duration ($O_1$ and $O_2$). On the other hand, the increase in the frequency of channel openings caused by benzodiazepines is attributed to either an enhancement of the association rate for GABA at the first, but not the second, binding site or a reduction in the rate of entry of the channel into its desensitized state.

Although the barbiturates and benzodiazepines effectively control numerous seizure types, the development of tolerance and unacceptable side-effects associated with their chronic clinical use has created an increasingly negative attitude with respect to their safety as therapeutic agents (Rogawski and Porter, 1990; Lader, 1995). In the case of the benzodiazepines, there is hope that novel avenues for development of benzodiazepine ligands may yet lead to efficacious compounds capable of controlling seizures while minimizing side effects and the development of tolerance. This optimism is based on the demonstration that partial benzodiazepine agonists, such as imidazenil, can produce an anticonvulsant action associated with little if any side effects or development of tolerance (Giusti et al., 1993; Zanotti et al., 1996). Finally, recent evidence suggests that epileptiform activity can produce important changes in the subunit composition of native $GABA_A$ receptors (Buhl et al., 1996; Gibbs, et al., 1997; Schwarzer et al., 1997; Sperk et al., 1997) resulting in the expression of receptors displaying an altered sensitivity to benzodiazepine ligands with relatively subunit-selective actions. Therefore, these results suggest that novel benzodiazepines capable of selectively acting upon the aberrant $GABA_A$ receptors expressed in epileptic tissues could be developed which, by nature of

their specificity, would be expected to produce a relatively selective anticonvulsant action.

### 2.3.2 $GABA_B$ Receptors: Structure and Function

The existence of $GABA_B$ receptors was first proposed based on the selective ability of a population of bicuculline-insensitive GABA receptors to bind with high affinity the radiolabeled compound β-p-chlorophenyl-GABA (baclofen). In contrast to the $GABA_A$ receptor which, as previously mentioned, directly gates an intrinsic $Cl^-$-permeable ion channel and mediates fast synaptic inhibition, the $GABA_B$ receptor modulates both pre and postsynaptic conductances through an indirect coupling of the receptor to its effector channels via pertussis toxin-sensitive heterotrimeric G proteins. The modulation of ion channel function by these receptors has been shown to result in either an increase in the conductance of K+ channels or a decrease in the conductance of those that are permeable to calcium. In general, the postsynaptic $GABA_B$ receptor is positively coupled to K+ channels and its physiological activation is thought to underlie the slow component of IPSPs. In contrast, negative coupling of the presynaptic receptor with calcium channels is believed to be the mechanism which allows $GABA_B$ receptors to control the release of various neurotransmitters. The $GABA_B$ receptor has, in addition, been shown to modulate the activity of both adenyl cyclase (Wojcik and Neff, 1984; Knight and Bowery, 1996) and phospholipase $A_2$ (Duman et al., 1986) as well as the turnover of phosphotidyl inositol (Crawford and Young, 1988; Smith and Li, 1991).

Several lines of evidence demonstrating the G protein coupling of the $GABA_B$ receptor to its effector sites strongly suggested that this receptor was a member of a family of metabotropic receptors which characteristically possess seven putative transmembrane spanning domains. The recent expression cloning of two $GABA_B$ receptor variants, believed to be derived from the same gene through alternative splicing and termed $GABA_BR1a$ and $GABA_BR1b$ (Kaupmann et al., 1997), has allowed a greater insight into the structure of this

receptor. While $GABA_BR1a$ encodes a protein of 960 amino acids with a predicted molecular weight of 106K, $GABA_BR1b$ encodes for a protein of 844 amino acids with a predicted molecular weight of 92K. The predicted molecular weights derived from each cDNA clone closely corresponded to those of two putative, widely expressed $GABA_B$ receptor proteins of 130K and 100K identified by photoaffinity labeling using a novel high affinity antagonist [$^{125}$I]CGP71872 (Kaupmann et al., 1997). Hydropathy profiles derived from the amino acid sequence of each cDNA clone confirmed the presence of seven transmembrane domains and indicated that the predicted transmembrane topology of the receptor was very similar to that of the metabotropic glutamate receptors (mGluR). The cloned cDNAs were demonstrated to share 18-23% amino acid sequence identity and 43-48% related residues with the mGluR suggesting that both of these receptors form a common gene family. In addition to its seven transmembrane domains, $GABA_BR1a/b$ are predicted to have a large N-terminal extracellular domain containing several *N*-glycosylation sites and proposed to be the site of agonist binding. *In situ* hybridization revealed that $GABA_BR1a/b$ is ubiquitously distributed with abundant levels of transcripts observed in all layers of cerebral cortex as well in the pyramidal and granule cell layers of the hippocampus.

### *A) Pharmacology of $GABA_B$ receptors*

As mentioned above, the use of baclofen, a selective agonist of the $GABA_B$ receptor, was instrumental in first identifying this GABA receptor subtype. Baclofen, a structural analog of GABA, was first synthesized in 1962 and later introduced into clinical practice as an effective treatment against spasticity resulting from multiple sclerosis or spinal cord injury. Although baclofen remains the most commonly utilized agonist of $GABA_B$ receptors, others have been synthesized which display a higher affinity for this receptor. The compounds (3-aminopropyl)phosphinic acid (3-APA) (Hills et al., 1989) and its methyl derivative (3-aminopropyl)methylphosphinic acid (Howson et al., 1993) were recently

developed and shown to possess three- to seven-fold greater potency at $GABA_B$ receptors than baclofen. Most recently the compound (3-amino-2(*S*)-hydroxypropyl)methylphosphinic acid was synthesized and demonstrated to be more potent than baclofen in producing muscle relaxation while producing fewer side effects in rats and was thus chosen as a development compound for the treatment of spasticity (Froestl et al., 1995).

Many more antagonists than agonists of the $GABA_B$ receptors have been synthesized over the years. The first of these was the phosphinic analog of baclofen, phaclofen (Kerr et al., 1987). Although phaclofen had a relatively low affinity for the receptor, it was effectively utilized to characterize the slow-component of IPSPs as being mediated by $GABA_B$ receptors (Dutar and Nicoll, 1988; Soltesz et al., 1988). The introduction of phaclofen was quickly followed by that of 2-hydroxy-saclofen (2-OH-saclofen), the sulphonic acid derivative of baclofen (Curtis et al., 1988; Lambert et al., 1989). Although 2-OH-saclofen proved to be a much more potent antagonist of the $GABA_B$ receptor than phaclofen, its usefulness as a possible therapeutic agent was limited due to its inability to cross the blood-brain-barrier. Recently, this limitation was overcome with the development of CGP35348, which is structurally related to the $GABA_B$ receptor agonist 3-APA (Olpe et al., 1990). Capable of penetrating the CNS following its i.p. administration, CGP35348 was not however active when administered orally and was of relatively low potency. Numerous antagonists have now been introduced which have very high affinity (1-2 nM) and are active after oral administration. The development of these compounds, as will be discussed in the following section, has greatly expanded our understanding of the $GABA_B$ receptor.

## *B) Pre- and postsynaptic $GABA_B$ receptor-mediated mechanisms*

### *Postsynaptic $GABA_B$ receptors*

Early intracellular recordings from hippocampal CA1 pyramidal neurons maintained in slices revealed that orthodromic stimulation of their afferent fibers by an electrode situated in the stratum radiatum evoked a stereotyped pattern of postsynaptic responses. These consisted of: (1) an EPSP which, if of sufficient amplitude, could trigger the firing of a single action potential, (2) a GABA-mediated bicuculline-sensitive IPSP with a time-to-peak of approximately 10-20 ms, and (3) a late (or slow) hyperpolarizing potential with its peak occurring at about 150-200 ms (Nicoll and Alger, 1981; Alger and Nicoll, 1982a; Alger and Nicoll, 1982b). Similar delayed hyperpolarizations had also been observed in granule cells of the dentate gyrus in response to orthodromic stimulation (Thalmann and Ayala, 1982). The late hyperpolarizing response was resistant to what are now recognized as antagonists of the $GABA_A$ receptor (had been referred to as GABA antagonists) and appeared to be generated through an increase in K+ permeability. Although initially proposed to be generated through an increase in $Ca^{2+}$-dependent $K^+$ conductance as a result of the preceding EPSP, experimental manipulations which reduce depolarization-induced rises in intracellular $Ca^{2+}$ (i.e. intracellular application of EGTA) had no effect on the evoked late hyperpolarization (Lancaster and Wheal, 1984; Newberry and Nicoll, 1984). On the basis of these and other experimental findings, the late hyperpolarization was proposed to be a slow-IPSP, generated following the release of a transmitter substance from inhibitory interneurons (Newberry and Nicoll, 1984). Comparison of the properties of the slow-IPSP with those of bicuculline-resistant responses evoked following the iontophoretic application of either GABA or baclofen revealed numerous similarities which suggested that the activation of $GABA_B$ receptors by synaptically released GABA may underlie the slow-IPSP (Newberry and Nicoll, 1985). More definitive evidence of the involvement of $GABA_B$ receptors in mediating the slow-IPSP came with the

demonstration of the ability of phaclofen to selectively antagonize (1) the postsynaptic bicuculline-resistant actions of both baclofen and GABA and (2) the slow-IPSP (Dutar and Nicoll, 1988). The dependence of the slow-IPSP on the activation of postsynaptic $GABA_B$ receptors has since been confirmed in many parts of the CNS from different species using more recently developed high-affinity $GABA_B$ receptor antagonists (Lambert et al., 1989; Olpe et al., 1990; Davies et al., 1993a; Jarolimek et al., 1993; Olpe et al., 1993; Bon and Galvan, 1996; Schmidt and Perkel, 1998).

The involvement of G-proteins in the postsynaptic actions of the $GABA_B$ receptor was suggested from several experimental manipulations expected to disrupt G-protein mediated interactions. In their inactivated state, G proteins are bound to GDP. Binding of agonist to G protein-coupled receptors causes the exchange of GDP for GTP. The resulting activated G protein may then interact with its effectors. Application of non-hydrolysable analogues of GDP (GDP-β-S) and GTP (GTP-γ-S) have thus been used to irreversibly inactivate and activate G proteins, respectively. Some of the first evidence suggesting that the coupling of $K^+$ channels to the $GABA_B$ receptor occurs through a G protein-mediated mechanism came from experiments demonstrating that application of GDP-β-S reduces the action of baclofen, while in contrast, GTPγ-S could mimic the effects of baclofen (Andrade et al., 1986; Thalmann, 1988). Further evidence came with the demonstration of the ability of pertussis toxin, which prevents receptor-G protein interactions, to block both the effects of applied agonists (GABA and baclofen) as well as the slow-IPSP (Andrade et al., 1986; Colmers and Williams, 1988; Thalmann, 1988; Thompson and Gähwiler, 1992a). Pertussis toxin specifically inactivates G proteins of the $G_i$ and/or $G_o$ type, suggesting that these specific G proteins are responsible for $GABA_B$ receptor-mediated events.

Few studies have investigated the properties of the $K^+$ channels to which $GABA_B$ receptors are coupled. Using the cell-attached configuration of the patch clamp technique, single-channel recordings from cultured hippocampal neurons were recently performed in order to study the properties of the channels involved

(Premkumar et al., 1990). Under these conditions, single-channel $K^+$ currents could be recorded following exposure of the neuron to baclofen. Although highly variable, the amplitude of the smallest current resulting from the application of baclofen corresponded to a conductance of 5-6 pS. The channels were highly selective for $K^+$ ions ($P_{Na}/P_k$ ratio of 0.03-0.04) consistent with the previously measured reversal potential of the slow-IPSP which predicted the involvement of a $K^+$ selective conductance. The channels displayed a bursting behavior and opened to numerous subconductance states which were multiple integers of the elementary conductance of 5-6 pS. More recently, the properties of $K^+$ channels activated in response to synaptically released GABA were examined using nonstationary fluctuation analysis of synaptic currents recorded from dentate gyrus granule cells of hippocampal slices (De Koninck and Mody, 1997). Such an analysis revealed a single-conductance of 5-12 pS, similar to the elementary conductance previously estimated from single-channel recordings. Thus, contrary to the $GABA_A$ receptor where, as mentioned previously, most of the synaptic current was shown to be carried through the larger of multiple conductance states, the synaptic current elicited following the activation of $GABA_B$ receptors appears to be carried by the smallest of observed conductance states.

*Presynaptic $GABA_B$ receptors*

Presynaptic $GABA_B$ receptors have been identified at the terminals of numerous neurotransmitter systems where they regulate transmitter release. Thus, the $GABA_B$ receptor has been shown to act as both an autoreceptor, affecting the release of its own endogenous ligand GABA, and a heteroreceptor, the activation of which reduces transmitter release from glutamatergic, catecholaminergic, serotonergic, peptidergic and acetylcholinergic terminals. However, since heteroreceptor function has predominantly been examined in response to the exogenous application of agonists, including baclofen and GABA, it is unclear whether transmitter release from GABAergic synapses can

affect these more distant receptors. Although the possibility exists that these heteroreceptors could be activated by GABA acting in a paracrine fashion following its release, the physiological role of these receptors largely remains to be determined. In contrast, the physiological role of $GABA_B$ autoreceptors in the presynaptic control of GABA release is more firmly established. Their existence at GABAergic terminals was first suggested based on the ability of baclofen to depress GABA-mediated inhibition in various CNS regions (Cain and Simmonds, 1982; Misgeld et al., 1984). Since then, numerous studies using highly specific $GABA_B$ receptor antagonists have investigated the consequences of their physiological and pathophysiological activation by synaptically released GABA. Results from these studies will be discussed in the following section.

The mechanisms by which $GABA_B$ autoreceptors cause a reduction in the release of GABA in different areas of the CNS as well as within a particular brain structure appear heterogeneous. As with postsynaptic $GABA_B$ receptor-mediated responses, the presynaptic actions of GABA, with the possible exception of magnocellular neurons of the rat supraoptic nucleus (Mouginot et al., 1998), can generally be abolished by pretreatment with pertussis toxin (Thompson and Gähwiler, 1992a; Potier and Dutar, 1993; Pitler and Alger, 1994; Santos et al., 1995; Chen and Van den Pol, 1998). Although these results would suggest that the presynaptic receptors are generally coupled to the same G protein ($G_i/G_o$), heterogeneity appears to exist with respect to the effectors to which these receptors are coupled. For example, experiments with barium have suggested that whereas the $GABA_B$ autoreceptors located in the dentate gyrus and CA3 region of the hippocampus appear to couple with a similar $K^+$ conductance as the postsynaptic receptor (Misgeld et al., 1989; Thompson and Gähwiler, 1992a), those located in CA1 do not (Lambert et al., 1991; Pitler and Alger, 1994). Therefore, at least in the dentate gyrus and CA3 region, it appears as though activation of autoreceptors may decrease transmitter release by hyperpolarizing GABAergic terminals and thus reducing the influx of $Ca^{2+}$ during nerve terminal depolarization. In contrast, recent evidence from experiments utilizing the $Ca^{2+}$ channel blocker, cadmium, suggests that in the CA1 region autoreceptors may

reduce GABA release by causing a direct reduction in the influx of $Ca^{2+}$ through voltage-dependent channels (Doze et al., 1995). Furthermore, the use of subtype selective $Ca^{2+}$ channel antagonists suggested that N- and P/Q-type, but not L-type channels, may mediate the presynaptic actions of GABA in area CA1. Consistent with these findings, whole-cell recordings from inhibitory interneurons located in the stratum radiatum of CA1, revealed that the inhibitory effects of $GABA_B$ receptor activation on voltage-dependent $Ca^{2+}$ channels could be preferentially blocked by the selective antagonist of N-type channels, omega-conotoxin-GVIA (ω-CgTx-GVIA) (Lambert and Wilson, 1996). Thus, it appears as though at least two mechanisms underlie the ability of $GABA_B$ autoreceptors to cause a reduction in the release of GABA: reduction of $Ca^{2+}$ influx into nerve terminals due to (1) nerve terminal hyperpolarization resulting from the activation of a presynaptic $K^+$ conductance, and (2) due to a direct inhibition of a voltage-dependent $Ca^{2+}$ conductance. Based on the ability of baclofen to affect GABA release despite the presence of both cadmium and barium, a third mechanism, most likely involving an action at a site downstream of $Ca^{2+}$ influx, has recently been proposed (Jarolimek and Misgeld, 1997).

### *C) Physiological role of $GABA_B$ receptors*

As described in the previous section, $GABA_B$ receptors, located on both the pre and postsynaptic neuronal membrane, couple to diverse effector systems allowing them to mediate highly divergent functions. In the following section, the physiological functions of the $GABA_B$ receptor will be discussed separately in terms of its pre and postsynaptic-mediated actions.

#### *Postsynaptic $GABA_B$ receptors*

The development of highly specific antagonists possessing a high affinity for $GABA_B$ receptors has allowed a more detailed study of the physiological role of these receptors. In striking contrast to the effects produced following the

antagonism of $GABA_A$ receptors, block of $GABA_B$ receptors following the administration of these compounds does not result in the development of epileptiform activity in brain slices maintained *in vitro*, nor does it generally result in any overt behavioral changes. The inability of these compounds to induce a hyperexcitable state both *in vivo* and *in vitro* underlines numerous important differences in the role of $GABA_B$ and $GABA_A$ receptor-mediated mechanisms in the control of CNS function. Firstly, the more rapid onset of $GABA_A$ receptor-mediated IPSPs results in a significant overlap of this inhibitory event with incoming fast non-NMDA receptor-mediated EPSPs. Thus, the relatively large increase in input conductance and the associated hyperpolarization resulting from the activation of $GABA_A$ receptors will severely limit the temporal extent of neuronal excitation. Recently, $GABA_A$ receptor-mediated events of particularly rapid onset have been identified that occur at or near the somatic region of CA1 pyramidal cells and appear to be especially effective in limiting EPSP-induced action potential firing (Pearce, 1993). Similar $GABA_A$ receptor-mediated events with more rapid kinetics have recently been described in the piriform cortex (Kapur et al., 1997). In contrast, the much slower kinetics of $GABA_B$ receptor-mediated IPSPs more closely resemble those of NMDA receptor-mediated depolarizations. Therefore, although $GABA_B$ receptor activation may regulate excitation under conditions in which NMDA receptors are strongly activated (Olpe and Karlsson, 1990; Morrisett et al., 1991), the relatively small contribution of these receptors to fast excitatory transmission may partially explain the absence of excitability following $GABA_B$ receptor blockade.

A number of further possibilities may explain the absence of hyperexcitability following $GABA_B$ receptor blockade. Whereas $GABA_A$ receptors are tonically activated by ambient levels of GABA and thus play a role in determining the resting membrane potential of neurons, as evidenced by the depolarizing shift in membrane potential resulting from the block of these receptors, the $GABA_B$ receptors are not (Otis et al., 1991; Otis and Mody, 1992). In addition, the spontaneous action potential-dependent and -independent release of GABA, which underlies spontaneous IPSP/Cs (sIPSP/Cs) and

miniature IPSP/Cs (mIPSP/Cs), respectively, contributes to the resting inhibitory tone. The resulting postsynaptic responses generated by such continuous bombardment of the target neuron can be fully blocked by the application of $GABA_A$ receptor antagonists. Thus, $GABA_B$ receptors do not appear to be involved in the generation of spontaneously occurring responses under control conditions. They have, however, been reported to contribute to these events under conditions which increase the probability of transmitter release (Otis and Mody, 1992). Finally, application of $GABA_B$ receptor antagonists will likely produce a block of both pre-and postsynaptic receptors. Thus, a decrease in postsynaptic inhibition resulting from the antagonism of $GABA_B$ receptors may be compensated for by an increase in the extent of $GABA_A$ receptor-mediated inhibition resulting from an increase in GABA release due to a block of the presynaptic autoreceptor.

*Presynaptic $GABA_B$ receptors*

$GABA_B$ receptor antagonists have been successfully utilized to determine the contribution of presynaptic autoreceptor activation to the well characterized phenomenon of activity- (or frequency-) dependent depression of inhibition. First observed as a decrease in the effectiveness of GABA-mediated inhibition with repeated stimulation at frequencies as low as 0.2 Hz, but subsequently observed during even paired stimulation of inhibitory pathways (paired-pulse-depression) as well, this phenomenon is believed to importantly contribute to the development of seizures by facilitating the spread and generalization of epileptiform activity. Numerous mechanisms have been proposed to account for this phenomenon. These include, in no particular order: (1) desensitization of postsynaptic $GABA_A$ receptor-mediated responses following the prolonged exposure of these receptors to GABA (Ben-Ari et al., 1979), (2) collapse of the transmembrane $Cl^-$ gradient which results in a decrease in the driving force for this ion and thus reduces the ability of $GABA_A$ receptors to cause hyperpolarization (McCarren and Alger, 1985; Thompson and Gähwiler, 1989a),

and (3) reduced release of GABA following presynaptic $GABA_B$ autoreceptor activation (Deisz and Prince, 1989; Thompson and Gähwiler, 1989c). Although any of these mechanisms could potentially contribute under a variety of conditions to the activity-dependent depression of inhibition, most of the available data has suggested that the latter two mechanisms may be more important contributors to this phenomenon. However, although shifts in the transmembrane $Cl^-$ gradient likely contribute to the depression observed following the prolonged repetitive stimulation of inhibitory pathways in the hippocampus, this mechanism was reported to contribute little, if at all, to the depression of GABA-mediated responses in the neocortex (Deisz and Prince, 1989). Regardless of the possible contribution of $Cl^-$-dependent mechanisms to the depression of inhibition, a consistent finding in these early studies was the observation of a large reduction in the $GABA_A$ receptor-mediated conductance which coincided with the period of depression.

A consistent finding in experiments using prolonged repetitive stimulation is that a large portion of the depression of inhibitory events occurs between the first and second stimulus (Deisz and Prince, 1989), at a time when the $Cl^-$ reversal potential is unlikely to have changed considerably. This observation would appear to validate the use of the paired-pulse paradigm in the study of mechanisms contributing to activity-dependent depression. The marked depression of GABA-mediated synaptic responses observed under these conditions confirms that changes in the gradient of $Cl^-$ plays a minor role in the use-dependent depression of inhibition (Davies et al., 1990; Nathan and Lambert, 1991). By utilizing specific antagonists of both non-NMDA and NMDA receptors, Davies et al. (1990) were able to study the mechanisms contributing to paired-pulse depression under conditions that avoid the possible confounding issue of changes in the strength of excitatory transmission during paired stimulation. These experiments, and others since, have all reported that a block of $GABA_B$ receptors with its specific antagonists results in the near complete abolition of paired-pulse depression. Furthermore, with $GABA_B$ receptors blocked, the amplitude of IPSP/Cs are well maintained throughout the entire

duration of the low-frequency repetitive stimulation. These results have thus suggested that $GABA_B$ receptors play a predominant role in the phenomenon of activity-dependent depression of inhibition. Studies of long-term potentiation have suggested that this autoreceptor-mediated mechanism may operate under physiological conditions during memory formation.

### *D) Anticonvulsant therapies aimed at $GABA_B$ receptors*

From the preceding experimental findings concerning the physiological role of both pre- and postsynaptic $GABA_B$ receptors, one can clearly propose two contrasting mechanisms through which a compound could produce an anticonvulsant effect by acting upon the $GABA_B$ receptor: (1) enhancement of inhibition through a direct agonist action at the postsynaptic receptor, and (2) increased release of GABA by an antagonism of the presynaptic receptor. Yet, despite reports of the ability of certain agents to produce a relatively selective action at either the pre- or postsynaptic receptors, pharmacological distinction between these two mechanisms has yet to be achieved. Thus, the outcome of a pharmacological intervention aimed at the $GABA_B$ receptor will depend on the relative effect of the compound on the pre and postsynaptic receptor. Consistent with this suggestion, the nonselective $GABA_B$ receptor agonist baclofen was reported to have proconvulsant as well as anticonvulsant effects on experimental and clinical seizures (Löscher and Schmidt, 1994b).

Nevertheless, $GABA_B$ receptor antagonists have been shown to be capable of controlling absence seizures in numerous animal models (Hosford et al., 1992; Smith and Fisher, 1996; Snead, 1996; Aizawa et al., 1997; Vergnes et al., 1997). This is thought to be due to an interaction between $GABA_B$ receptor-mediated IPSPs and T-type $Ca^{2+}$ currents generated in thalamic neurons. The rhythmic activation of these $Ca^{2+}$ currents is thought to underlie the spike-and-wave discharge (SWD) pattern which is characteristically recorded in the EEG of patients suffering from this form of epilepsy. Such a rhythmic discharge pattern has been shown to follow the activation and inactivation of the T-type current. In

this respect, the hyperpolarization provided by $GABA_B$ IPSPs is thought to play an important role in deinactivating the T-type current. Thus, block of $GABA_B$ IPSPs by the $GABA_B$ receptor antagonists is believed to reduce the priming of $Ca^{2+}$ currents and thereby abolish the SWD.

Although, as detailed above, postsynaptic $GABA_B$ receptors play a relatively minor role under physiological conditions, their activation appears to significantly reduce the duration of postsynaptic depolarization during episodes of CNS hyperexcitability. Under these conditions, $GABA_B$ receptor activation has been reported to result in the generation of prolonged (0.5-2 s) postsynaptic hyperpolarizations having a maximal amplitude of 30 mV which may act as a compensatory mechanism under conditions of partial disinhibition. Their elimination following the application of $GABA_B$ receptor antagonists resulted in the development of intense and sustained epileptic discharges lasting for up to 1 min (Scanziani et al., 1991). Consistent with a role of $GABA_B$ receptors in controlling epileptic events, $GABA_B$ receptor antagonists reportedly accelerate the development of amygdala kindling (Karlsson et al., 1992) and facilitate the elicitation of sound-induced tonic seizures (Vergnes et al., 1997) following their systemic injection in rats. Therefore, in contrast to the therapeutic potential of $GABA_B$ receptor antagonists in the treatment of absence seizure, the development of selective agonists of the postsynaptic receptor may prove effective in other forms of epilepsy.

## 2.4 Reuptake of GABA

Following their release from presynaptic terminals, several neurotransmitters are rapidly removed from the extracellular space by an active transport process mediated by a family of membrane bound proteins. Neurotransmitter transporters have been identified at all central synapses with the exception of those mediating cholinergic transmission where transmitter removal is accomplished by the extracellular metabolizing enzyme, acetylcholinesterase. These transporters have been proposed to serve a

number of functions. These include: (1) the termination of synaptic events by the rapid removal of the transmitter substance from the synaptic cleft; this action limits not only the duration of the activation of pre and postsynaptic receptors but also the spread of transmitter to neighboring synapses, (2) the replenishment of neurotransmitter pools, and (3) the release of transmitter by a $Ca^{2+}$-independent mechanism involving reversed transporter activity.

### 2.4.1 $Na^+/Cl^-$-dependent transporters of GABA

A subfamily of transporters has been identified which specifically mediates the uptake of GABA into neurons and glia. The GABA transporters belong to a much larger family of $Na^+/Cl^-$ coupled transporters whose activities, as their name implies, have an absolute requirement for the presence of $Na^+$ and $Cl^-$ which are co-transported along with their respective substrates. The first cloned member of this family was a GABA transporter, designated GAT-1 (Guastella et al., 1990), which became the prototypical member of a family that now includes transporters for norepinephrine, serotonin, dopamine, glycine, and taurine. Additional GABA transporters have since been cloned (Borden et al., 1992; Yamauchi et al., 1992). Besides GAT-1, the family of GABA transporters now includes GAT-2, GAT-3 as well as the betaine/GABA transporter, BTG-1. Homologues of each have been identified in several species, including man.

Within the GABA transporter subfamily the various members display approximately 50-70% amino acid identity. The cDNAs are predicted to encode proteins of 600-630 amino acids with molecular weights of 65-70 kDa, in good agreement with the size of the purified unglycosylated transporters. They share, along with the other members of the $Na^+/Cl^-$ coupled transporters, numerous structural features, most of which are predicted by hydropathy analyses of their deduced amino acid sequence and can be summarized as follows: (1) 12 hydrophobic putative membrane spanning α-helices, (2) intracellularly located N- and C-terminal regions, (3) a large extracellular loop between transmembrane regions 3 and 4, and (4) several consensus sites for *N*-linked glycosylation

located within the large extracellular loop. A similar membrane topology has recently been proposed based on the protease sensitivity of various regions of the GAT-1 transporter expressed in *Xenopus* oocytes (Clark, 1997).

### *A) Mechanism of transporter-mediated GABA uptake*

The process of concentrative transport into both neurons and glia is made possible by coupling the transport of GABA to the movement of inorganic ions down their concentration gradient. Thus, for each GABA molecule, two $Na^+$ and one $Cl^-$ ions are co-transported. The transport process is thus electrogenic resulting in the transfer of one net charge per transporter cycle. The inorganic anions are furthermore proposed to play an important role in preparing the transporter for the binding of GABA by transforming it to a state having a high affinity for the substrate (Mager et al., 1996).

Various models have been proposed in order to explain transporter function. The generally accepted model by which the GABA transporter shuttles its substrates across the membrane is termed the alternating-access model. In this model, the transporter is viewed as an aqueous pore to which access is restricted due to the presence of two gates on the extracellular and intracellular surfaces of the protein. Within the pore region are acceptor sites to which the permeant substrates bind. The model predicts that in its resting state the extracellular gate is left opened allowing the substrates access to their binding sites. Once these sites are occupied, the gates undergo a conformational change which causes the extracellular gate to close and the intracellular gate to open. Once the substrates are released to the intracellular solution, the transporter reverts to its resting state where it is now ready to repeat the cycle. Recently, in heterologous expression systems, electrophysiological techniques have been used to record the currents resulting from the binding and unbinding of $Na^+$ and $Cl^-$ to the GAT-1 transporter as well as from the translocation of substrates across the plasma membrane (Cammack et al., 1994; Mager et al., 1996). The recorded currents revealed complexities of the transport process

which cannot be explained by the alternating-access model. On the basis of these findings a second model has been proposed, termed the multi-substrate single-file transport model (Su et al., 1996). The model assumes substrate-substrate interactions at multiple binding sites but makes no assumptions with regards to global conformation changes. The multiple binding sites within the pore formed by the transporter are aligned in a single file. Given the absence of gates, substrates have free access to the transporter lumen and thus bind at a rate partially determined by mass action. Substrates are assumed to hop from the solutions to the binding sites as well as from one binding site to the next. Coupling ratios between inorganic and organic substrates arise from substrate-substrate interactions (attraction or repulsion) which favor translocation of substrates from one side of the membrane to the other. More detailed knowledge concerning the structure of the transporters and the residues which actually line the pore region will be needed in order to select between these models.

***B) Mechanism of transporter-mediated GABA release***

The GABA transporter can mediate transmitter release by at least two mechanisms: (1) homo- and heteroexchange, and (2) reversed transporter activity, leading to a net efflux of GABA. The two processes are observed to occur in the absence of any external $Ca^{2+}$ and thus do not involve the exocytosis of GABA from synaptic vesicles. The former of these release processes was observed in neurochemical experiments which demonstrated that [$^3$H]GABA, preloaded into membrane synaptosomes, can exchange extensively with both exogenously applied GABA (homoexchange) as well as with substances which can serve as substrates for the GABA transporter (heteroexchange), such as nipecotic acid (Johnston et al., 1976b; Bernath and Zigmond, 1988) and diaminobutyric acid (DABA) (Sitges et al., 1993). Although independent of $Ca^{2+}$, this process does however depend on the presence of extracellular $Na^+$ (Sitges et al., 1993).

The second means through which the transporter can cause a release of GABA stems from its dependence on the transmembrane electrochemical gradients for $Na^+$ and $Cl^-$. Thus, reversed transporter activity will occur under conditions, such as strong membrane depolarization or the intracellular accumulation of $Na^+$, which favor a reversal of the electrochemical gradient for these ions. Based on the recognized stoichiometry of the transporter (2 $Na^+$:1 $Cl^-$ :1 GABA) the following thermodynamic equation has been proposed which governs the extracellular concentration of GABA (Attwell et al., 1993):

$$[GABA]_o = [GABA]_i([Na^+]_i/[Na^+]_o)^2([Cl^-]_i/[Cl^-]_o)\exp[VF/RT]$$

where $[\,]_o$ and $[\,]_i$ refers to the extracellular and intracellular concentrations of each molecular species, respectively, and where V, F, R, and T have their usual meanings. Based on the dependence of transporter activity on the presence of two $Na^+$ ions, the equation predicts that depolarizing events which lead to an influx of $Na^+$ will more effectively trigger a transporter-mediated release of GABA than the presence of depolarization alone. Such a prediction is consistent with experimental observations (Santos et al., 1992). From this it follows that such a mechanism will more significantly contribute to the release of GABA during a relatively sustained depolarization of neurons, as might be expected to occur in neurons involved in the generation and propagation of epileptiform activity. In support of this, a recent microdialysis study in patients suffering from temporal-lobe epilepsy demonstrated a reduction in the glutamate-stimulated $Ca^{2+}$-independent release of GABA which was attributed to a reduction in the number of GABA transporters (During et al., 1995). The loss of transporter function in epileptogenic brain areas was thus suggested to contribute to the maintenance of the epileptic state in these patients.

### *C) GABA transporter heterogeneity in the CNS*

Although not definitively confirmed until the members of the GABA transporter family had been cloned, the existence of at least two distinct transporters for the GABAergic neurotransmitter had been proposed based on the relatively selective ability of a series of compounds to inhibit the transport of GABA into either cultured neurons or astrocytes (Schousboe et al., 1979; Larsson et al., 1981; Larsson et al., 1983). Compounds such as hydroxynipecotic acid and β-alanine inhibited with higher potency the uptake of GABA into glia whereas the compounds (1RS, 3RS)-3-aminocyclohexanecarboxylic acid (ACHC) and L-DABA preferentially inhibited uptake into neurons. The most potent inhibitors of the uptake of GABA were the monocyclic amino acids, nipecotic acid and guvacine. However, these compounds displayed only a modest degree of selectivity (~3-4 fold) for the glial transporter. The cloning of the GABA transporters has revealed that the actions of these compounds are more complex than was originally proposed based on the differential pharmacology of the glial versus neuronal transporter. In fact, characterization of the distribution and pharmacology of the cloned transporters has failed to confirm the existence of clearly distinguishable neuronal versus glial transporters.

GAT-1 (GABA Transporter 1) possesses properties which most closely approximates those expected for a neuronal transporter and is the most widely distributed member of the GABA transporter family (Durkin et al., 1995). The pharmacological profile of GAT-1 is consistent with those of the proposed neuronal transporter (Guastella et al., 1990). However, although it has been reported to be highly expressed in cells with a neuronal morphology (Itouji et al., 1996), numerous reports have found GAT-1 to be present in astrocytes as well (Ribak et al., 1996). In contrast, the properties of the much less widely distributed GAT-3 most closely resemble those of the glial transporter. As such, GAT-3 displays a higher affinity for β-alanine than GAT-1 and shows little affinity

for the proposed neuronal selective compound ACHC. However, the finding that GAT-3 displays a lower affinity for hydroxynipecotic acid than did GAT-1, is inconsistent with GAT-3 being designated as the previously characterized glial transporter, although GAT-3 is reported to be preferentially expressed in astrocytes (Durkin et al., 1995; Itouji et al., 1996). In situ hybridization has shown that the distribution of mRNA of GAT-2 which is closest to GAT-3 with respect to its deduced amino acid sequence and pharmacological profile, is limited to cells forming the meninges (Durkin et al., 1995). Finally, although BTG-1 (Betaine/GABA Transporter 1) mRNA has been detected in the CNS of both humans and rats, its distribution does not correlate with GABAergic pathways and thus does not appear to play a role in terminating the action of GABA at the synapse (Borden et al., 1995).

### 2.4.2 High affinity lipophilic inhibitors of the GABA transporter: Development of tiagabine

Although the inhibition of GABA uptake by both nipecotic acid and guvacine was demonstrated to produce an anticonvulsant effect, due to their high degree of hydrophilicity these compounds were unable to permeate the blood-brain-barrier and thus could only produce an anticonvulsant effect following their intracerebroventricular administration. Another marked disadvantage of these compounds is the fact that they can act as a substrate for the neuronal and glial transporter (Johnston et al., 1975; Johnston et al., 1976a). As a consequence of its ability to be taken up into presynaptic terminals, nipecotic acid has been reported to produce a concentration- and perfusion time-dependent disinhibitory effect *in vivo* (Lerma et al., 1985). Evidence from neurochemical studies has suggested at least two possible mechanisms by which nipecotic acid may have produced such an effect. The first is based on the observation that following its uptake into neurons, nipecotic acid can be released in a $Ca^{2+}$-dependent manner from slices of rat cerebral cortex (Johnston et al., 1976b). Since nipecotic acid has no appreciable effect on the postsynaptic

GABA receptors, the storage and subsequent release of this compound will reduce the effectiveness of GABA-mediated inhibition. Therefore, nipecotic acid would be acting as a false transmitter (Lerma et al., 1985; Roepstorff and Lambert, 1992). An additional explanation for the disinhibitory effects of nipecotic acid is based on this compound's ability to cause an increase in the release of GABA through a transporter-mediated heteroexchange process (Szerb, 1982a; Solís and Nicoll, 1992). Thus, a depletion of the presynaptic GABA stores may also contribute to the observed disinhibitory actions of nipecotic acid.

In an attempt to develop novel GABA uptake blockers capable of producing a CNS action following their systemic administration, Ali et al. (1995) examined whether the addition of a large lipophilic moiety (4,4-diphenyl-3-butenyl, DPB) to the nitrogen atoms of nipecotic acid, guvacine and other inhibitors of GABA uptake would facilitate the penetration of the resulting compounds through the blood-brain-barrier. It had previously been shown that the introduction of small substituents to the amino groups of nipecotic acid or guvacine normally results in compounds with a diminished affinity for the GABA transporter (Wood et al., 1979; Krogsgaard-Larsen et al., 1985). Therefore, it was somewhat surprising that many of the substituted derivatives demonstrated a higher potency at inhibiting GABA transport than their respective parent compounds. In particular, SKF 899976-A and SKF 100330-A displayed a 20-fold increase in potency compared to nipecotic acid and guvacine, respectively. In contrast to their parent compounds, these blockers act as non-competitive inhibitors and cannot serve as substrates for the transporter. They are believed to produce their inhibitory effects by preventing the disassociation of $Na^+$ ions, thereby locking the transporter in a intermediate step of its cycle (Mager et al., 1996). Importantly, when tested in rats and mice, these compounds were shown to produce an anticonvulsant effect following systemic administration.

Using a similar strategy, numerous compounds have since been synthesized including CI-966 (Bjorge et al., 1990), NNC-711 (Suzdak et al., 1992a), and tiagabine (TGB) (Andersen et al., 1993). Although all of these

compounds have been demonstrated to produce an anticonvulsant effect in laboratory animals, only TGB has proven to be useful in the treatment of epilepsy in humans. Clinical trials with CI-966 were discontinued following reports of the development, in healthy human volunteers, of severe physical abnormalities including tremor, myoclonus and short- and long-term memory loss, as well as mental disturbances consisting of symptoms indicative of mania and schizophrenia (Sedman et al., 1990). An examination of the effect of these compounds on the GABA transporters, GAT-1 and GAT-3, revealed that all of these inhibitors display a much higher affinity for GAT-1 over GAT-3 and in this respect, they are 50-200-fold more potent than their parent compounds, nipecotic acid and guvacine (Clark et al., 1992; Clark and Amara, 1994; Borden et al., 1994). Thus, the anticonvulsant actions of these compounds can most likely be attributed to their selective inhibition of GAT-1-mediated GABA transport.

### *A) Preclinical pharmacology of tiagabine*

TGB exerts potent anticonvulsant activity in numerous chemical and electrically induced convulsion models as well as in genetic epilepsy models. The administration (i.p.) of TGB was demonstrated to fully protect animals against pentylenetetrazol- (PTZ-) induced tonic seizures and death while affording a 50-60% protection against the associated clonic seizures. Similarly, methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate- (DMCM-) induced clonic seizures and death were completely prevented by TGB. The observed anticonvulsant effect was generally dose-dependent with the exception of PTZ- and DMCM-induced clonic seizures where a reduction in the anticonvulsant efficacy became apparent at higher doses (Nielsen et al., 1991). Animals were only partially protected from bicuculline-induced seizures and protection from maximal electrostimulation (MES) was only observed at relatively higher doses of TGB. In addition, TGB was shown to suppress both the development of amygdala kindling epileptogenesis and the expression of fully kindled seizures (Suzdak and Jansen, 1995; Dalby and Nielsen, 1997; Morimoto et al., 1997).

Amygdala kindling is believed to be useful in predicting anticonvulsant effectiveness against previously drug resistant complex partial epilepsy (Löscher and Rundfeldt, 1991). In genetic models, TGB was shown to dose-dependently inhibit sound-induced tonic seizures in DBA/2 mice (Nielsen et al., 1991) and audiogenic seizures in the genetically epilepsy-prone rat (Faingold et al., 1994). Finally, in evaluating the possible neurological side-effects resulting from treatment with TGB it was observed that inhibition of locomotor activity in mice and rats occurred at doses 10-14 times higher than those necessary to produce an anticonvulsant effect (Nielsen et al., 1991). In this respect, the therapeutic window for TGB was considerably larger than that observed for compounds such as carbamazepine, diazepam, phenobarbital, phenytoin and valproic acid. Interestingly, although tolerance to the anticonvulsant effects did not develop during 21 day treatment with TGB, tolerance to the already slight sedative and motor impairing effects was observed resulting in an increase in the therapeutic window for TGB from approximately 14 to 28 (Suzdak, 1994). In addition, no sign of withdrawal or physical dependence was seen upon discontinuation of drug treatment. These results would therefore suggest that treatment of patients with TGB may be better tolerated than the currently established antiepileptics.

***B) Human studies with tiagabine***

As a general rule, the results from clinical trials with TGB are consistent with the seizure protection profile predicted on the basis of preclinical animal testing. When TGB was administered as add-on therapy for patients suffering from partial seizures (simple, complex and secondarily generalized) that were unsatisfactorily controlled with existing antiepileptic drugs, approximately 30% of patients experienced at least a 50% reduction in the frequency of their seizures. For particularly difficult to treat complex partial seizures this number was increased to 42% with optimal TGB dosing (Ben-Menachem, 1995a). The effectiveness of TGB in these trials was clearly dose-dependent. Although results from more trials will be needed, early indications suggest that TGB can

also be effective in monotherapy (Biton et al., 1996). Consistent with results from animal testing, treatment of patients with TGB was generally well tolerated and adverse effects were usually mild and transient. The most commonly reported side-effects were dizziness, asthenia, nervousness and tremor (Ben-Menachem, 1995a; Leppik, 1995). Furthermore, the anticonvulsant effectiveness of TGB was well maintained, and in some cases increased, during long-term treatment of up to 12 months (Ben-Menachem, 1995a).

### 2.4.3 Mechanisms of action of tiagabine

The effects of TGB on the uptake of GABA have been tested in numerous *in vitro* preparations including synaptosomal membranes, neuronal as well as glial cultures, and heterologous expression systems (Braestrup et al., 1990; Borden et al., 1994). As previously mentioned, TGB preferentially inhibits the activity of the GAT-1 transporter. Using either stable or transient transfections of both human and rat clones of GAT-1, the affinity of the various lipophilic transport inhibitors were tested. The highest potency at inhibiting GAT-1 was observed with NNC-711. This was followed by TGB, SKF 89976-A, and CI-966. The rank order of potency was identical regardless of species. TGB was equally potent at inhibiting the uptake of [$^3$H]GABA into cultured neurons ($IC_{50}$ = 446 nM) and glia ($IC_{50}$ = 182 nM) demonstrating a lack of preference for either of these uptake sites. The inhibition of uptake was stereoselective as the *S*-enantiomer of TGB was approximately fourfold less potent. Uptake was inhibited with similar potency in synaptosomes prepared from various species (rat, pigeon, rabbit, guinea pig and mouse) as well as in various rat brain regions (pons-medulla, cerebellum, cortical regions, corpus striatum, midbrain and hippocampus). These experiments also confirmed the inability of TGB to act as a substrate for the transporter.

*In vivo* and *in vitro* binding experiments were performed using [$^3$H]TGB in order to better characterize the specific target with which this compound interacts (Braestrup et al., 1990; Suzdak et al., 1992b). These experiments demonstrated

that TGB binds with high affinity to a single class of binding sites in a manner which was dependent on the presence of both $Na^+$ and $Cl^-$. The binding of [$^3$H]TGB was saturable, could be inhibited by various known inhibitors of GABA transport and was well correlated with the regional distribution of [$^3$H]GABA uptake *in vitro* with the highest concentration of binding sites found in the hippocampus. These results strongly suggest that [$^3$H]TGB binding represented binding to the GABA uptake carrier. Following the administration of a dose (1.2 mg/kg) of TGB which corresponded to $ED_{50}$ for inhibiting DMCM-induced clonic seizures, *in vivo* receptor occupancy was estimated at 30%. In contrast, receptor occupancy following a dose (6 mg/kg) of TGB corresponding to the $ED_{50}$ for inhibiting rotarod activity was estimated at 60% suggesting that a different degree of receptor occupancy is needed for TGB to produce its anticonvulsant effects and ataxia.

Finally, the interaction of TGB with the GABA transporter was shown to be highly selective. TGB had no appreciable affinity for dopamine, adrenergic, muscarinic, serotonin, histamine (H2 and H3), opiate, glycine, glutamate, GABA, or sigma receptors. Similarly, TGB had essentially no effect on the uptake of dopamine, norepinephrine, serotonin, glutamate or choline. In addition, TGB did not interfere with the binding of blockers of both calcium and sodium channels. Although weak affinity was demonstrated for the histamine H1 receptor and the benzodiazepine receptor, the concentration of TGB at which these interactions were seen was over 98-fold greater than the $IC_{50}$ for inhibition of [$^3$H]GABA uptake (Braestrup et al., 1990; Suzdak and Jansen, 1995).

### 2.4.4 Electrophysiological studies of tiagabine's actions

The actions of TGB on GABA-mediated IPSP/Cs are generally consistent with those reported previously for the non-substrate, high affinity, lipophilic blockers of GABA uptake and are in striking contrast to those of nipecotic acid. Unlike nipecotic acid which moderately prolonged the decay phase of evoked IPSCs while causing a more pronounced reduction in their amplitudes, TGB had

no consistent effect on the amplitude of the evoked inhibitory events and greatly prolonged their duration such that the decay time constant of evoked IPSCs was increased from 16 to 250 ms (Rekling et al., 1990; Roepstorff and Lambert, 1992; Thompson and Gähwiler, 1992b). Furthermore, in contrast to the smooth exponentially decaying monosynaptic IPSPs that were recorded following the complete block of excitatory postsynaptic receptors, monosynaptic responses evoked in the presence of TGB displayed a prominent, more delayed, second component. Application of the specific $GABA_B$ receptor antagonist, CGP 35 348, abolished this delayed component indicating that it was generated as a result of the activation of postsynaptic $GABA_B$ receptors. Thus, the prolonged duration of IPSPs evoked in the presence of TGB is in part due to a greater contribution of $GABA_B$ receptors to the postsynaptic response. Given the previously reported role of $GABA_B$ receptors in controlling the duration of epileptiform activity (see 2.3.2 section D), these results would suggest that such an increase in $GABA_B$ receptor-mediated IPSPs could contribute to the anticonvulsant actions of TGB. In contrast to its effect on evoked IPSP/Cs, inhibition of GABA uptake by TGB was reported to have no effect on the mean decay time constant of action potential-independent miniature IPSCs (mIPSCs)(Thompson and Gähwiler, 1992b). This suggests that the clearance of GABA from the synaptic cleft following its spontaneous quantal release is determined by diffusion rather than by uptake. Nevertheless, as expected from its ability to prolong the postsynaptic actions of GABA, TGB was shown to inhibit the spontaneous epileptiform bursting induced in the presence of a high (8 mM) extracellular concentration of $K^+$.

# Results

## Preface to Section 3

In contrast to the activity-dependent depression of GABA-mediated inhibition which is characteristically produced by the repetitive stimulation of inhibitory interneurons at low-frequency (0.5-10 Hz), high-frequency stimulation (HFS; 100 Hz, 200ms) has been demonstrated to greatly increase the strength of evoked inhibition above that which is produced by a single stimulus (Čapek and Esplin, 1993). Furthermore, GABA uptake blockers with demonstrated anticonvulsant properties were shown to produce a relatively selective increase in the inhibition resulting from HFS of hippocampal recurrent inhibitory pathways suggesting that the actions of these drugs are frequency-dependent. Tiagabine is a recently developed selective GABA uptake inhibitor which, in contrast to previously developed structurally related compounds, has proven to be effective in treating patients suffering from certain forms of epilepsy. The following study was undertaken with the aim of examining the mechanisms which underlie the frequency-dependent effects of uptake blockers on GABA-mediated inhibition.

**Frequency-dependent enhancement of hyperpolarizing and depolarizing GABAergic synaptic responses following inhibition of GABA uptake by tiagabine**

Epilepsy Research (under revision)

Michael F. Jackson, Barbara Esplin and Radan Čapek

*Department of Pharmacology and Therapeutics*
*McGill University, Montreal, Que. (Canada)*

*Correspondence*: Dr. Radan Čapek
Department of Pharmacology and Therapeutics
McGill University
3655 Drummond Street
Montreal, Que. H3G IY6, CANADA
Tel.: (514) 398-3607
FAX: (514) 398-6690

**Abstract**

The effects of the GABA uptake blocker tiagabine on isolated inhibitory postsynaptic potentials (IPSPs), elicited by high frequency (100 Hz, 200 ms) stimulation (HFS) of inhibitory interneurons, were examined in CA1 pyramidal cells in the hippocampal slice preparation. We report that HFS produced a large increase in both fast and slow hyperpolarizations resulting from the activation of $GABA_A$ and $GABA_B$ receptors, respectively. In addition, incremental increases in the intensity (80-550 μA) of HFS caused the appearance of a depolarizing response (DR) of progressively larger amplitude which occurred between, and overlapped with, the fast and slow hyperpolarizing components of the IPSP. The amplitude and duration of the DR was dependent upon the intensity and duration of HFS and could be blocked by bath application of bicuculline methiodide (40 μM), suggesting that $GABA_A$ receptors were involved in its generation. The consequences of tiagabine (20 μM) application on GABA-mediated responses evoked by HFS were dependent on the intensity of the electrical stimulation used. Thus, measurements of the time integral of evoked responses show that with weak (60 μA) HFS tiagabine caused a 3.6-fold increase in the area of hyperpolarization while in contrast, with strong (530 μA) HFS tiagabine produced a 13.5-fold increase in the depolarizing actions of GABA. Our results suggest that tiagabine, a therapeutically effective anticonvulsant, may paradoxically produce an increase, through a $GABA_A$ receptor-mediated mechanism, of neuronal depolarization during the high-frequency discharge of neurons involved in epileptiform activity.

*Keywords:* Anticonvulsant; GABA; Uptake, Tiagabine; Hippocampus; evoked IPSPs

## Introduction

The neurotransmitter γ-aminobutyric acid (GABA) plays a critical role in controlling the spread of excitation in the central nervous system (CNS)(Traub et al., 1987a; Traub et al., 1987b; Miles and Wong, 1987). Although GABAergic inhibitory interneurons represent as little as 10% of the neuronal population in the hippocampus (Olbrich and Braak, 1985; Woodson et al., 1989), due to their extensive dendritic and axonal arborizations (Li et al., 1992; Sik et al., 1995; Miles et al., 1996) they are estimated to account for 20 to 50% of all CNS synapses (Bloom and Iversen, 1971; Decavel and Van den Pol, 1990). Following its release from inhibitory interneurons, GABA activates postsynaptic ligand-gated $GABA_A$ and G-protein coupled $GABA_B$ receptors which mediate fast- and slow-inhibitory postsynaptic potentials (f- and s-IPSPs), the amplitude and duration of which determines the strength of GABAergic inhibition in the CNS. For neurotransmitter systems devoid of extracellular metabolizing enzymes, the duration of postsynaptic events will be determined by (1) intrinsic channel properties, (2) the rate of diffusion of the transmitter from the synaptic cleft, as well as by (3) the reuptake of transmitter into neurons and glia through an active transport process. Although the decay of both spontaneous IPSPs as well as those evoked with weak stimulation of GABAergic neurons appears to be uniquely determined by intrinsic channel properties and diffusion of GABA out of the synapse, the use of GABA uptake inhibitors has demonstrated that transporter activity plays an important role in the termination of responses evoked with either exogenous GABA application or strong stimulation of the GABAergic neurons (Thompson and Gähwiler, 1992b; Isaacson et al., 1993; Oh and Dichter, 1994). These results would suggest that uptake inhibitors will have a more pronounced effect on events resulting from a relatively large release of GABA.

In contrast to pyramidal cells, interneurons display a high rate of spontaneous activity, short action potential duration and pronounced brief afterhyperpolarizations following a single action potential. Furthermore, they fire

trains of non-adapting action potentials in response to sustained depolarizing current injections (Schwartzkroin and Mathers, 1978; Knowles and Schwartzkroin, 1981; Lacaille et al., 1987; Williams et al., 1994). Although the membrane properties of interneurons would clearly allow the firing of high frequency bursts of action potentials during epileptiform activity, very little has been done in order to characterize GABA-mediated postsynaptic responses evoked following the high frequency activation of interneurons. Repetitive stimulation of inhibitory pathways at low frequencies (0.5-10 Hz) is known to cause the activity-dependent depression of $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) due to a combination of factors including a rundown of the transmembrane $Cl^-$ gradient as well as a reduced release of transmitter following the activation of presynaptic $GABA_B$ autoreceptors (McCarren and Alger, 1985; Thompson and Gähwiler, 1989a). In contrast, we have recently demonstrated that high frequency stimulation (HFS) of recurrent inhibitory pathways in the hippocampus produces an increase in the strength and duration of GABA-mediated inhibition. Furthermore, the GABA uptake blockers SKF 89976A and SKF 100330A, derivatives of nipecotic acid and of guvacine respectively, caused a much greater increase in the inhibition produced by the HFS of recurrent inhibitory pathways compared with that resulting from a single stimulus (Čapek and Esplin, 1993).

The aim of the present study was therefore to investigate the effects of tiagabine, a novel GABA uptake inhibitor recently approved in the US for the adjunctive treatment of partial seizures, on monosynaptic IPSPs recorded from CA1 pyramidal cells following the stimulation of hippocampal inhibitory interneurons at a frequency (100 Hz) intended to mimic the activation seen in seizures. We report that such HFS results in a large increase in the amplitude and duration of $GABA_A$ and $GABA_B$ receptor-mediated IPSPs. Consistent with previous reports (Staley et al., 1995), such stimulation also caused the appearance of $GABA_A$ receptor-mediated depolarizations. Tiagabine application resulted in a stimulus intensity- and frequency-dependent increase in the postsynaptic actions of GABA resulting in a predominantly large increase in

GABA-mediated depolarizing responses (DRs). Preliminary accounts of these results have been presented (Jackson et al., 1995; Jackson et al., 1996).

**Methods**

Male Sprague-Dawley rats (100-200 g, Charles River) were decapitated, their brains rapidly removed and placed in cold (4°C) oxygenated (95% $O_2$ and 5 % $CO_2$) artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl, 124; KCl, 3.3; $CaCl_2$, 2.5; $MgSO_4$, 2.4; $NaHCO_3$, 25.6; $KH_2PO_4$, 1.25; glucose, 10. The brains were then glued to the stage of a vibratome and coronal whole-brain slices (400 µm) were prepared. Slices containing transverse sections of the hippocampus were incubated at room temperature in a holding chamber for 1 hour in oxygenated aCSF. For experiments, slices were transferred to a recording chamber and maintained at 31°C at the interface between moist 95 % $O_2$ and 5 % $CO_2$ and oxygenated aCSF perfused through the chamber at a rate of 2-3 ml/min. Using a blind approach (Blanton et al., 1989), tight seal whole cell recordings were obtained from CA1 pyramidal cells maintained, by appropriate current injection, at a constant membrane potential. Electrodes, prepared from borosilicate glass (WPI) using a Brown-Flaming micropipette puller (Sutter Instruments, P-80), had a resistance of 2-4 MΩ when filled with a solution containing (in mM): 140-150 $KMeSO_4$, 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and 2 $MgCl_2$. Intracellular solutions were adjusted to pH 7.2-7.3 with KOH and filtered through a 0.2 µm pore size filter (Nalgene). Total osmolality for all intracellular solutions ranged from 270 to 290 mOsm. Monosynaptic IPSPs (Davies et al., 1990) were evoked in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX,10-20 µM) and 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 5 µM) by a bipolar stimulating electrode positioned in either the stratum radiatum or alveus in close proximity to the recording electrode. Stimulation was by either a single stimulus (50-600 µA, 100 µs) or a train of 20 stimuli delivered at high-

frequency (100 Hz, 200 ms). When reported, the significance of the changes induced following the bath application of tiagabine were tested using a two-tailed Student's *t*-Test. Signals were recorded with an Axoclamp 2A amplifier (Axon Instruments) in bridge mode, digitized at 3-5 kHz and stored on disk using a PC based acquisition and analysis system (Théorêt et al., 1984).

*Drugs*

Bicuculline methiodide was purchased from Sigma Chemical Co. (St-Louis, MO). The specific $GABA_B$ receptor antagonist, 3-[[(3,4-dichlorophenyl)methyl]amino]propyl](diethoxy-methyl) (CGP 52 432) and (R)-*N*-[4,4-di-(3-methylthien-2-yl)but-3-enyl] nipecotic acid hydrochloride (tiagabine) were kind gifts of Novartis Pharma AG (Basel, Switzerland) and Abbott Laboratories (Chicago, Illinois), respectively. Drugs used were dissolved in aCSF and added to the perfusing medium when required.

**Results**

*Monosynaptic IPSPs evoked by high-frequency stimulation*

Monosynaptic IPSPs, consisting of both fast and slow hyperpolarizing components, were evoked following a single stimulus in stratum radiatum. Their amplitude and duration increased with the stimulus intensity over the range of 80 to 550 µA (Fig. 1A, upper traces). When the inhibitory interneurons were stimulated at high-frequency (100Hz, 200ms), the evoked hyperpolarizations were of much greater amplitude and duration. HFS furthermore caused the appearance of a DR which, at low stimulus intensity (80 µA), typically consisted of a depolarizing "hump" interposed between the fast and slow hyperpolarizations. Its amplitude increased with the stimulus intensity (Fig 1A, lower traces) as well as with the number of pulses delivered (Fig. 1B) and could evoke a burst of action potentials (Fig 1B, bottom trace). The HFS-evoked DR

could be abolished following the bath application of the $GABA_A$ receptor antagonist, bicuculline methiodide (40 μM) (Fig. 2A, bottom traces). Such $GABA_A$ receptor-mediated depolarizations have previously been observed under conditions which produce a strong activation of the postsynaptic receptors (Andersen et al., 1980; Thalmann et al., 1981; Perreault and Avoli, 1992; Grover et al., 1993) and are most likely generated by the efflux of bicarbonate anions ($HCO_3^-$) through the receptor channel (Staley et al., 1995; Perkins and Wong, 1996). In addition to producing a block of the DR, bath application of bicuculline (40 μM) abolished the initial fast hyperpolarization of HFS-evoked responses thus revealing a pronounced increase in the amplitude and duration of the slow hyperpolarization (Fig. 2A, bottom traces). Slow hyperpolarizations were blocked by the $GABA_B$ receptor antagonist CGP 52 432 (10 μM) indicating that they are $GABA_B$ receptor-mediated. In the presence of both bicuculline and CGP 52 432, the remaining small depolarization was most likely due to a rise in extracellular $K^+$ resulting from the intense activation of the inhibitory interneurons since it persisted even in the absence of extracellular $Ca^{2+}$ and was not associated with an increase in conductance (data not shown). The decrease in the transmembrane potential changes evoked by injection of constant current pulses and superimposed on the trace indicated that a conductance increase was associated with each of the three components of the HFS-elicited response (Fig. 2C).

*Effects of GABA uptake block on whole-cell recorded IPSPs evoked by either single stimulus or by HFS*

Consistent with the previously reported effects of GABA uptake blockers (Isaacson et al., 1993), bath applied tiagabine (20 μM) greatly prolonged the duration of only those IPSPs which were evoked following a single strong (200-400 μA) stimulation while producing little or no effect on the amplitude of responses evoked using either low (30-50 μA) or high (200-400 μA) stimulus intensities (Fig. 3A and B). In contrast to these relatively small changes, the

blockade of GABA uptake by tiagabine resulted in a much more pronounced effect on responses elicited with HFS. As previously mentioned, compared with responses elicited with a single stimulus, the amplitude and duration of the hyperpolarizing components increased with HFS. Regardless of the stimulus intensity used, application of tiagabine caused further increases in both the amplitude as well as the duration of hyperpolarizing IPSPs. In addition, tiagabine application greatly facilitated the generation of DRs following HFS. This effect was especially prominent at high stimulus intensities, where HFS could now evoke large (>10 mV) depolarizations which dominated the postsynaptic response and obscured the slow hyperpolarizing component of the IPSP. Such large evoked depolarizations could furthermore trigger the burst firing of action potentials (as in Fig. 5A).

The frequency-dependent actions of tiagabine were further tested by examining the effects of tiagabine application on the time integral of responses evoked with either a single stimulus or a train of stimuli delivered at high frequency (Fig. 4). With a baseline which corresponded to the resting membrane potential, such an analysis allowed for a better determination of the overall contribution of hyperpolarizing and depolarizing components to the evoked responses. In control, responses evoked following a single stimulus were always hyperpolarizing with the measured negative area becoming larger as the stimulus intensity was increased from 60 to 530 μA. Following the bath application of tiagabine the duration of evoked hyperpolarizing IPSPs was greatly enhanced producing a 3.2-fold increase in the area of hyperpolarization at a stimulus intensity of 340 μA (Fig. 4A). In contrast to the straightforward increase in hyperpolarizing IPSPs elicited with a single stimulus, the effects of tiagabine on responses evoked with HFS were more complex. With weak HFS (60 μA), application of tiagabine resulted in a 3.6-fold increase in the area of hyperpolarization. However, as the stimulus intensity was increased, block of GABA uptake resulted in an overwhelmingly large increase in the amplitude and duration of DRs such that at a stimulus intensity of 340 μA the time integral of the response was increased 13.5-fold. Therefore, when using HFS, tiagabine application produced a biphasic effect on postsynaptic responses such that an

increase in hyperpolarization was seen at low stimulus intensities while in contrast, an increase in $GABA_A$ receptor-mediated depolarization was observed at higher stimulus intensities (Fig. 4B).

*Differences in the effects of tiagabine on responses elicited by HFS stimulation of proximal and distal interneuronal populations*

The increase in HFS-induced depolarizing responses following the application of tiagabine suggests that inhibition of GABA uptake may result in an increase in excitability. Yet our studies of the effects of SKF 89976A and SKF 100330A (Čapek and Esplin, 1993) as well as of tiagabine (Čapek, 1997) demonstrated that block of GABA uptake results in a robust increase in inhibition produced by high-frequency activation of inhibitory pathways. However, these experiments involved the activation of recurrent inhibitory pathways by a stimulating electrode located in the alveus rather than in the stratum radiatum. Since electrical stimulation at each of these sites is likely to have activated different interneuronal populations which possess diverse properties and serve distinct functional roles in the hippocampus (Buhl et al., 1994; Miles et al., 1996), we examined the effects of tiagabine on HFS-evoked monosynaptic responses from both the alveus and the stratum radiatum.

As in Fig. 3, bath applied tiagabine caused the appearance of large DRs following HFS delivered to both the stratum radiatum and alveus (Fig. 5A). However, the response of the cell to the DRs elicited from each stimulation site differed substantially. Although the elicited DRs were of similar amplitude (21.8 ± 2.6 and 16.0 ± 2.6 mV, $p = 0.17$) (Fig. 5B), those generated from stimulation of the stratum radiatum could preferentially trigger the firing of action potentials. In only one of five cells did alvear stimulation evoke action potential firing. Overall, the mean number of APs triggered by DRs from the stratum radiatum was 12.4 ± 3.4 compared to only 0.4 ± 0.4 for those elicited from the alveus (Fig. 5C). Therefore, in the presence of tiagabine, direct excitation occurs only with large

$GABA_A$ receptor-mediated depolarizations generated in the distal dendrites of CA1 pyramidal cells following HFS delivered to the stratum radiatum.

## Discussion

In the present study, we report that the electrical stimulation of hippocampal slices at a frequency intended to mimic the activation of inhibitory interneurons during epileptiform activity produced two important effects on GABA-mediated postsynaptic potentials: (1) a large increase in the amplitude and duration of both $GABA_A$ and $GABA_B$ receptor-mediated hyperpolarizations and (2) the appearance of $GABA_A$ receptor-mediated depolarizing responses. The occurrence of these two contrasting effects depended on the duration and intensity of HFS, with increases in one and/or the other favoring the generation of GABA-mediated depolarizations. The monosynaptic activation of inhibitory interneurons at high-frequency revealed that the effects of tiagabine on GABA-mediated postsynaptic responses are dependent on both the frequency and intensity of stimulation. Furthermore, inhibition of GABA uptake greatly facilitated the generation of $GABA_A$ receptor-mediated DRs following HFS.

In the adult rat, GABA-mediated DRs have previously been reported to occur following the application of an exogenous GABAergic agonist (Andersen et al., 1980; Alger and Nicoll, 1982a), HFS of interneurons (Grover et al., 1993; Staley et al., 1995) as well as following any intervention that produces a large activation of postsynaptic $GABA_A$ receptors (Xie and Smart, 1991; Perreault and Avoli, 1992). A recently proposed model (Staley et al., 1995) suggests that GABA-mediated depolarizations are generated by an efflux of $HCO_3^-$, to which the $GABA_A$ receptor ionophore is permeable (Kaila, 1994), following the activity-induced collapse of the transmembrane $Cl^-$ gradient.

DRs have generally been much easier to evoke following the activation of $GABA_A$ receptors on the distal dendritic membrane of pyramidal cells. This was initially believed to be due to the maintenance of a higher intracellular $Cl^-$ concentration in distal dendrites resulting in an equilibrium potential more positive

than the resting potential (Misgeld et al., 1986). However, the ability to evoke hyperpolarizing responses following dendritic GABA application or activation of dendritic inhibitory synapses is inconsistent with such an explanation. In contrast, the $Cl^-$ accumulation model suggests that GABA-mediated depolarizations are more readily observed in distal dendrites because of the lower intracellular volume, relative to the soma, which results in a more rapid collapse of the $Cl^-$ gradient following the sustained activation of $GABA_A$ receptors. Our results indicate that DRs can nevertheless be evoked by the activation of the more proximally located receptors following strong HFS delivered to the alveus. Furthermore, as with those evoked from the stratum radiatum, the amplitude and duration of proximally evoked DRs are greatly increased following the inhibition of GABA uptake by tiagabine. Generation of DRs following the application of tiagabine has similarly been observed to be facilitated in cortical wedges prepared from audiogenic seizure-prone DBA/2 mice (Hu and Davies, 1997). Such a facilitation is likely produced as a result of the much stronger and more prolonged activation of postsynaptic $GABA_A$ receptors which may cause a more rapid and complete collapse of the transmembrane $Cl^-$ gradient. The tiagabine-induced increase in the amplitude of the $GABA_A$ receptor-mediated fast hyperpolarization, which always precedes the DR, is consistent with such an explanation (Fig. 5A).

In addition to having been shown to produce an anticonvulsant effect in animal seizure models (Faingold et al., 1994; Suzdak, 1994; Halonen et al., 1996; Pfeiffer et al., 1996), tiagabine has proven itself to be particularly effective in the treatment of patients suffering from partial complex seizures (Ben-Menachem, 1995a; Shinnar, 1997; Sachdeo et al., 1997). Our previous demonstration of the frequency-dependent enhancement of inhibition by the GABA uptake inhibitors SKF 89976A and SKF 100330A (Čapek and Esplin, 1993) suggested that this class of anticonvulsant compounds may selectively strengthen GABA-mediated inhibition during the high-frequency synchronous discharge of neurons involved in the initiation and/or propagation of epileptic brain activity. Such a mechanism of action would be expected to minimize the occurrence of CNS depressant side effects normally associated with drugs which produce an indiscriminate

enhancement of inhibition. However, the demonstration that in the presence of tiagabine the depolarizing actions of GABA following HFS are enhanced, seems contrary to expected actions of an anticonvulsant. Despite the occurrence of these depolarizations, the effects of tiagabine during synchronized high frequency neuronal activity may nevertheless reinforce inhibition. Indeed, burst firing was only observed in response to tiagabine-enhanced GABA-mediated depolarizations generated in the distal dendrites of pyramidal cells. Furthermore, we have recently demonstrated that in cells from which a DR could be evoked following HFS in the alveus, an inhibitory period of 200-400 ms was observed during which time stratum radiatum-evoked excitatory postsynaptic potentials (EPSPs) were of reduced amplitude and no longer capable of generating action potentials. This inhibitory period was extended to 800-1600 ms following the bath application of tiagabine, an effect which paralleled the increase in the duration of GABAergic DRs (Jackson et al., 1997). This inhibition most likely resulted from a reduction in the depolarizing effects of the dendritic conductance underlying the EPSP due to the drop in proximal membrane resistivity and dendritic length constant resulting from the concurrently active conductance responsible for the depolarizing response (Qian and Sejnowski, 1990; Staley and Mody, 1992). Similarly, the inability of depolarizations evoked following HFS in the alveus to trigger action potential firing may have been related to the more proximal increase in conductance resulting from the large activation of $GABA_A$ receptors located on the initial segment and soma of CA1 neurons. The activation of inhibitory cells with synaptic targets preferentially distributed on the perisomatic regions of pyramidal cells has previously been shown to suppress the repetitive discharge of sodium-dependent action potentials (Miles et al., 1996). In contrast, in the absence of a proximal increase in conductance, the passive propagation of more distally generated GABA-mediated depolarizations may more readily trigger the firing of a burst of action potentials.

Finally, the effects of tiagabine on GABA-mediated postsynaptic responses were clearly dependent on both the frequency and intensity of stimulation. Increasing the intensity of electrical stimulation will cause the synchronous

activation of an increasingly large population of interneurons. However, such a high degree of neuronal synchronization is unlikely to occur under physiological conditions. Therefore, even though the biophysical properties of interneurons favors the firing of high-frequency bursts of action potentials, the frequency-dependent effects of tiagabine are unlikely to affect normal GABA-mediated neurotransmission. The dependence of this drug's effects on synchronized, high-frequency neuronal activity may therefore allow a more selective anticonvulsant action to be achieved.

**Acknowledgements**

This work was supported by the Medical Research Council of Canada. We thank Dr. K. Krnjević for his helpful advice with the whole-cell recording technique. M. Jackson was supported by an FCAR studentship and by a McGill Faculty of Medicine Internal Studentship.

## Figure Legends

**Fig. 1. Effects of high frequency stimulation (HFS) (100 Hz, 200 ms) on monosynaptic IPSPs elicited in the presence of CNQX (20 μM) and CPP (5 μM).** A: With stimulation by a single pulse, increases in the stimulus intensity (SI) evoked biphasic IPSPs of increasing amplitude and duration (upper traces). HFS using a low SI resulted in a further large increase in both the amplitude and duration of hyperpolarizing IPSPs. With HFS, similar increases in SI caused the appearance of a depolarizing response (DR) of increasingly large amplitude (lower traces). B The amplitude of the evoked DR also depended on the duration of HFS. From top to bottom, responses evoked by a single stimulation followed by responses elicited with HFS of 20, 50, 100, 200 and 1000 ms duration, using an SI of 450 μA. Time scale of 1000 ms in B applies only to lowermost response evoked with HFS of 1000 ms. Inverted triangles in this and subsequent figures indicate the timing of each stimulus.

**Fig. 2. Pharmacological profile of monosynaptic IPSPs evoked by HFS.** A: With stimulation by a single pulse (upper traces), application of the $GABA_A$ receptor antagonist, bicuculline (40 μM), blocked the fast hyperpolarizing component and resulted in the isolation of a slow hyperpolarization which could be blocked by the addition of the $GABA_B$ receptor blocker, CGP 52 432 (10 μM). Application of bicuculline also blocked the fast hyperpolarization as well as the depolarization evoked by HFS (lower traces). Under these conditions, a large slow hyperpolarization was observed, which was blocked by addition of CGP 52 432. B: The reduction in the amplitude of the membrane's response to hyperpolarizing current injections superimposed upon a HFS-elicited postsynaptic response indicates that an increased level of conductance is associated with each component of the response.

**Fig. 3. Effects of tiagabine on monosynaptic IPSPs elicited by low and high stimulus intensities.** Application of tiagabine (20 μM) had no effect on IPSPs elicited by a single stimulus of low intensity (A, upper) while causing an increase in the duration of those elicited using a higher stimulus intensity (B, upper). With low intensity HFS (A, lower) tiagabine caused an increase in the amplitude of the fast hyperpolarization and the appearance of both a depolarizing response and a prominent slow hyperpolarization. With high intensity HFS (B, lower), tiagabine caused the appearance of a large depolarization (10 mV) which dominated the postsynaptic response.

**Fig. 4. Tiagabine's effects on monosynaptic responses evoked from the stratum radiatum** with either a single stimulus (A) or a train of stimuli delivered at high frequency (B) were quantified by measuring the time integral of elicited responses from a baseline corresponding to the resting membrane potential. Traces to the right show examples of the measurements made. A: Bath application of tiagabine (20 μM) caused an increase in the area of hyperpolarization following stratum radiatum stimulation. B: Similarly, with HFS using a low stimulus intensity (60 μA) tiagabine (20 μM) significantly increased the resulting hyperpolarizing area. However, as the stimulus intensity was increased, block of GABA uptake by tiagabine resulted in an overwhelmingly large increase in the amplitude and duration of DRs such that this component of the response now dominated the area measurement. (* $p < 0.03$ and ** $p < 0.01$; control: $n = 7$, tiagabine: $n = 4$-5)

**Fig. 5. Tiagabine-induced changes in the responses evoked following HFS in either the stratum radiatum or alveus.** (A) Following the application of tiagabine (20 μM) large DRs could be evoked by HFS of both the alveus and stratum radiatum. Although the DRs elicited from either location were of similar amplitude (B) induction of action potential firing was typically only seen in response to DRs from the stratum radiatum (A). In the presence of tiagabine, the mean number of APs triggered by DRs from the stratum radiatum was 12.4 ± 2.0 compared to only 0.4 ± 0.4 for those elicited from the alveus ($n = 5$) (C). (** $p < 0.01$, $n = 5$, n.s. = not significantly different from control)

**Fig. 1. Effects of high frequency stimulation (HFS) (100 Hz, 200 ms) on monosynaptic IPSPs elicited in the presence of CNQX (20 μM) and CPP (5 μM).**

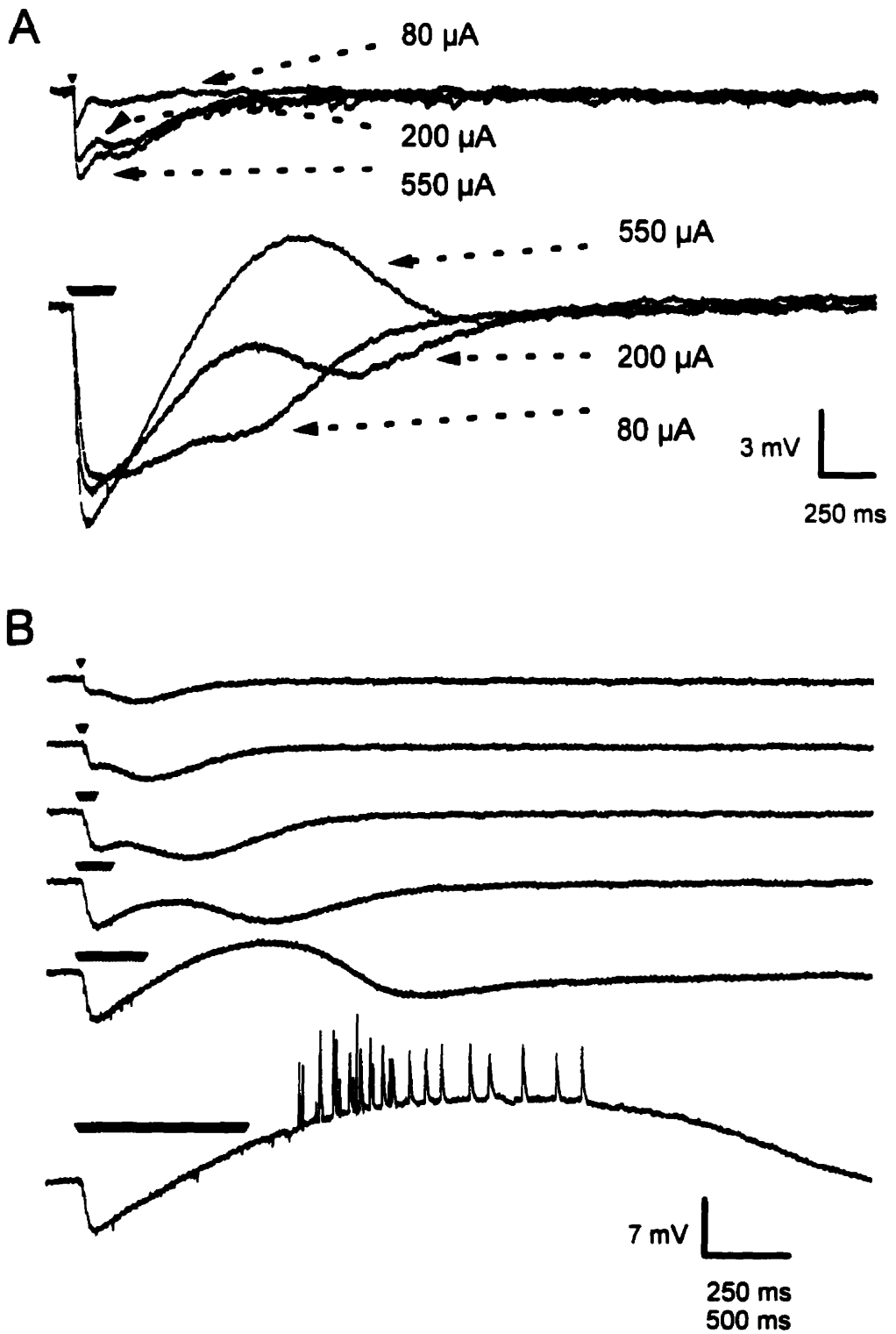


**Fig. 2. Pharmacological profile of monosynaptic IPSPs evoked by HFS.**

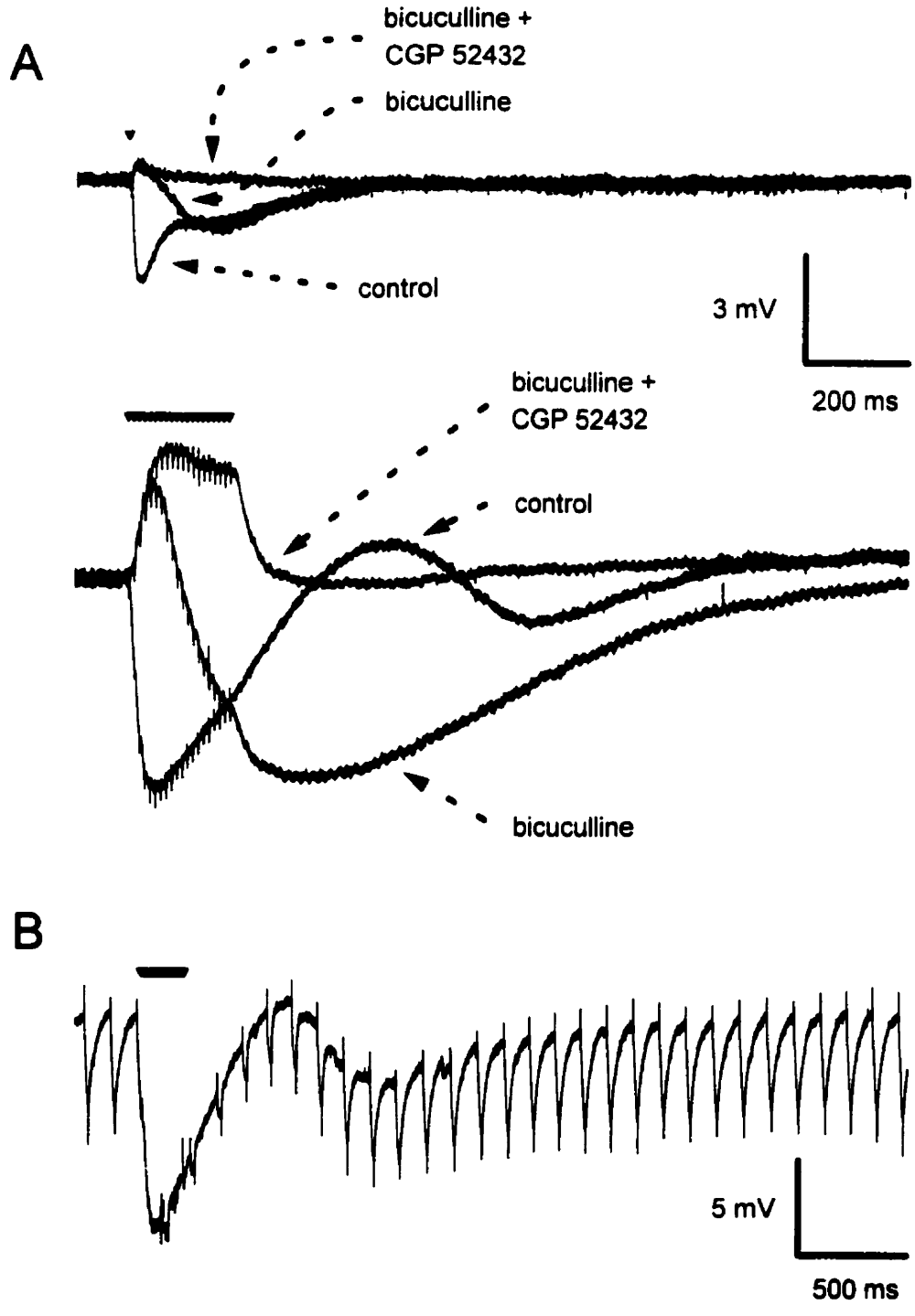


**Fig. 3. Effects of tiagabine on monosynaptic IPSPs elicited by low and high stimulus intensities.**

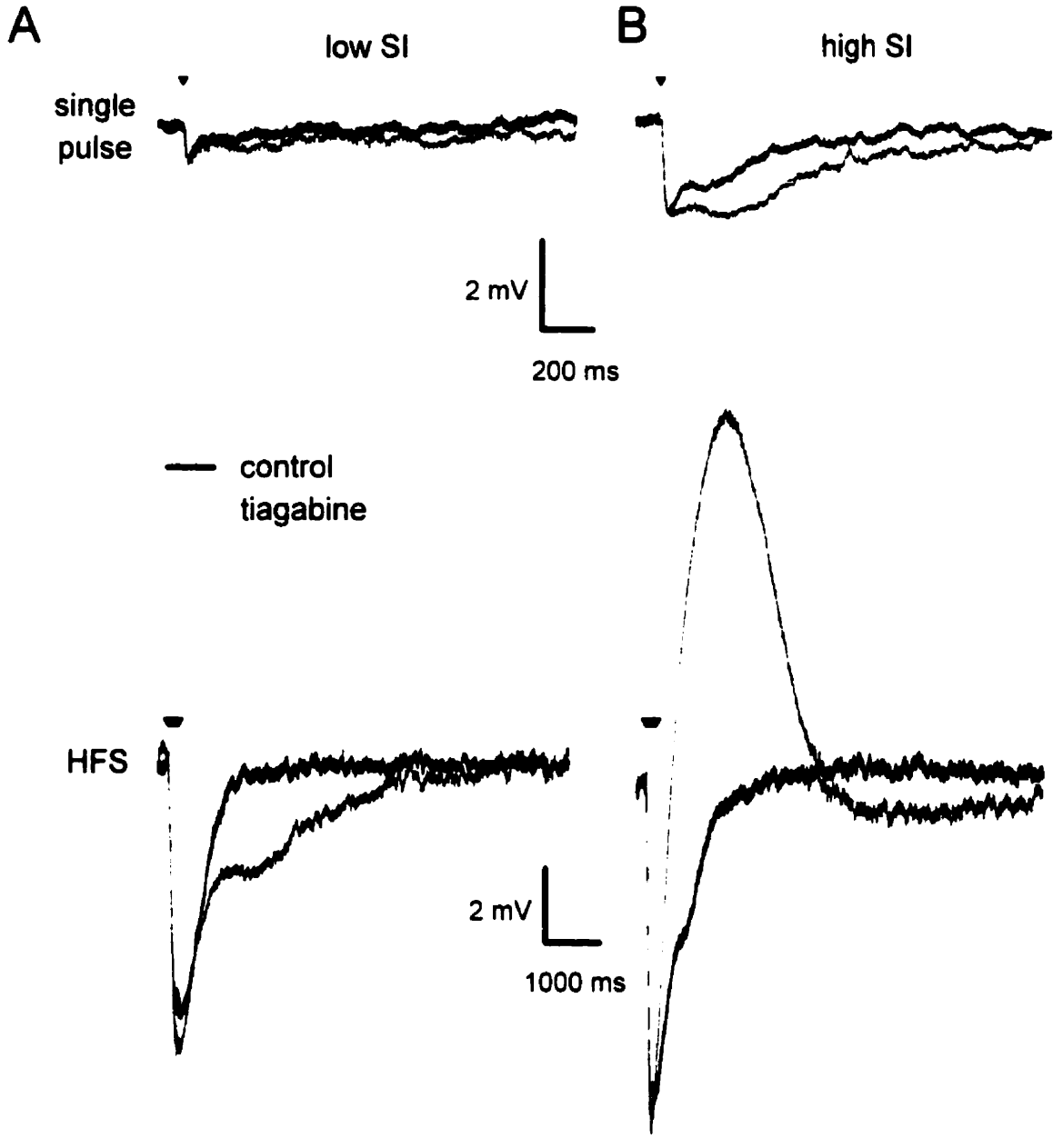


**Fig. 4. Tiagabine's effects on monosynaptic responses evoked from the stratum radiatum**

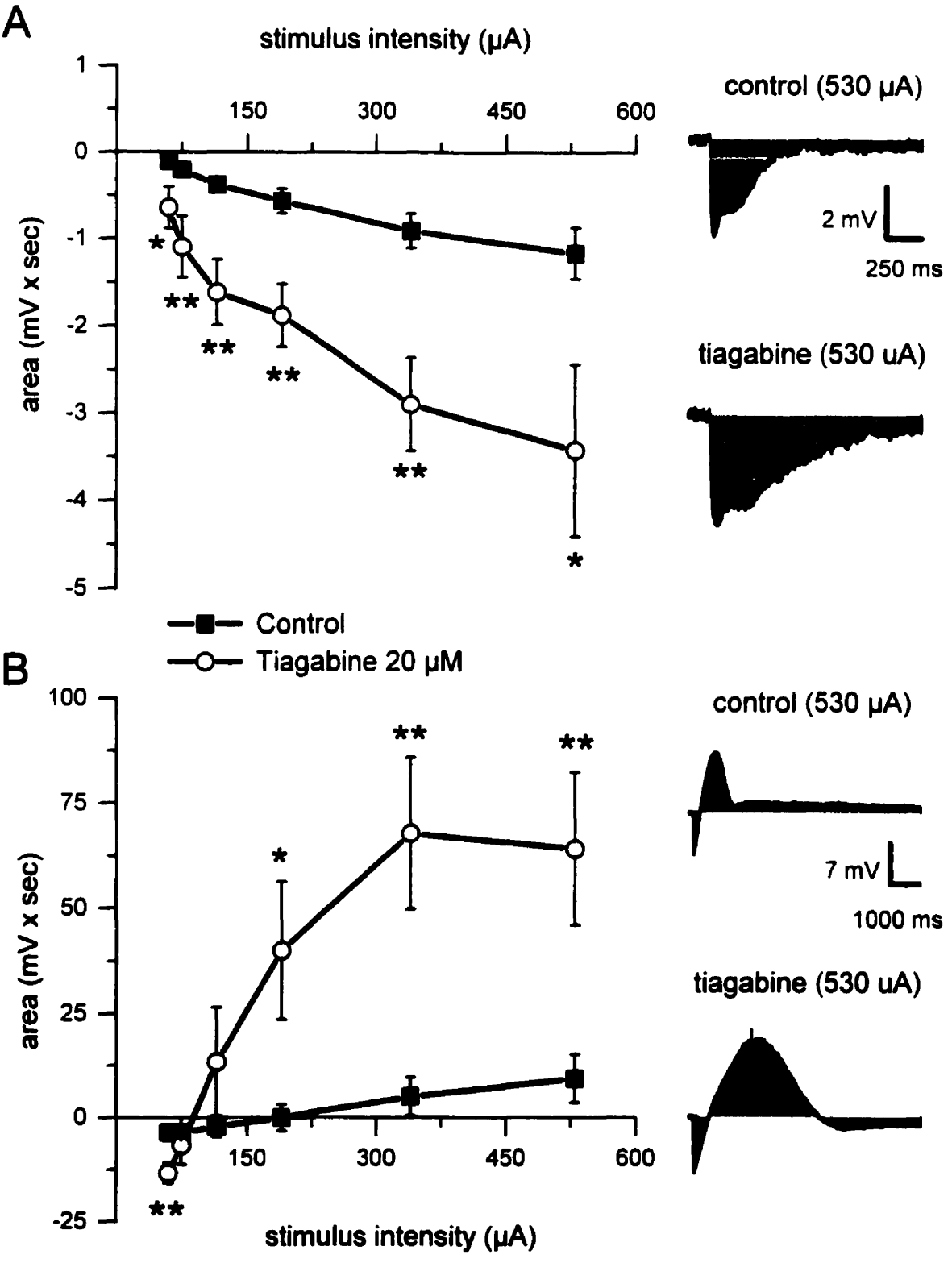
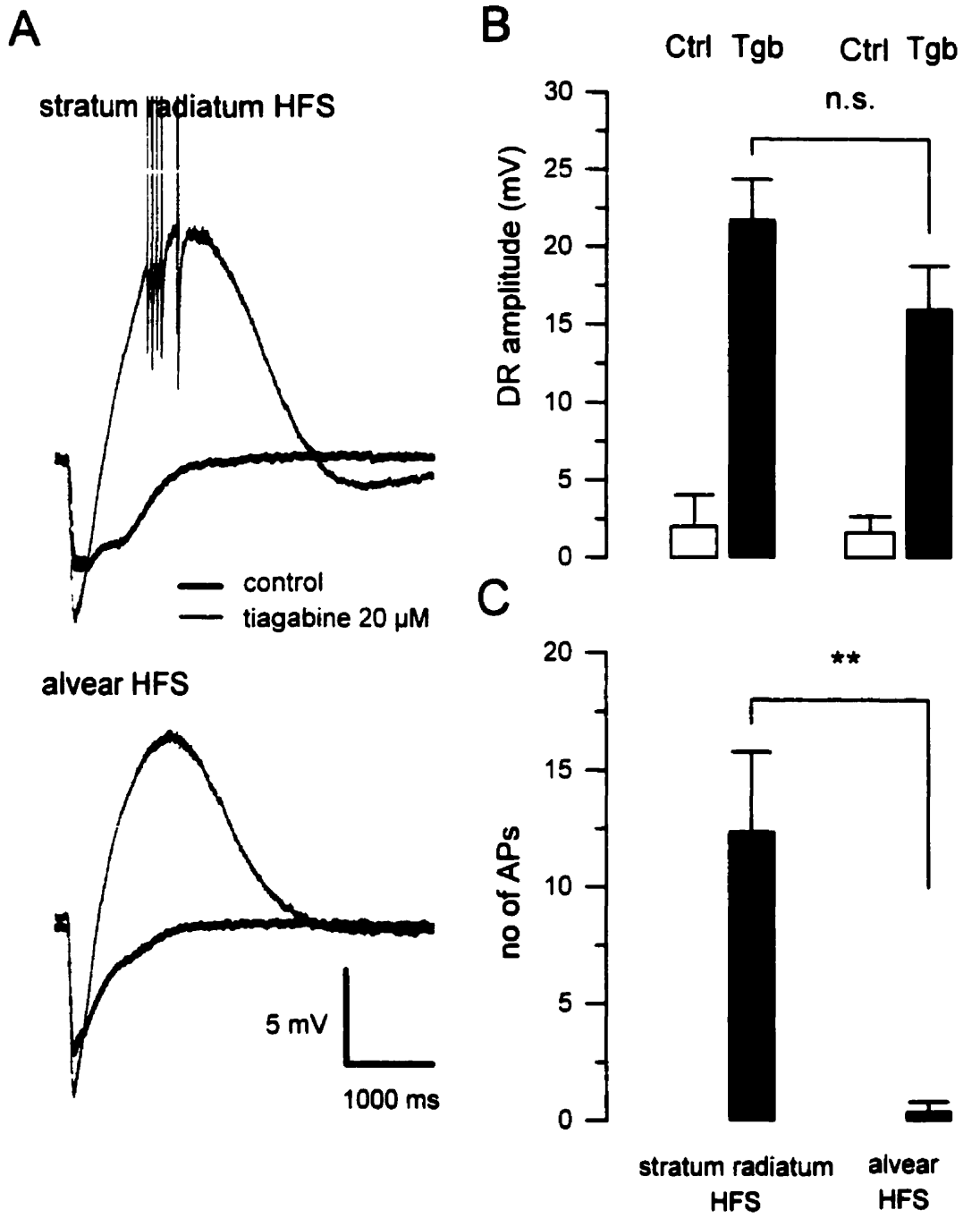


**Fig. 5. Tiagabine-induced changes in the responses evoked following HFS in either the stratum radiatum or alveus.**



## Preface to Section 4

Consistent with the previously demonstrated frequency-dependent actions of GABA uptake blockers, the results presented in the preceding section demonstrated that tiagabine has a more pronounced effect on GABA-mediated synaptic responses evoked by HFS than on those elicited with a single stimulus. However, the frequency-dependent enhancement of GABA-mediated transmission by tiagabine was unexpectedly associated with a facilitation of the depolarizing actions of GABA, which have previously been described in the adult mammalian CNS following the HFS of inhibitory interneurons (Grover et al., 1993; Staley et al., 1995). The ability of tiagabine-augmented DRs to occasionally fire bursts of action potentials suggested that under certain circumstances this anticonvulsant could paradoxically produce a proconvulsant effect. The functional consequences of tiagabine-augmented DRs were therefore assessed in the following study by examining whether these GABA-mediated responses facilitate or inhibit the depolarizing influence of excitatory synaptic transmission.

**The inhibitory nature of tiagabine-augmented $GABA_A$ receptor-mediated depolarizing responses (DRs) in hippocampal pyramidal cells**

Journal of Neurophysiology (under revision)

abbreviated title: Role of tiagabine-augmented GABAergic depolarizations

Michael F. Jackson, Barbara Esplin and Radan Čapek

*Department of Pharmacology and Therapeutics*
*McGill University, Montreal, Que. H3G 1Y6 (Canada)*

*Correspondence*: Dr. Radan Čapek
Department of Pharmacology and Therapeutics
McGill University
3655 Drummond Street
Montreal, Que. H3G IY6, CANADA
Tel.: (514) 398-3607
FAX: (514) 398-6690
E-mail: rcapek@pharma.mcgill.ca

**Abstract**

Tiagabine is a potent GABA uptake inhibitor with demonstrated anticonvulsant activity. GABA uptake inhibitors are believed to produce their anticonvulsant effects by prolonging the postsynaptic actions of GABA, released during episodes of neuronal hyperexcitability. However, tiagabine has recently been reported to facilitate the depolarizing actions of GABA in the CNS of adult rats following the stimulation of inhibitory pathways at a frequency (100 Hz) intended to mimic interneuronal activation during epileptiform activity. In the present study, we performed extracellular and whole-cell recordings from CA1 pyramidal neurons in rat hippocampal slices in order to examine the functional consequences of tiagabine-augmented GABA-mediated depolarizing responses. Orthodromic population spikes (PSs), elicited from the stratum radiatum, were inhibited following the activation of recurrent inhibitory pathways by antidromic conditioning stimulation of the alveus which consisted of either a single stimulus or a train of stimuli delivered at high-frequency (100 Hz, 200 ms). The inhibition of orthodromic PSs produced by high-frequency conditioning stimulation (HFS), which was always of much greater strength and duration than that produced by a single conditioning stimulus, was greatly enhanced following the bath application of tiagabine (2-100 μM). Thus, in the presence of tiagabine (20 μM), orthodromic PSs, evoked 200 and 800 ms following HFS, were inhibited to 7.8 ± 2.6% (mean ± SE) and 34.4 ± 18.5% of their unconditioned amplitudes compared to only 35.4 ± 12.7% and 98.8 ± 12.4% in control. Whole-cell recordings revealed that the bath application of tiagabine (20 μM) either caused the appearance or greatly enhanced the amplitude of GABA-mediated depolarizing responses (DR). Excitatory postsynaptic potentials (EPSPs) evoked from stratum radiatum during time points which coincided with the DR were inhibited to below the threshold for action potential firing. Independently of the stimulus intensity with which they were evoked, the charge carried to the soma by excitatory postsynaptic currents (EPSCs), elicited in the presence of tiagabine (20 μM) during the large (1428 ± 331 pA) inward currents which underlie the DRs, was decreased by an average

of 90.8 ± 1.7%. Such inhibition occurred despite the presence of the $GABA_B$ receptor antagonist, CGP 52 432 (10 μM), indicating that $GABA_B$ heteroreceptors, located on glutamatergic terminals, do not mediate the observed reduction in the amplitude of excitatory postsynaptic responses. The present results suggest that despite facilitating the induction of GABA-mediated depolarizations, tiagabine application may nevertheless increase the effectiveness of synaptic inhibition during the synchronous high frequency activation of inhibitory interneurons through a postsynaptic action.

## Introduction

γ-Aminobutyric acid- (GABA-) mediated inhibition plays a critical role in the control of central nervous system (CNS) excitability as demonstrated by the convulsions resulting from the administration, in both animals and humans, of compounds which decrease the efficacy with this neurotransmitter system (for review see Gale, 1992). The development of pharmacological agents capable of potentiating GABAergic neurotransmission has therefore been an important strategy in the search for novel anticonvulsants. Such a mechanism is thought to underlie or contribute to the effectiveness of the barbiturates and benzodiazepines (Rogawski and Porter, 1990), both allosteric modulators of $GABA_A$ receptor function, as well as to the more recently developed antiepileptic vigabatrin (Grant and Heel, 1991), an inhibitor of GABA metabolism.

The therapeutic potential of GABA uptake inhibitors in the treatment of epilepsy has been recognized since the early demonstrations of the ability of small amino acids, such as nipecotic acid and guvacine, to enhance GABA mediated responses evoked either synaptically (Matthews et al., 1981; Dingledine and Korn, 1985) or by iontophoretic application of the neurotransmitter (Dingledine and Korn, 1985; Curtis et al., 1976). However, being highly polar, these compounds do not readily cross the blood-brain barrier and therefore can only produce an anticonvulsant effect when administered intracerebroventricularly (Croucher et al., 1983; Frey et al., 1979). The addition of lipophilic anchors to these simple structures increases not only their ability to permeate the blood-brain-barrier but also their affinity for the GABA transporter and thus allows them to exert an anticonvulsant effect following their systemic administration (Yunger et al., 1984; Braestrup et al., 1990; Suzdak et al., 1992a).

We have previously reported that in hippocampal slices, bath application of two of these novel uptake blockers, SKF 89976A and SKF 100330A, results in a relatively selective and prolonged enhancement in the inhibition produced following the high-frequency stimulation (100 Hz, 200 ms) of recurrent inhibitory pathways (Čapek and Esplin, 1993). Such a frequency-dependent mechanism

of action should result in a selective strengthening of synaptic inhibition during episodes of high-frequency activity and allow these compounds to control seizures while minimizing the occurrence of side effects normally associated with drugs which produce an indiscriminate enhancement of inhibition. Paradoxically, recent evidence has demonstrated that high-frequency activation of inhibitory pathways, similar to that used in our previous study, evokes large (5-20 mV) $GABA_A$-receptor mediated depolarizing responses (DRs) capable of triggering a burst of action potentials in hippocampal pyramidal cells, suggesting that GABAergic mechanisms may contribute to the generation and/or propagation of seizure activity (Staley et al., 1995; Jackson et al., 1995; Grover et al., 1993). Furthermore, we have recently demonstrated that tiagabine, a novel GABA transport inhibitor with demonstrated anticonvulsant properties in animal models of epilepsy (Faingold et al., 1994) as well as in human partial epilepsy (Schachter, 1995; Ben-Menachem, 1995a), greatly facilitates the depolarizing actions of GABA following the stimulation of hippocampal interneurons at high (100 Hz) frequency (Jackson et al., 1996).

We therefore decided to further investigate the mechanisms by which GABA-uptake blockers produce frequency-dependent enhancement of inhibition by studying the effects of tiagabine-augmented GABA-mediated depolarizations on excitatory responses evoked at various (100-1600 ms) time intervals following the high frequency stimulation of hippocampal recurrent inhibitory pathways.

**Methods**

Extracellular field and whole-cell recordings were made from the CA1 region of hippocampal slices obtained as follows: male Sprague-Dawley rats (100-200 g) were decapitated, their brains quickly removed and placed in cooled, oxygenated (95% $O_2$, 5% $CO_2$) artificial cerebrospinal fluid (aCSF) of the following composition (in mM): 124 NaCl, 3.3 KCl, 2.5 $CaCl_2$, 2.4 $MgSO_4$, 25.6 $NaHCO_3$, 1.25 $KH_2PO_4$ and 10 glucose. Transverse slices (400-500 µm) were then prepared using either a McIlwain tissue chopper or vibratome and allowed to

recover at room temperature for at least 1 hour in oxygenated aCSF. For recordings, slices were transferred to a tissue chamber and maintained at 31°C at the interface between humidified 95% $O_2$ and 5% $CO_2$, and oxygenated aCSF perfused through the chamber at a flow rate of 1.5 -3.5 ml/min.

The effects of tiagabine on GABA-mediated inhibition were assessed using the antidromic-orthodromic test of inhibition as previously described (Čapek and Esplin, 1993; Jackson et al., 1994). Briefly, field potentials were recorded with a 3 M NaCl-filled micropipette (5-15 MΩ) located in the pyramidal cell layer of the CA1 region. Constant-current stimuli (100 μs) delivered through bipolar stimulating electrodes to the Schaffer-collaterals of stratum radiatum elicited orthodromic population spikes (PSs). These were inhibited by preceding antidromic conditioning stimulation delivered to the alveus which causes the activation of inhibitory interneurons and the subsequent release of GABA onto pyramidal cells. Conditioning alvear stimulation was by either a single stimulus or by a train of 20 stimuli delivered at a high (100 Hz) frequency. The delay between the conditioning stimulus and subsequent orthodromic test response is referred to as the interstimulus interval (ISI) and was in the range of 10-1600 ms. Orthodromic stimulus intensity was adjusted to produce a half-maximal PS. At an ISI of 10 ms, antidromic stimulus intensity was increased until it produced a 70-90% reduction in the amplitude of the orthodromic PS. In this paradigm, inhibition is measured indirectly as a reduction in the amplitude of the test PS expressed as a percentage of the unconditioned PS. For each ISI, responses were obtained before and after drug treatment. Results were expressed as means ± SE and the significance of the drug-induced change was evaluated using Student's *t*-test.

Tight seal (>3 GΩ) whole-cell recordings were obtained from CA1 pyramidal cells using patch pipettes pulled from borosilicate glass (WPI) with a Brown-Flaming micropipette puller (Sutter Instruments, P-80). Patch electrodes had a resistance of 2-4 MΩ when filled with an internal solution of the following composition (in mM): 140-150 $KMeSO_4$ (or $CsMeSO_4$ where indicated) 10 *N*-2-

hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES), 2 $MgCl_2$. The solution was buffered with KOH (or CsOH) to a pH of 7.2-7.3 and filtered through a 0.2 µm pore size filter (Nalgene). Total osmolality for all intracellular solutions ranged from 270 to 290 mOsm. Current-clamp recordings were made with an Axoclamp 2A amplifier (Axon Instruments) operated in bridge mode whereas an Axopatch 200A (Axon Instruments) was used for voltage-clamp recordings. For current clamp recordings, cells were maintained, by appropriate current injection, at a constant membrane potential. All signals were digitized at 18 kHz (Instrutech Corp., VR-10A) and stored unfiltered on videotape for later retrieval and analysis using a PC based acquisition and analysis system functionally similar to that described previously (Théorêt et al., 1984).

In all recordings, drugs used were dissolved in aCSF and superfused over slices. Tiagabine ((R)-*N*-[4,4-di-(3-methylthien-2-yl)but-3-enyl] nipecotic acid hydrochloride) and CGP 52 432 (3-[[[(3,4-dichlorophenyl)methyl]amino]propyl](diethoxy-methyl)) were generously supplied by Abbott Laboratories (Chicago, Illinois) and Novartis Pharma AG (Basel, Switzerland), respectively.

**Results**

Bath application of tiagabine (20 µM) had no effect on the amplitude of the unconditioned PS (Fig. 1A) and produced no change in the inhibition resulting from a single antidromic conditioning stimulation at short interstimulus intervals (ISI) (10-20 ms) (Fig. 1A and B). At longer ISIs (40-160 ms), inhibition was however increased resulting in a further 34.6% reduction in PS amplitude at an ISI of 160 ms (Fig. 1B). The frequency-dependence of drug action was tested by stimulating the recurrent inhibitory pathways with a train of stimuli delivered at 100 Hz. The inhibition produced by such high frequency stimulation was of greater strength and duration than that resulting from a single stimulus. Thus, although the PS had fully recovered by 160 ms following conditioning stimulation by a single pulse, with HFS it was inhibited to below 35% of its original amplitude

at a more delayed ISI of 200 ms and recovered from inhibition over the next several hundred milliseconds (Fig. 1B). When tiagabine was added to the perfusate, inhibition was increased to the extent that high frequency antidromic conditioning stimulation could now produce a near complete inhibition of the population spike at an ISI of 800 ms (Fig. 1A). The recovery from inhibition was also greatly prolonged such that the PS recovered to its original amplitude over the course of several thousand milliseconds rather than the hundreds of milliseconds time needed in control (Fig. 1B). The effects of tiagabine on recurrent inhibition were concentration-dependent over a range of 2 to 100 μM.

Given its ability to increase the depolarizing actions of GABA released following the HFS of inhibitory interneurons (Jackson et al., 1996), tiagabine could easily have been expected to produce an increase in pyramidal cell excitability. However, consistent with our previous findings with the SKF series of GABA uptake blockers (Čapek and Esplin, 1993), bath application of tiagabine resulted in a large increase in the functional inhibition resulting from the HFS of recurrent inhibitory pathways. Using the same stimulation paradigm, whole-cell recordings were made from CA1 pyramidal cells in order to investigate the basis for enhanced inhibition following the application of tiagabine. In the absence of preceding alvear HFS, EPSP-IPSP sequences were evoked following the single stimulation of Schaffer-collaterals in stratum radiatum (Fig. $2A_1$ and $2B_1$). The evoked IPSPs were comprised of both fast and slow hyperpolarizing components (f- and s-IPSPs respectively). As illustrated in the insets of figure $2A_1$ and $B_1$, a current intensity for Schaffer-collaterals stimulation was chosen which could reliably elicit unconditioned EPSPs capable of triggering the firing of a single AP. When evoked 400 ms following the high-frequency activation of recurrent inhibitory pathways, the resulting EPSPs could no longer trigger an AP (Fig. $2A_2$). This, in spite of the fact that the amplitude and duration of the EPSPs appeared enhanced due to a reduction in the amplitude of the overlapping f-IPSP. Such a reduction most likely occurred as a result of a decrease in the driving force for $Cl^-$ following its intracellular accumulation during the sustained activation of postsynaptic $GABA_A$ receptors resulting from high-frequency alvear stimulation

(Thompson and Gähwiler, 1989a; McCarren and Alger, 1985). In contrast, when the interstimulus interval was 800 ms, preceding high frequency alvear stimulation no longer prevented the firing of APs following orthodromic stimulation (Fig. $2B_2$). Consistent with previous reports of the effects of GABA uptake inhibitors on evoked IPSPs (Thompson and Gähwiler, 1992b; Roepstorff and Lambert, 1992), bath application of tiagabine greatly prolonged the duration of IPSPs while producing little or no effect on their amplitude (Fig. $2A_1$ and $B_1$). Rather than producing a qualitatively similar change in the duration of hyperpolarization following HFS, tiagabine either caused the appearance or greatly increased the amplitude and duration of a GABA-mediated depolarization. The presence of the DR coincided with a prolonged period of inhibition as demonstrated by the suppression of action potential firing and large reduction in the amplitude of EPSPs superimposed at various time points along the different phases of the DR (Fig. $2A_2$ and $B_2$). Thus, in control, inhibition of action potential firing was observed in only 4 of 9 and 4 of 10 cells at ISIs of 200 and 400 ms respectively. Following the addition of tiagabine to the superfusate, inhibition could now be seen in all cells at these same ISIs, with action potential firing now observed in only 1 of 10 cells at 800 and in only 1 of 7 at 1600 ms following HFS of the alveus (Table 1). It is interesting to note that, under control conditions, inhibition of cell firing was observed in only those cells from which a DR could be elicited. Furthermore, following the application of tiagabine, the change in the time course of the inhibition of EPSP-induced AP firing was similar to that of the inhibition of stratum radiatum evoked PSs (compare with Fig. 1)

The observed reduction in the amplitude of EPSPs following the HFS of recurrent inhibitory pathways could be explained by two possible mechanisms: 1) reduced activation of postsynaptic excitatory amino acid receptors due to a decrease in transmitter release caused by the activation of presynaptic $GABA_B$ heteroreceptors present at glutamatergic terminals, and 2) shunting of the dendritically evoked EPSP by the concurrently active conductance responsible for the DR. These possibilities were investigated in cells voltage-clamped near the reversal potential for $GABA_A$ receptor-mediated inhibitory postsynaptic currents

(IPSCs) and superfused with aCSF containing 20 μM tiagabine and 10 μM of the specific $GABA_B$ receptor antagonist CGP 52 432, a concentration which prevents the inhibition of glutamate release by GABA (Waldmeier et al., 1994). Under these conditions relatively pure excitatory postsynaptic currents (EPSCs) could be elicited following the single stimulation of Schaffer-collaterals. HFS of the alveus evoked large (1428 ± 331 pA) inward currents with a time course similar to that of the DRs recorded in current-clamp and which reversed at a membrane potential of -51 mV (Fig. 3A), a value identical to the previously reported reversal potential of GABA-mediated DRs (Staley et al., 1995; Perkins and Wong, 1996). As with the EPSPs, EPSCs evoked 800 ms following HFS of the alveus (Fig. 3B, conditioned) were of much reduced amplitude, this in spite of the presence of CGP 52 432, indicating a lack of involvement of presynaptic $GABA_B$ receptors in the reduction of excitatory responses. This effect was quantified by measuring the percent reduction of charge transferred to the soma during EPSCs that were superimposed upon the large HFS-evoked inward currents (Fig. 3C). The inhibition produced following HFS of the alveus was independent of the stimulus intensity used to evoke the EPSCs and averaged 90.8 ± 1.7% ($n$ =5) across all stimulus intensities used. In an attempt to improve the quality of the space clamp, and thus record a larger fraction of the distally generated EPSC, recordings were made using a $CsMeSO_4$ based electrode solution. Nevertheless, under these recording conditions, the mean percent reduction of EPSCs was identical to that observed in the absence of intracellular $Cs^+$ ions and was equal to 87.3 ±. 2.9% ($n$ = 4) across an identical range of stimulus intensities. For this reason, the data obtained under both recording conditions were pooled in order to generate the graph in Fig. 3C. The percent drop in membrane resistivity, estimated from a 10 mV, 200 ms step superimposed upon the large GABA-mediated inward currents, was 83.4 ± 1.2% (data not shown). This result is strikingly similar to the percent reduction of charge carried by evoked EPSCs suggesting that the observed inhibition of excitatory responses, at time points which coincide with the occurrence of DRs, is most likely due to a shunting mechanism.

## Discussion

In the adult rat, biphasic $GABA_A$ receptor-mediated postsynaptic responses consisting of an initial hyperpolarization followed by a depolarization have been observed under a number of circumstances which have in common the prolonged activation of postsynaptic $GABA_A$ receptors. These include: 4-aminopyridine-induced epileptiform activity (Perreault and Avoli, 1992; Traub et al., 1995; Perkins and Wong, 1996), iontophoretic application of GABA (Thalmann et al., 1981; Andersen et al., 1980; Alger and Nicoll, 1982a), evoked synaptic responses in the presence of barbiturates (Alger and Nicoll, 1982b; Thalmann et al., 1981) or zinc (Xie and Smart, 1991), and responses evoked by high frequency stimulation of inhibitory interneurons (Grover et al., 1993; Staley et al., 1995; Jackson et al., 1995). In neurons from immature animals, GABA also produces depolarizing responses which appear to be due to the maintenance of a $Cl^-$ gradient which is positive to the cells resting membrane potential (RMP) (Mueller et al., 1984; Cherubini et al., 1990). In the adult, however, the DR appears to be mediated by bicarbonate ($HCO_3^-$) anions, which in addition to $Cl^-$ are known to permeate the $GABA_A$ ionophore (Kaila, 1994). However, given the fact the $Cl^-$ reversal potential in CNS neurons is typically more negative than the RMP (with dorsal root ganglion and hippocampal granule cells being exceptions) and that $GABA_A$ receptors are approximately 5 times more permeable to $Cl^-$ anions than to $HCO_3^-$ (Bormann et al., 1987), the inward flow of $Cl^-$ normally dominates postsynaptic currents leading to a hyperpolarization of the neuronal membrane. As mentioned above, DRs typically occur following the decay of an initial hyperpolarization of variable amplitude and duration. A recently proposed model (Staley et al., 1995) suggests that during the sustained activation of $GABA_A$ receptors the inward flow of $Cl^-$, which underlies the initial hyperpolarization, causes a partial collapse of the gradient for this anion resulting in a diminished flow of $Cl^-$. This allows a greater net flow of $HCO_3^-$, whose reversal potential is more positive than the RMP, leading to the generation of a depolarizing potential.

The amplitude and duration of GABA-mediated DRs are critically dependent on both the duration of HFS as well as on the stimulus intensity used (Jackson et al., 1995). Furthermore, they are typically much larger when evoked monosynaptically following the blockade of excitatory amino acid receptors with a combination of CNQX and CPP, which antagonizes AMPA/kainate and NMDA receptors (unpublished observation). The activation of a greater number of inhibitory interneurons due to the necessity, under these conditions, of positioning the stimulating electrodes much closer to the cell from which monosynaptic GABAergic responses are to be recorded, is most likely responsible for this. Given the fact that the DRs are generated only after such strong, sustained activation of postsynaptic $GABA_A$ receptors, which is unlikely to occur during normal synaptic transmission, the exact physiological role of GABA-mediated depolarizations is unclear. Evidence has been presented suggesting that the transient transformation of the postsynaptic actions of GABA from mainly hyperpolarizing to depolarizing may contribute to synaptic plasticity by relieving the voltage-dependent $Mg^{2+}$ block of NMDA receptors (Staley et al., 1995). Although such an interaction may occur in dendritic compartments in which both $GABA_A$ and NMDA receptors are activated simultaneously, our results suggest that a large proximal increase in input conductance resulting from the activation of postsynaptic $GABA_A$ receptors, such as would be expected to occur following the intense activation of inhibitory interneurons during epileptiform activity, results in a transient suppression in the ability of excitatory transmission to depolarize the soma following the activation of distal excitatory amino acid receptors.

Although the inhibition of EPSPs could have been due to the activation of $GABA_B$ heteroreceptors located at glutamatergic terminals, the suppression of EPSCs which occurred despite the presence of the $GABA_B$ receptor blocker CGP 52 432 argues against the involvement of such a mechanism. The evidence presented here suggests that shunting inhibition of evoked EPSPs most likely underlies the much stronger inhibition of stratum radiatum evoked population spikes resulting from the activation of recurrent inhibitory pathways with HFS compared with that produced following the activation with a single stimulus (Fig.

1). In order for a conductance to effectively shunt the potential generated by the activation of another, it should be sufficiently large and generated more proximally (Koch et al., 1983; Qian and Sejnowski, 1990; Vu and Krasne, 1992). The conductance increase resulting from the activation of $GABA_A$ receptors following HFS stimulation was very large as demonstrated by 83.4% reduction in input resistance which was observed in our experiments. Furthermore, either directly or through the recurrent axon collaterals of pyramidal cells, the electrical stimulation of the alveus which we used will have caused the activation of interneurons predominantly located within the alveus and stratum oriens, and to a lesser extent, stratum pyramidale. Among the different types of interneurons responding to such stimulation, GABAergic basket and axo-axonic cells have been shown to preferentially make synaptic contacts with the soma, proximal dendrites and initial segment of CA1 pyramidal cells. The electrical stimulation of these interneuronal populations from an electrode positioned in the alveus is therefore likely to have caused a proximal increase in conductance following the activation of $GABA_A$ receptors. Furthermore, our demonstration that the percent reduction in the charge transferred to the soma was independent of the strength of the excitatory input is consistent with predictions of the consequences of proximal shunting inhibition on distally evoked EPSPs (Vu and Krasne, 1992).

Due to their ability to directly trigger the firing of bursts of action potentials, DRs have also been implicated in the generation of epileptiform activity. This is further supported by experiments demonstrating a reduction in the duration of afterdischarges following the blockade of $GABA_A$ receptors by the specific antagonist bicuculline (Higashima et al., 1996; Traub et al., 1995). In light of this, the large increase in GABA-mediated depolarizations, observed following the application of tiagabine, would seem contrary to the expected actions of an anticonvulsant. However, strong inhibition was always observed in response to the HFS of recurrent inhibitory pathways in the presence of tiagabine (Fig. 1). Furthermore, our results suggest that during the synchronous, high-frequency activation of proximally located interneurons, tiagabine increases the $GABA_A$ receptor-mediated conductance to such a large extent that the ability of cells to

fire action potentials in response to dendritically-evoked EPSPs is suppressed, indicating that tiagabine-facilitated DRs can retain the inhibitory character typical of GABA action. Therefore, despite producing a facilitation of GABA-mediated depolarizations, the potentiation of GABA-mediated shunting inhibition following tiagabine administration may allow this drug to produce an anticonvulsant effect by suppressing the generation of burst discharges during epileptiform activity.

**Acknowledgement**

We wish to thank Ms. A. Constantin for her competent technical assistance and Dr. Yves De Koninck for his helpful advice as well as for the use of his recording equipment. This work was supported by the Medical Research Council of Canada. M. Jackson was supported by an FCAR studentship and by a McGill Faculty of Medicine Internal Studentship.

**Table 1.** Summary of tiagabine effects on the inhibition of action potential firing in response to Schaffer-collateral stimulation following HFS of recurrent-inhibitory pathways.

| | Interstimulus interval (ms) | | | |
|---|---|---|---|---|
| | 200 | 400 | 800 | 1600 |
| Control | 4/9 | 4/10 | 10/10 | 8/8 |
| Tiagabine (20 μM) | 0/9 | 0/10 | 1/10 | 1/7 |

Values indicate the number of cells under each condition that fired an AP in response to evoked EPSPs over the number of cells examined.

## Figure Legends

**Fig. 1. Effects of tiagabine (20 μM) on recurrent GABA-mediated inhibition.** A: Bath applied tiagabine had no effect on unconditioned orthodromic PSs, nor on the recurrent inhibition produced by a single pulse. The recurrent inhibition produced by HFS (100 Hz, 20 pulses) was greatly enhanced by tiagabine resulting in a complete inhibition of the PS elicited at 800 ms. B: Summary of the effects of tiagabine (20 μM) on recurrent inhibition produced by stimulation with either a single pulse (- - - -) or with HFS (------). Inhibited PSs, expressed as a % of the unconditioned PS at various interstimulus intervals (ISI) are shown. Each point represents the mean of values obtained from 5 experiments. * $p < 0.05$; ** $p < 0.01$ compared to controls.

**Fig. 2. Effects of the tiagabine-induced increase of DRs on superimposed evoked EPSPs in a typical CA1 pyramidal neuron.** EPSP-IPSP sequences were evoked by a single stimulation in stratum radiatum. While tiagabine produced no effect on evoked EPSPs, an increase in the duration of IPSPs was observed following its application ($A_1$ and $B_1$). With HFS of the alveus, action potential firing in response to evoked EPSPs was inhibited at 400 ms ($A_2$ and inset), but not at 800 ms ($B_2$ and inset). Application of tiagabine resulted in the appearance of DRs following HFS ($A_2$ and $B_2$). EPSPs, superimposed upon the DRs, were of reduced amplitude and the action potential firing was inhibited at both 400 and 800 ms. In this and the subsequent figure the bars below or above traces indicate timing and duration of HFS in the alveus while filled triangles show timing of stratum radiatum stimulation.

**Fig. 3. Large proximally evoked inward currents underlying GABA-mediated depolarizations inhibit distally evoked EPSCs.** Recordings were made in the presence of 20 μM tiagabine and 10 μM CGP 52 432. A: The reversal potential of $GABA_A$ receptor-mediated currents evoked by HFS in the alveus was determined from responses generated at membrane potentials of -30 to -70 mV. Dashed line indicates the time point at which the amplitude of evoked inwards was measured (1100 ms following HFS). Amplitudes (mean ± SE, $n$ = 2) were plotted vs. membrane potential and the data points were fitted with a straight line obtained by linear regression. B: The influence of concurrently active inward $GABA_A$-receptor currents on EPSCs evoked from stratum radiatum was tested 800 ms following HFS. Traces show EPSCs evoked with (conditioned) or without (unconditioned) preceding HFS in the alveus. The region of the traces selected by the dashed box are shown to the right on an expanded time base. C: The charge carried to the soma by EPSCs evoked using a range of stimulus intensities (70-310 μA) was determined by measuring the time integral of recorded currents. The percent reduction in the charge transferred to the soma by EPSCs (mean ± SE, $n$ = 7-9) evoked 800 ms following HFS in the alveus was plotted against the intensity of stratum radiatum stimulation used to evoke EPSCs.

**Fig. 1. Effects of tiagabine (20 μM) on recurrent GABA-mediated inhibition.**

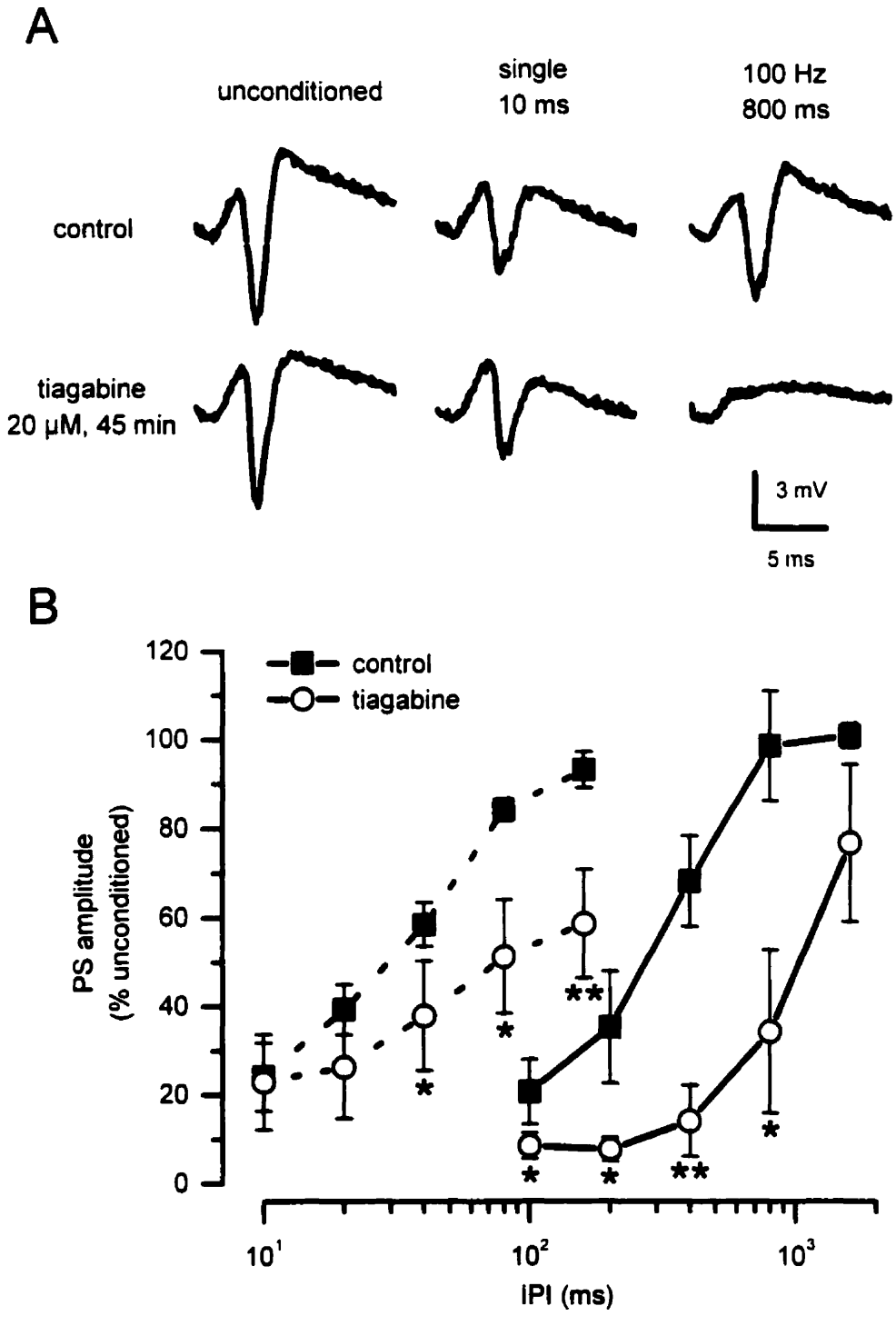


**Fig. 2. Effects of the tiagabine-induced increase of DRs on superimposed evoked EPSPs in a typical CA1 pyramidal neuron.**

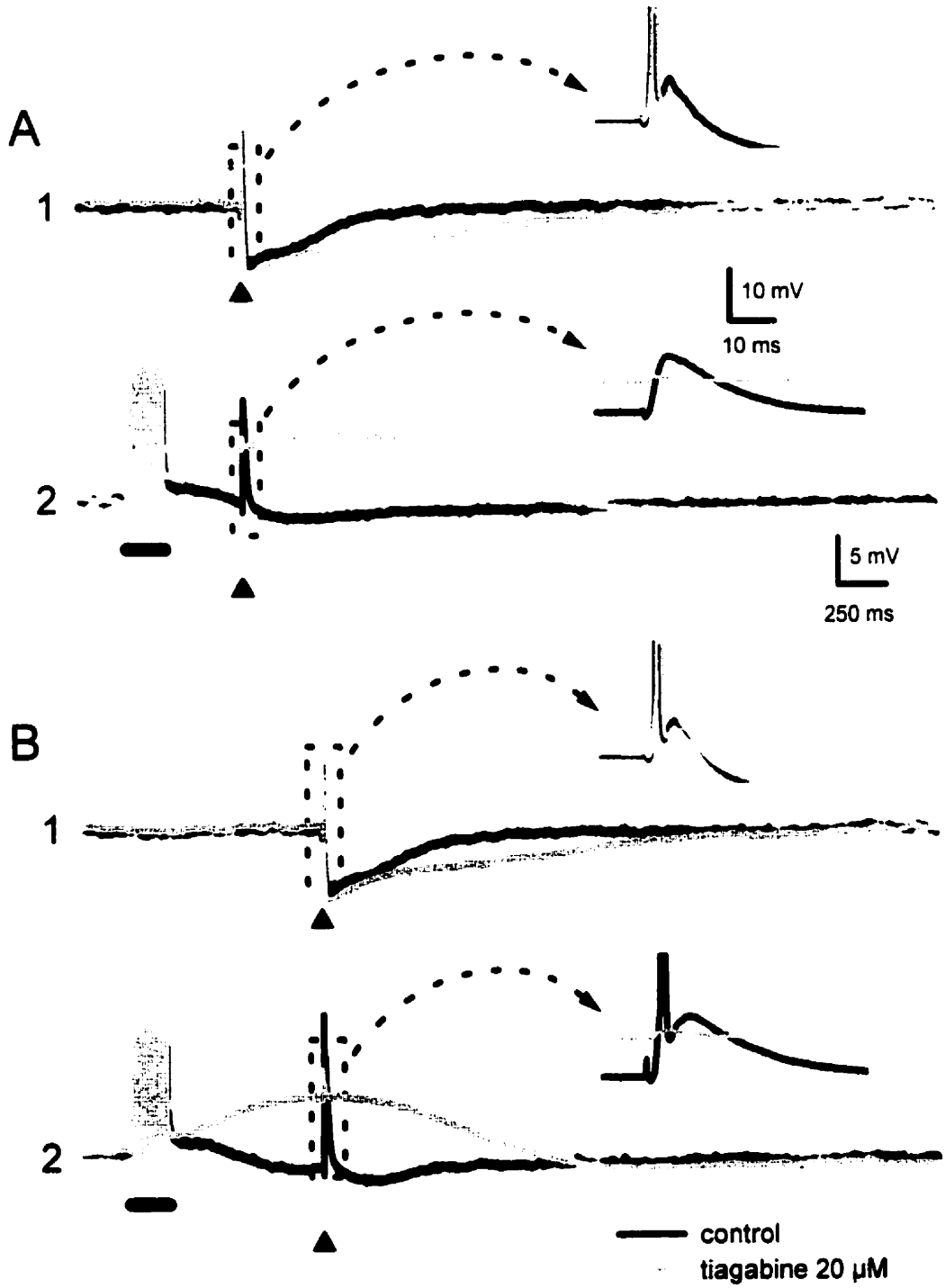
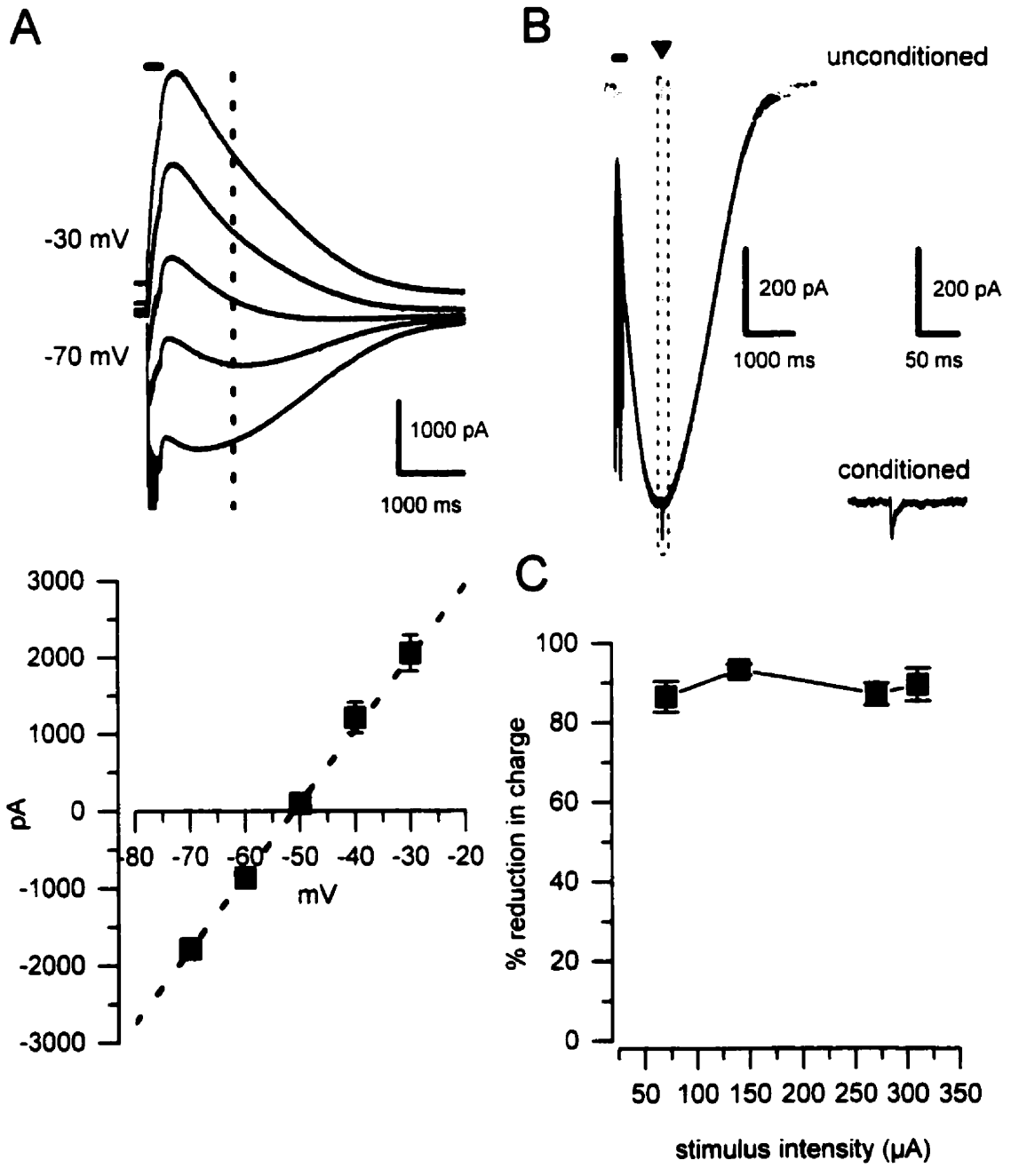


**Fig. 3. Large proximally evoked inward currents underlying GABA-mediated depolarizations inhibit distally evoked EPSCs.**



**Preface to Section 5**

Numerous neurochemical studies have demonstrated the ability of GVG to elevate the CNS levels of GABA. This would seem to support the widely accepted mechanism whereby GVG produces its anticonvulsant actions by increasing the effectiveness of synaptic inhibition. However, such evidence is somewhat circumstantial since these studies cannot demonstrate that the increased levels of neurotransmitter cause an increase in the activation of postsynaptic receptors through which GABAergic inhibition is mediated. The only previous report which has attempted to examine the effects of GVG pretreatment on GABA-mediated inhibition failed to observe any significant change with several treatment regimens previously demonstrated to protect against experimentally-induced seizures (Rak and Lothman, 1988). As with GABA uptake blockers, the possibility exists that the actions of GVG on the GABA neurotransmitter system are frequency-dependent. Such an action could not have been detected due to the design of this previous study which used only single conditioning stimulation to evaluate the strength of GABA-mediated inhibition. In the following experiments, the effects of GVG on GABA-mediated inhibition were reexamined using single, low- (2.5-10 Hz) , and high-(100Hz) frequency conditioning stimulation of recurrent inhibitory pathways in hippocampal slices prepared from drug (GVG, 1500 mg/kg) or saline pretreated rats.

**Reversal of the activity-dependent suppression of GABA-mediated inhibition in hippocampal slices from γ-vinyl GABA (vigabatrin)-pretreated rats**

(to be submitted)

*Running title*: GVG reverses use-dependent disinhibition

Michael F. Jackson, Barbara Esplin and Radan Čapek

*Department of Pharmacology and Therapeutics*
*McGill University, Montreal, Que. (Canada)*

*Keywords*: GABA; Anticonvulsant; Vigabatrin; Inhibition; Paired-pulse; IPSPs

*Correspondence*: Dr. Radan Čapek
Department of Pharmacology and Therapeutics
McGill University
3655 Drummond Street
Montreal, Que. H3G IY6, CANADA
Tel.: (514) 398-3607
FAX: (514) 398-6690

## Summary

γ-Vinyl GABA (GVG, vigabatrin) is an effective anticonvulsant designed to irreversibly inhibit GABA-transaminase, the enzyme responsible for the breakdown of GABA. In hippocampal slices prepared 24 hours following the pretreatment of rats with either an anticonvulsant dose of GVG (1500 mg/kg) or saline, extracellular and whole-cell recordings were used in order to examine the effects of GVG pretreatment on GABA-mediated inhibition as well as on inhibitory postsynaptic responses. Although little or no difference in the strength of inhibition was observed between the two population of slices when the inhibitory pathways were activated by a single stimulus, much stronger inhibition was consistently found in slices from GVG pretreated rats when the inhibitory pathways were activated repetitively by a train of stimuli delivered at low frequency. This enhancement of inhibition by GVG was greater as the frequency of stimulation was increased over the range of 2.5 to 10 Hz and contrasted with the marked activity-dependent depression of the strength of inhibition typically observed in slices from saline pretreated rats when using identical stimulation frequencies. Consistent with these findings, GVG pretreatment prevented the progressive decline in the amplitude of monosynaptic IPSPs during low-frequency stimulation of inhibitory interneurons. Thus, in slices from GVG pretreated rats, the amplitudes of both the fast and slow components of the last of a series of IPSPs evoked by a 5 Hz, 4 sec train were maintained at 91.5 ± 6.6% and 87.7 ± 6.5%, respectively, compared to 61.1 ± 3.9% and 57.1 ± 5.0% in control slices. Given the previously demonstrated involvement of $GABA_B$ autoreceptors in the activity-dependent depression of inhibition, we examined whether a reduction in the function of these receptors may underlie the presently observed effects of GVG pretreatment. Consistent with such a hypothesis, in slices from GVG pretreated animals we observed a reduction in the ability of the $GABA_B$ receptor agonist, baclofen, to decrease the amplitude of monosynaptic inhibitory postsynaptic currents. Our findings indicate that GVG pretreatment

results in a frequency-dependent reinforcement of inhibition which most likely contributes to the anticonvulsant effectiveness of this compound.

## Introduction

Despite its continued spontaneous release from inhibitory interneurons, low extracellular levels of GABA are maintained in the central nervous system due to the presence at inhibitory synapses of a high-affinity uptake system capable of the concentrative transport of GABA into neurons and glia (for review see Borden, 1996). Neurochemical studies have demonstrated that a large fraction of the intracellular pool of GABA formed as a result of the activity of the GABA-transporter is destined to be degraded through a transamination reaction catalyzed by the enzyme γ-aminobutyrate-α-oxoglutarate aminotransferase (GABA transaminase, GABA-T, E.C. 2.6.1.19)(Abe and Matsuda, 1983; Wood et al., 1988). Compounds capable of inhibiting the enzymatic activity of GABA-T are expected to facilitate GABAergic transmission by increasing the presynaptic availability and subsequent release of GABA. Given the importance of GABA in the control of CNS excitability, inhibitors of GABA-T are therefore expected to possess anticonvulsant properties. The enzyme-activated irreversible inhibitor of GABA-T, GVG, was rationally designed with the aim of developing a novel anticonvulsant drug capable of facilitating GABA-mediated inhibition (Jung et al., 1977a; Lippert et al., 1977). Studies have shown that GVG can effectively control seizures in animal models (Schechter et al., 1977; Shin et al., 1986) as well as in humans suffering from previously drug-resistant partial epilepsy (Michelucci and Tassinari, 1989; Arzimanoglou et al., 1997). Furthermore, compared with the anticonvulsant benzodiazepines and barbiturates, the effectiveness of GVG is generally better maintained over time and fewer CNS depressant side effects are associated with its prolonged use (Upton, 1994; Brodie and Dichter, 1996).

Neurochemical studies in both animals and humans have generally demonstrated that the anticonvulsant actions of GVG are well correlated with the increased brain GABA levels resulting from the irreversible inhibition of GABA-T (Schechter et al., 1977; Riekkinen et al., 1989b; Ben-Menachem, 1989). Although these results are consistent with the suggestion that GVG produces its

therapeutic effects by facilitating GABA-mediated transmission, convincing direct evidence of this drug's ability to cause an increase in synaptic inhibition has not yet been presented. In fact, the only previous attempt at characterizing the effects of GVG on GABA-mediated inhibition (Rak and Lothman, 1988) failed to demonstrate an increase in synaptic inhibition at several time points following GVG pretreatment at which an anticonvulsant effect had previously been reported. Specifically, no enhancement of inhibition was observed 8 and 24 hours following the administration of a single i.p. injection of GVG at a dose (1500 mg/kg) previously shown to protect animals from kindling-induced seizures (Shin et al., 1986). In this *in vivo* electrophysiological study, inhibition was assessed indirectly by examining the effects of conditioning stimulation, which is expected to activate GABAergic inhibitory pathways, on the subsequent extracellularly recorded response of a population of neurons to stimulation of their afferent fibres. The rather unimpressive effects of GVG on GABA-mediated inhibition observed under these testing conditions may have been due to the failure of the single conditioning stimulation used to reflect the hypersynchronous and repetitive activation of inhibitory interneurons expected to occur during a seizure. The importance of using a pattern of stimulation which more accurately mimics seizure activity when testing the effects of an anticonvulsant drug on synaptic inhibition is highlighted by the well characterized dependence of the effectiveness of GABA-mediated transmission on the frequency at which it is activated. Thus, stimulation at low-frequencies (0.2-20 Hz) causes an activity-dependent depression in the strength of inhibition produced as a result of both pre- and postsynaptic mechanisms (Ben-Ari et al., 1980; McCarren and Alger, 1985; Thompson and Gähwiler, 1989a; Nathan and Lambert, 1991; Davies and Collingridge, 1993b). In contrast, compensation for the effects of repetitive stimulation occurs with higher stimulation frequencies (>20 Hz) (Davies and Collingridge, 1993b) such that an enhancement in the strength of inhibition can be observed (Esplin and Čapek, 1990). The effects of an anticonvulsant drug acting specifically to either prevent the use-dependent depression of inhibition or

paradoxically to augment its frequency-dependent facilitation would therefore not be detected using a simple paired-pulse protocol.

Using single, low- and high-frequency conditioning stimulation, the aim of the present study was therefore to determine whether changes in the strength of GABA-mediated synaptic inhibition can be detected in hippocampal slices prepared from animals having previously received a single anticonvulsant dose of GVG. A preliminary account of these experiments has been presented (Jackson et al., 1993a).

## Methods

### *Preparation of slices*

Hippocampal slices were prepared from male Sprague-Dawley rats 24 hours after they had been given a single intraperitoneal injection of GVG (1500 mg/kg) or saline by an experimenter blinded to the kind of treatment. Slices (400-500 μm) were prepared with the use of either a tissue chopper or vibratome and allowed to recover at room temperature for at least 1 hour in a holding chamber filled with artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl, 124; KCl, 3.3; $CaCl_2$, 2.5; $MgSO_4$, 2.4; $NaHCO_3$, 25.6; $KH_2PO_4$, 1.25; glucose, 10. For recordings, slices were transferred to a recording chamber where they rested on either a nylon mesh or piece of lens paper at the interface between warm (33-35°C) 95 % $O_2$ and 5 % $CO_2$ and oxygenated aCSF perfused through the chamber at a rate of 2-3 ml/min.

### *Extracellular recordings*

Extracellular recordings of population spikes (PSs) were made using a 3 M NaCl filled micropipette (5-15 MΩ) positioned in the CA1 pyramidal cell layer. Constant-current stimuli (100 μs), applied through bipolar stimulating electrodes

positioned in the stratum radiatum and alveus, evoked orthodromic and antidromic PSs, respectively. Orthodromically evoked PSs were inhibited by conditioning stimulation delivered to either the stratum radiatum (ortho-ortho paired-pulse test) or alveus (anti-ortho paired-pulse test). Inhibition evoked by orthodromic conditioning stimulation can be attributed to the activation of both feed-forward and recurrent inhibitory pathways, while inhibition evoked by antidromic conditioning is produced as a result of the activation of only the latter. The frequency-dependence of GVG's effects were tested using repetitive antidromic conditioning stimulation which consisted of a train of 20 stimuli delivered at either a low (2.5, 5 or 10 Hz) or high (100 Hz) frequency. The delay between the end of a single conditioning stimulus or of the last stimulus in a train and the subsequent orthodromic test stimulus was varied between 10 and 1600 ms and was termed the interstimulus interval (ISI). In each slice, a stimulus intensity for orthodromic stimulation was selected which produced a half-maximal PS. For the ortho-ortho paired-pulse test, the same stimulus intensity was used for both conditioning and testing, while for the anti-ortho paired-pulse test, the intensity of antidromic conditioning stimulation was adjusted so that the amplitude of the half-maximal orthodromic test PS was reduced to between 10 and 30% of its original amplitude at an ISI of 10 ms. The reduction in the amplitude of the test PS, expressed as a percentage of the unconditioned response, was used as a measure of the strength of GABA-mediated inhibition. Responses were obtained at each ISI in slices prepared from the GVG treated and control animals, and were compared using the two-tailed Student's *t*-test.

*Whole-cell recordings*

Tight-seal (>3 GΩ) whole-cell recordings were obtained from CA1 pyramidal cells using patch pipettes which had a resistance of 2-4 MΩ when filled with an internal solution consisting of (in mM): 135-145 of either potassium methylsulfate ($KMeSO_4$, used for current-clamp recordings) or cesium

methanesulfonate ($CsCH_3SO_3$, used for voltage-clamp recordings), 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 2 $MgCl_2$. The solutions were buffered with KOH or CsOH to a final pH of 7.2-7.3 and total osmolality was 270-290 mOsm. With excitatory transmission blocked by the addition of the excitatory amino acid receptor antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) and 3-((R)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 5 μM) to the aCSF, monosynaptic inhibitory postsynaptic potentials (IPSPs) or currents (IPSCs) were recorded following stimulation delivered to the stratum radiatum through a bipolar stimulating electrode. In order to directly activate the inhibitory interneurons, stimulating electrodes were positioned in close proximity to the pyramidal cells from which recordings were made. Stimulation by single pulses was at a frequency (0.03-0.05 Hz) which assured a stable response throughout the recording period. Current-clamp recordings were made with an Axoclamp 2A amplifier (Axon Instruments) operated in bridge mode whereas an Axopatch 200A (Axon Instruments) was used for the voltage-clamp recordings. For current clamp recordings, cells were maintained, by appropriate current injection, at a constant membrane potential. All signals were filtered at 1 kHz, digitized at 3-5 kHz and stored on disk using a PC based acquisition and analysis system functionally similar to that described previously (Théorêt et al., 1984). Baclofen (0.5-2 μM), used to examine changes in the sensitivity of the presynaptic $GABA_B$ autoreceptors, was dissolved in aCSF and superfused over slices. The significance of GVG induced changes were evaluated using the two-tailed Student's *t*-test.

## Results

*Effects of GVG pretreatment on paired-pulse inhibition*

The effects of GVG pretreatment on GABA-mediated inhibition were first evaluated using the ortho-ortho (Fig 1A) and anti-ortho (Fig 1B) paired-pulse tests of inhibition. In control slices prepared from saline injected animals, both orthodromic and antidromic conditioning stimulation resulted in a strong reduction in the amplitude of subsequent orthodromic test PSs evoked at an ISI of 10 to 20 ms. With antidromic conditioning stimulation, the test PS gradually recovered to its unconditioned amplitude as the ISI was increased from 40 to 160 ms (Fig. 1B). In comparison, following orthodromic conditioning stimulation, the return of the test PS to its unconditioned amplitude was more rapid and occurred within an ISI of 40 ms (Fig. 1A). The much shorter inhibitory phase (10-20 ms) which was observed with orthodromic conditioning stimulation was followed by a period of marked facilitation which outlasted the range of ISIs which we tested (Fig 1A, 80-160 ms). The effects of GVG pretreatment on paired-pulse inhibition were limited to a decrease in the inhibition produced 10 ms following a single orthodromic conditioning stimulus (Fig. 1A).

*Effects of GVG pretreatment on the inhibition produced by low and high-frequency stimulation*

The effects of GVG on the activity-dependent depression of inhibition produced by low-frequency stimulation as well as on the inhibition produced following high-frequency stimulation were tested using trains of antidromic stimuli delivered at either low (5 Hz) or high (100 Hz) frequencies. Given that the strength of inhibition is measured indirectly as a reduction in the population response of CA1 pyramidal cells to a presumably constant excitatory input, repetitive orthodromic stimulation could not be used since this would have resulted in a change in the excitatory drive against which inhibition is measured.

It is for this reason that our analysis was restricted to a study of the frequency-dependent effects of GVG pretreatment on antidromically-evoked recurrent inhibition. In addition, special care was taken when positioning the alvear stimulating electrode so that no synaptically evoked PS, resulting from the activation of excitatory fibers in stratum oriens, could be detected following the direct antidromically evoked PS (see inset of Fig. 1B). Under these conditions, GVG pretreatment altered the pattern of inhibition typically observed in control slices with changes in the frequency of stimulation. Thus, in control slices, considerably less inhibition of the orthodromic PS was always observed following low-frequency conditioning stimulation (Fig. 2, control; 5 Hz, 4 sec [40 ms]) than following conditioning by a single stimulus (Fig. 2, control; single [40 ms]). However, in slices from GVG pretreated animals, there was no such activity-dependent depression of inhibition; the low-frequency conditioning stimulation now typically produced more inhibition of the PSs than conditioning with a single stimulus (Fig. 2, GVG; single [40 ms]). In control slices, high-frequency stimulation always resulted in a much stronger and longer lasting inhibition than that induced by a single stimulus; the PSs were greatly inhibited at ISIs exceeding by far the duration of inhibition induced by a single stimulus (Fig 2, control; 100 Hz, 200 ms [200 ms]). In GVG slices, the strong inhibition resulting from high-frequency stimulation (Fig. 2, GVG; 100 Hz, 200 ms [200 ms]) was qualitatively similar to that observed in control slices.

Since the activity-dependent depression of inhibition is typically observed at frequencies of 0.2 to 20 Hz, we decided to investigate the ability of GVG to reverse the depression of inhibition induced by a broader range of low-frequency stimulation. Therefore, in addition to using 5 and 100 Hz conditioning stimulation, the effects of GVG were tested at 2.5 and 10 Hz. Fig.3 illustrates the dependence of GVG's effects on the frequency of conditioning stimulation over the entire range of ISIs examined. In control slices, activity-dependent depression of inhibition was observed following repetitive stimulation at all of the low-frequencies tested. The resulting depression was greatest at ISIs of 10 to 40 ms and was slightly larger as the frequency of repetitive stimulation was

increased from 2.5 to 10 Hz. The effects of GVG pretreatment on the activity-dependent depression of inhibition were greater as the frequency of stimulation was increased over this range. At 2.5 Hz, GVG simply prevented the occurrence of depression such that the time course of inhibition was now identical to that resulting from conditioning with a single stimulus. In contrast, while the development of depression was similarly prevented at short time intervals of 10 to 20 ms after 5 and 10 Hz conditioning stimulation, GVG pretreatment not only prevented but actually reversed the depression of inhibition at ISIs of 40 to 160 ms such that significantly stronger inhibition than that produced by a single conditioning stimulus was now observed. The frequency-dependence of the resulting enhancement of inhibition produced following pretreatment with GVG is more directly evident in Fig. 4 where the opposing effects of frequency on the strength of inhibition in each population of slices can be clearly seen at two ISIs.

*Effects of GVG pretreatment on monosynaptic IPSPs evoked with repetitive stimulation*

The ability of GVG to prevent the activity-dependent depression of GABA-mediated neurotransmission was studied more directly by examining the consequences of low-frequency repetitive stimulation on monosynaptic IPSPs evoked in the presence of the excitatory amino acid receptor antagonists, CNQX and CPP. Low-frequency stimulation, which consisted of a train of 20 stimuli, was delivered at 5 Hz through a stimulating electrode positioned in the stratum radiatum. In control slices, a marked reduction in the amplitude of IPSPs was observed during such stimulation (Fig. 5A). The depression of IPSPs reached its maximum within the first five evoked responses (Fig. 5A2) and was then maintained at a relatively constant level through the remainder of the stimulation period (Fig. 5A1). The 20$^{th}$ IPSP superimposed over the 1$^{st}$ one (Fig. 5A3) clearly shows that repetitive low-frequency stimulation produced a reduction in the amplitude of both fast and slow components of evoked IPSPs. GVG pretreatment greatly reduced the extent to which IPSPs were depressed

following repetitive stimulation such that their peak amplitudes were relatively well maintained throughout the period of stimulation (Fig. 5B1). This effect was seen with both the fast (f-IPSP) and slow (s-IPSP) components of the evoked IPSPs (Fig. 5B3). Thus, with repetitive stimulation in GVG slices, the f-IPSPs and s-IPSPs were maintained at 91.5 ± 6.6% (mean ± se, $n$ = 9) and 87.7 ± 6.46% ($n$ = 9) of their original amplitude, respectively, compared to only 61.1 ± 3.9% ($n$ = 10) and 57.1 ± 5.0% ($n$ = 10) in control (Fig. 5C).

*Effects of GVG pretreatment on the baclofen-induced depression of IPSCs*

Two of the predominant mechanisms which are thought to underlie the activity-dependent depression of GABAergic transmission are (1) activation of $GABA_B$ autoreceptors and (2) shifts in the concentration gradients of the ionic species involved in the generation of IPSPs. The near identical effect of low-frequency repetitive stimulation on the amplitudes of both the fast, presumably $GABA_A$ receptor-mediated, and slow, presumably $GABA_B$ receptor-mediated, components of the evoked IPSPs suggests that the depression of IPSPs most likely occurred as a consequence of a reduced release of GABA following the activation of presynaptic $GABA_B$ autoreceptors rather than as a consequence of shifts in ionic equilibria. The frequency-dependent effects of GVG pretreatment on GABA-mediated neurotransmission may have therefore been due to a reduction in the sensitivity of $GABA_B$ autoreceptors following prolonged (i.e. 24 hours) exposure to the increased brain levels of GABA induced by the inhibition of GABA-T. This hypothesis was tested by examining the ability of the $GABA_B$ receptor agonist, baclofen, to cause a reduction in the amplitude of evoked IPSCs in slices from control and GVG pretreated animals. In control slices, baclofen caused a concentration-dependent reduction in the amplitude of evoked IPSCs, such that in the presence of 2.0 μM of the agonist the mean amplitude of IPSCs was reduced to 43.5 ± 2.1% ($n$ = 4) of responses evoked in the absence of baclofen. In contrast, application of baclofen to GVG slices produced

significantly less of an effect on the amplitude of IPSCs (Fig. 6). Thus, in the presence of 2.0 μM baclofen, the mean amplitude of IPSCs was 60.5 ± 3.9% (*n* = 7) of control responses. Partial recovery from the effects of baclofen was observed during washout of the agonist in slices from both control and GVG animals.

**Discussion**

Based on results from neurochemical studies, the coexistence of two distinct transmitter pools within GABAergic nerve terminals has been suggested (Abe and Matsuda, 1983; Sihra and Nicholls, 1987; Wood et al., 1988). The first of these pools is generated from the reuptake of GABA and is subjected to active degradation by GABA-T. Release from this pool, which has been termed cytoplasmic, occurs through a $Ca^{2+}$-independent mechanism involving the operation of the GABA transporter in a reversed direction. The second pool of GABA consists of newly synthesized transmitter, stored within synaptic vesicles. GABA within this pool, referred to as vesicular, is not subjected to the enzymatic activity of GABA-T and is released through a $Ca^{2+}$-dependent mechanism.

Intimate knowledge of the mechanism by which GABA-T catalyzes the degradation of GABA led to the rational design and synthesis of highly specific and irreversible catalytic (or suicide) inhibitors of this enzyme. Although both γ-acetylenic GABA (GAG) and GVG inhibit GABA-T (Löscher, 1980b), GAG was later demonstrated to also partially inhibit GAD (Sarhan and Seiler, 1979; Löscher, 1981), an action which is thought to underlie the proconvulsant properties of this compound and has precluded its further development as a novel anticonvulsant. In contrast, although some proconvulsant actions have been described for GVG (Löscher et al., 1989), it has generally proven itself to be highly effective in preventing seizures in animals (Schechter et al., 1977; Shin et al., 1986) as well as in previously drug-resistant epilepsy patients suffering from complex partial seizures (French et al., 1996).

The anticonvulsant properties of GVG have generally been attributed to its perceived ability to enhance GABA-mediated inhibition (Richens, 1989; Wilder, 1996). Although demonstrations of elevated brain GABA levels and enhanced transmitter release following the administration of GVG are strongly suggestive of enhanced GABAergic transmission, they do not constitute definitive evidence in favor of such a mechanism since the resulting functional consequences of the elevated GABA levels were not directly assessed. In the present study, we have demonstrated that pretreatment with a single acute injection of GVG (1500 mg/kg) causes a frequency-dependent enhancement of inhibition. The demonstration of a reduced sensitivity of monosynaptically evoked IPSCs to the inhibitory effects of baclofen suggests that the frequency-dependent actions of GVG are due to a reduction of presynaptic $GABA_B$ autoreceptor function. This conclusion is further supported by the inability of GVG to produce a change in the strength of the inhibition evoked with high-frequency stimulation. Indeed, a detailed study of the role of $GABA_B$ autoreceptors in the regulation of inhibitory synaptic responses evoked using a similar broad range of stimulation frequencies (0.1 to 100 Hz) demonstrated that the amplitude of IPSCs evoked during high-frequency (100 Hz) stimulation is unaffected by the activation of $GABA_B$ autoreceptors (Davies and Collingridge, 1993b).

Even though they were not directly assessed, previous studies of the neurochemical actions of GVG strongly suggest that elevated brain GABA levels were produced in our study following the pretreatment of animals with GVG. Although these increased levels most likely occur as a result of the inhibition of GABA-T, another source may also have contributed to raising the extracellular levels of the inhibitory transmitter in our study. Due to its very design as an enzyme-activated irreversible inhibitor, GVG was expected to interact highly specifically with GABA-T. However, since it is structurally quite similar to GABA, it has been possible to demonstrate further effects of GVG on the GABA neurotransmitter system. In addition to being a weak inhibitor ($IC_{50}$ > 1 mM) of the binding of [$^3$H]GABA to $GABA_A$ receptors (Löscher, 1980b; Jackson et al., 1994), GVG has been shown to inhibit the reuptake of GABA (Löscher, 1980b;

Abdul-Ghani et al., 1981; Jolkkonen et al., 1992). Furthermore, since it is a substrate for the GABA transporter (Schousboe et al., 1986), it has been possible to demonstrate that GVG can cause a release from rat cerebral cortical synaptosomes of both exogenous and endogenous GABA through a heteroexchange mechanism (Abdul-Ghani et al., 1981). In this respect, the neurochemical actions of GVG resemble those previously reported for nipecotic acid (Johnston et al., 1976b). The GABA releasing properties of GVG were demonstrated over a concentration range (0.25-0.5 mM) which has been estimated to occur in the CNS of animals given a single i.p. injection of GVG at a dose of 1500 mg/kg (Abdul-Ghani et al., 1981). It is therefore likely that the elevated brain GABA levels, which resulted in the observed down-regulation of $GABA_B$ autoreceptor function, were the result of both the GABA releasing as well as the GABA-T inhibiting properties of GVG.

Similar downregulation of autoreceptor function, resulting in a decrease of the baclofen-induced inhibition of evoked IPSPs, has previously been observed following the chronic treatment of animals with the $GABA_B$ receptor agonist, baclofen (Malcangio et al., 1995). Furthermore, in an animal model of temporal lobe epilepsy with a demonstrated deficit in $GABA_B$ receptor function, repetitive low-frequency activation was shown to produce no depression in the amplitude of monosynaptically evoked IPSPs (Mangan and Lothman, 1996). Such changes were proposed to serve as a compensatory mechanism designed to reduce the onset of epileptiform activity. Several mechanisms have been identified through which a reduction in receptor function can occur. These include changes in receptor phosphorylation, receptor sequestration, increased receptor degradation and reduction in receptor gene expression. Any of these potential mechanisms could have contributed to our observation of a reduced presynaptic autoreceptor function. However, a mechanism involving a change in $GABA_B$ receptor gene expression would be expected to operate on a somewhat longer time scale than the 24 hours of elevated GABA levels to which our animals were exposed following pretreatment with GVG.

Although a reduction in the baclofen-induced inhibition of monosynaptic IPSCs was demonstrated following GVG pretreatment, IPSCs were nevertheless inhibited by nearly 40% in the presence of 2 μM baclofen. Therefore, the reduction of $GABA_B$ autoreceptor function may only partially explain the observed effects of GVG on synaptic inhibition. Another explanation may be proposed based on the demonstration that the inhibition of GABA-T causes a large increase in the metabolic pool of GABA from which the transmitter can be released in a $Ca^{2+}$-independent manner (Szerb, 1982b; Wood et al., 1988), a process which is believed to be mediated through a reversal in the direction of GABA transport (Bernath, 1992; Attwell et al., 1993). The ability of the GABA transporter to operate in a reversed mode occurs as a result of the dependence of its activity on the electrochemical gradient of $Na^+$ and $Cl^-$ ions. Based on the recognized stoichiometry of the transporter (two $Na^+$ ions and one $Cl^-$ ion for each molecule of GABA transported) the following thermodynamic equation has been proposed which dictates the extent to which the transporter can cause an accumulation of GABA in the extracellular space (Attwell et al., 1993)

$$[GABA]_o = [GABA]_i([Na^+]_i/[Na^+]_o)^2([Cl^-]_i/[Cl^-]_o)\exp[VF/RT]$$

where $[\,]_o$ and $[\,]_i$ are the extracellular and intracellular concentrations of each molecular species, respectively, V is the voltage, F is Faraday's constant, R is the gas constant, and T is the absolute temperature. Thus, consistent with results from neurochemical studies (Haycock et al., 1978; Nelson and Blaustein, 1982), the contribution of a transporter-mediated mechanism to the evoked release of GABA will be strongly dependent on the extent to which the intracellular concentration of $Na^+$ is raised as a result of the activation of voltage-dependent $Na^+$ channels during the depolarization of nerve terminals. Since a much greater elevation of the intracellular concentration of $Na^+$ will be achieved as the frequency and duration of action potential firing is increased, the transporter-mediated release of GABA is therefore expected to depend on the frequency and duration of stimulation. Given the level of depression which we

observed under control conditions, it appears unlikely that the reversed transport of GABA contributed significantly to the inhibition resulting from low-frequency (2.5-10 Hz) stimulation. However, following pretreatment with GVG, the resulting elevated intracellular levels of GABA may facilitate the release of the neurotransmitter through a transporter-mediated process, even with repetitive stimulation at a relatively low-frequency of 2.5 Hz. As the frequency is increased to 5 and 10 Hz, the rise in intracellular $Na^+$ is expected to be greater, therefore increasing the relative contribution of this mechanism to the release of GABA. Such a mechanism would be consistent with the observed frequency-dependence of GVG's actions.

It should be noted that with repeated administrations of GVG, effective seizure control can be achieved with much lower doses ($ED_{50}$ = 36 mg/kg) than following a single injection ($ED_{50}$ = 990 mg/kg)(Schechter et al., 1977). It cannot be taken for granted that both or either of the mechanisms suggested to account for the efficacy of pretreatment with a single dose of GVG will contribute to the anticonvulsant effects of this drug when administered chronically. Although the high levels of GABA produced in the CNS during chronic treatment with GVG (Qume and Fowler, 1996) suggests that down-regulation of $GABA_B$ autoreceptors may equally occur during repetitive dosing with GVG, a similar investigation of the effects of GVG on GABA-mediated inhibition following chronic treatment would nevertheless be clearly desirable.

In acute models of focal epilepsy, synaptic inhibition importantly contributes to the inhibitory mechanisms which limit both the duration of epileptiform activity as well as the spatial extent of its propagation. The gradual fading and eventual disappearance of these inhibitory mechanisms, perhaps as a consequence of the repeated occurrence of focal epileptiform activity, is one of the initial events which are characteristically observed during the progressive development of full blown seizures (Dichter and Ayala, 1987). Therefore, the selective strengthening of inhibition by GVG during the repeated occurrence of epileptiform activity may allow a relatively selective anticonvulsant effect to be achieved while reducing the incidence of CNS side-effects.

**Acknowledgments**

We wish to thank Dr. B. Sasyniuk for kindly allowing us the use of her Axopatch 200A amplifier, Ms. Natalie Lavine for her assistance with the administration of the GVG and saline injection samples, and Marion Merrell Dow (Cincinnati, OH) for supplying us with samples of GVG. This work was supported by the Medical Research Council of Canada. M. Jackson was supported by a Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR) studentship and by a McGill Faculty of Medicine Internal Studentship.

## Figure Legends

**Fig.1. Effects of GVG on paired-pulse inhibition in slices prepared from pretreated animals.** GVG produced little or no change in the inhibition of orthodromically-elicited PSs produced by conditioning with either a single orthodromic (A) or antidromic (B) stimulus. Insets show examples of paired ortho-ortho (A) and anti-ortho (B) responses generated at an ISI of 10 ms. Inhibited (< 100%) or facilitated (> 100%) PSs were expressed as a % of the unconditioned test PS amplitude. Each point represents the mean ± SE of data ($n$ = 11-22) plotted on a semi-logarithmic scale against the ISIs (10-160 ms). * Significantly different from control at $p$ = 0.03.

**Fig. 2. GVG pretreatment reverses the activity-dependent depression of inhibition normally associated with the low frequency repetitive stimulation of recurrent inhibitory pathways.** Representative orthodromic responses recorded from control and GVG slices in either the absence of antidromic conditioning stimulation (unconditioned), 40 ms following antidromic conditioning by a either a single stimulus or a low frequency (5Hz), 4 sec train of 20 stimuli, as well as 200 ms following antidromic conditioning by a high frequency (100 Hz), 200 ms train of 20 stimuli.

**Fig. 3. Effects of GVG on the time course of inhibition produced by repetitive antidromic conditioning stimulation at low (2.5, 5 and 10 Hz) and high (100 Hz) frequencies.** In each graph, the inhibition resulting from a single antidromic conditioning stimulation (squares, dashed lines) was plotted in order to facilitate comparisons between the effects of repetitive stimulation (circles, continuous lines) on the inhibition produced in control (filled symbols) and GVG slices (open symbols). The means ± SE are shown. Results with repetitive stimulation were significantly different from control at: + $p < 0.05$, x $p < 0.02$ and * $p < 0.0001$, respectively ($n$ = 4-18).

**Fig. 4. Frequency-dependence of the enhancement of GABA-mediated inhibition following GVG pretreatment.** The amplitude of PSs recorded in slices prepared from control and GVG pretreated animals is plotted against the frequency (0-10 Hz) at which the repetitive conditioning stimulation was delivered with the effects of single conditioning stimulation plotted as a frequency of 0 Hz. When increasing the frequency of conditioning stimulation, a decrease in the effectiveness of inhibition was observed in slices from saline pretreated animals, while, in contrast, an increase in its effectiveness was observed in slices from GVG pretreated animals. The data for the graph is the same as that reported in Fig. 3. at ISIs of 40 ms.

**Fig. 5. GVG prevents the activity-dependent depression of monosynaptically evoked IPSPs.** In the presence of 20 μM CNQX and 5 μM CPP, repetitive stimulation consisting of a train of 20 stimuli delivered at a frequency of 5 Hz to the stratum radiatum of slices from control (A1) and GVG (B1) treated rats evoked a series of monosynaptic IPSPs. A2 and B2 shows initial five IPSPs in control and GVG, respectively. Most of the depression seen in control occurred between the first and second evoked IPSP. First and last ($20^{th}$) IPSPs were superimposed in A3 and B3, and shows the extent of depression in control as well as its prevention in GVG slices. The amplitudes of the fast (=) and slow (<) components of the $20^{th}$ evoked IPSPs were determined in control (A3, n = 10) and GVG slices (B3, n = 9) and expressed as a percentage of their respective original ($1^{st}$ IPSP) amplitudes (C). * Significantly different from control at $p < 0.01$.

**Fig. 6. GVG reduces the sensitivity of $GABA_B$ autoreceptors to the exogenous agonist, baclofen.** In cells voltage-clamped at 0 mV, monosynaptic IPSCs were continuously elicited at a frequency which did not exceed 0.02 Hz, thus insuring that a stable response amplitude was maintained throughout the recording period. After a stable control period, increasing concentrations of baclofen (0.5-2 μM) were added to the superfusate. The amplitude of baclofen-inhibited IPSCs were expressed as a percentage of responses obtained before the addition of the $GABA_B$ receptor agonist. Significantly less baclofen-induced inhibition of IPSCs was observed in GVG slices (n = 6-8) than in control (n = 4-7) at * $p < 0.01$.

**Fig.1. Effects of GVG on paired-pulse inhibition in slices prepared from pretreated animals.**

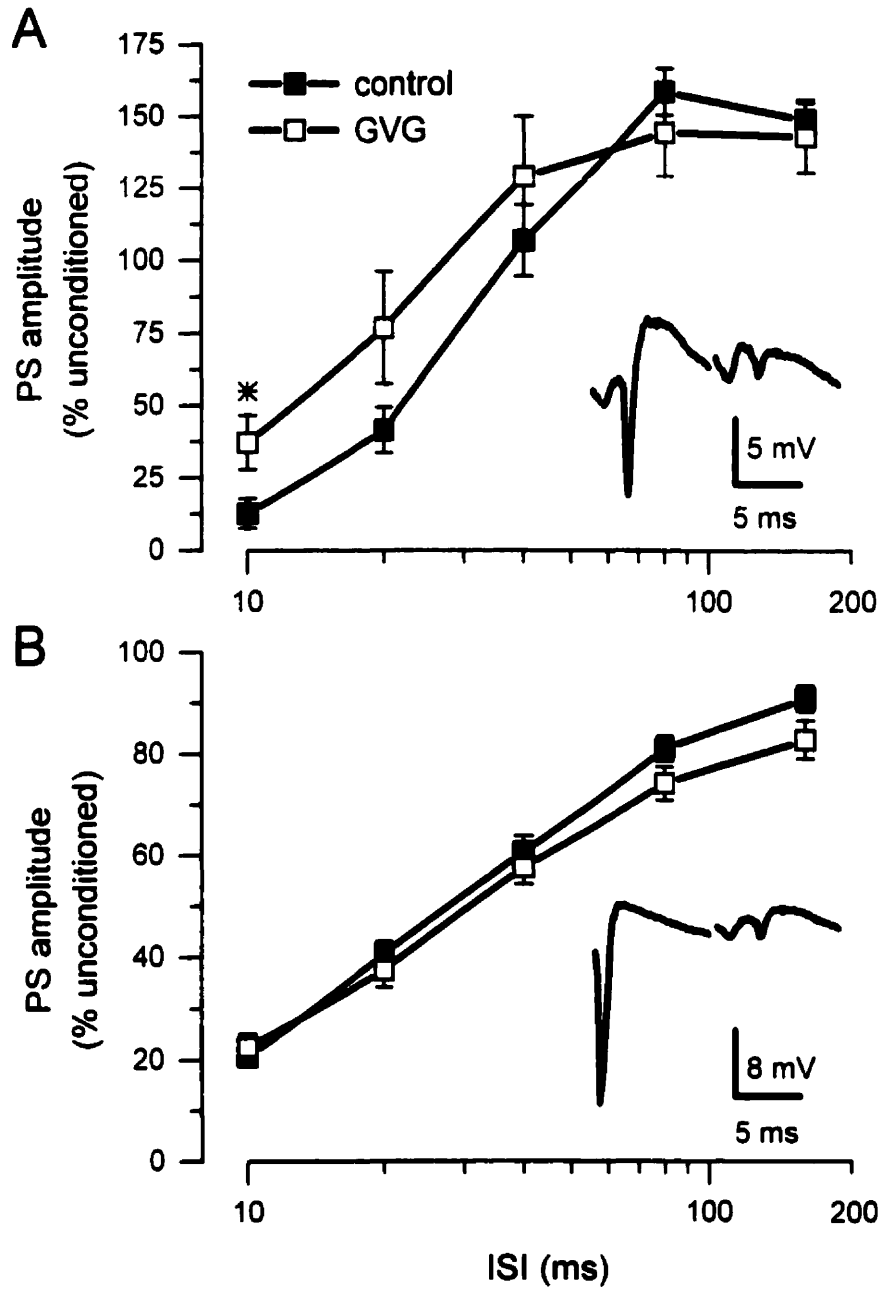


**Fig. 2. GVG pretreatment reverses the activity-dependent depression of inhibition normally associated with the low frequency repetitive stimulation of recurrent inhibitory pathways.**

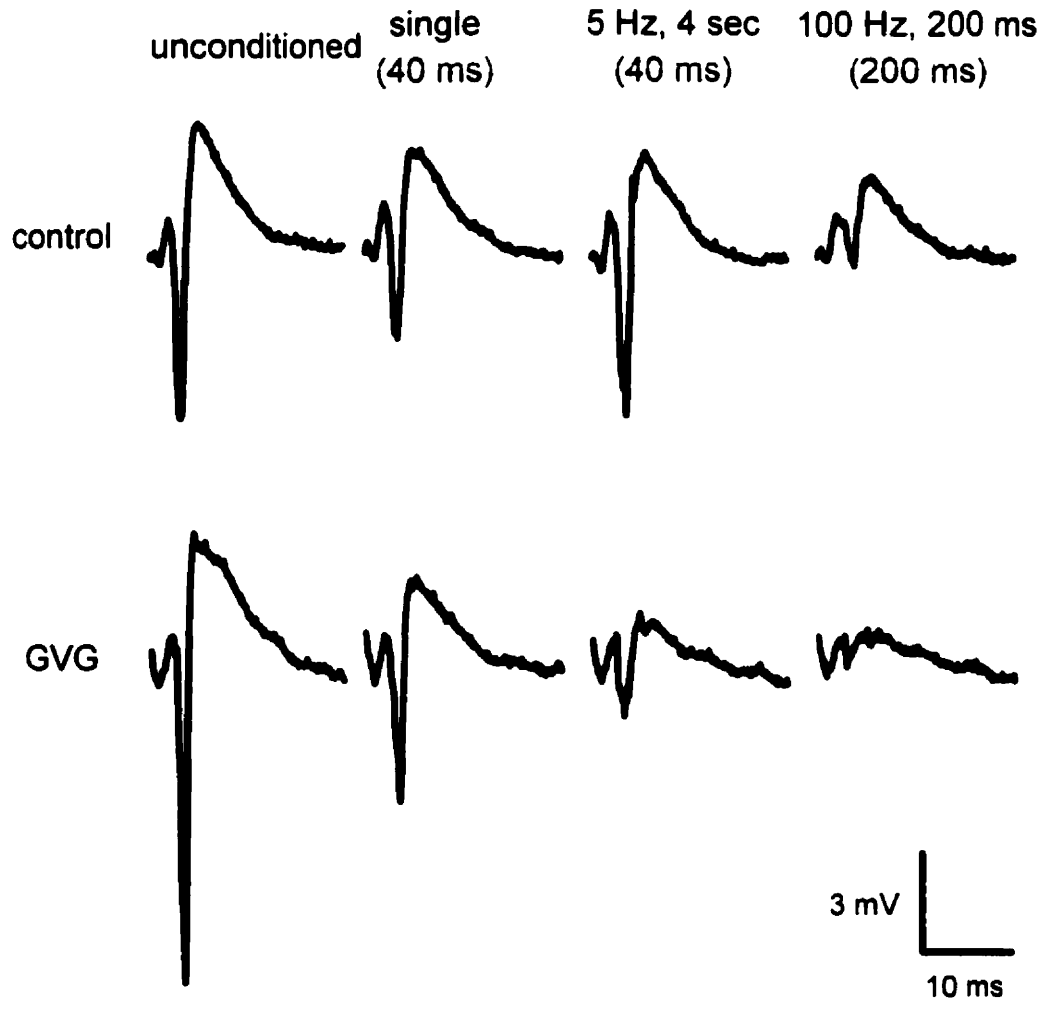


**Fig. 3. Effects of GVG on the time course of inhibition produced by repetitive antidromic conditioning stimulation at low (2.5, 5 and 10 Hz) and high (100 Hz) frequencies.**

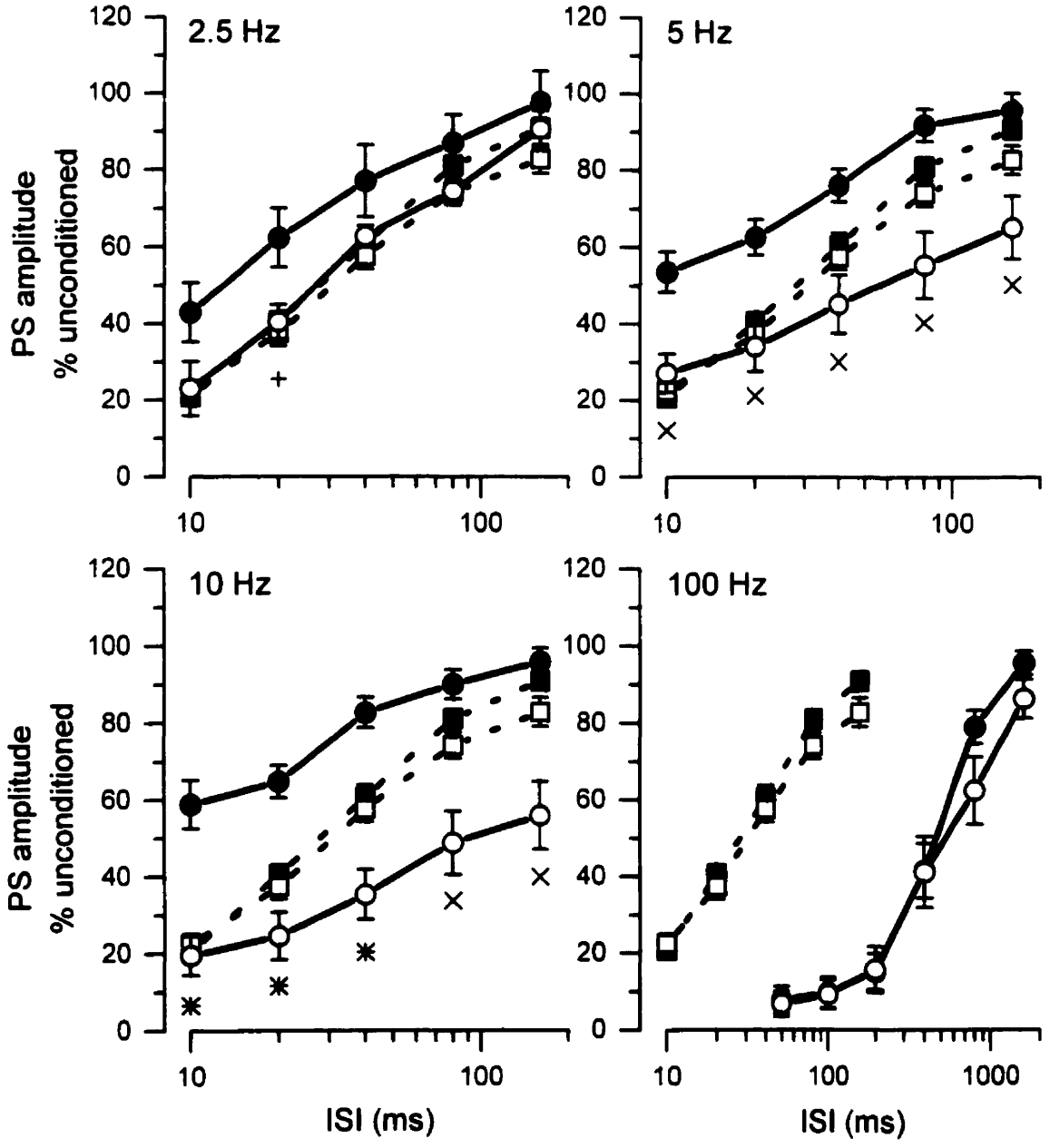


**Fig. 4. Frequency-dependence of the enhancement of GABA-mediated inhibition following GVG pretreatment.**

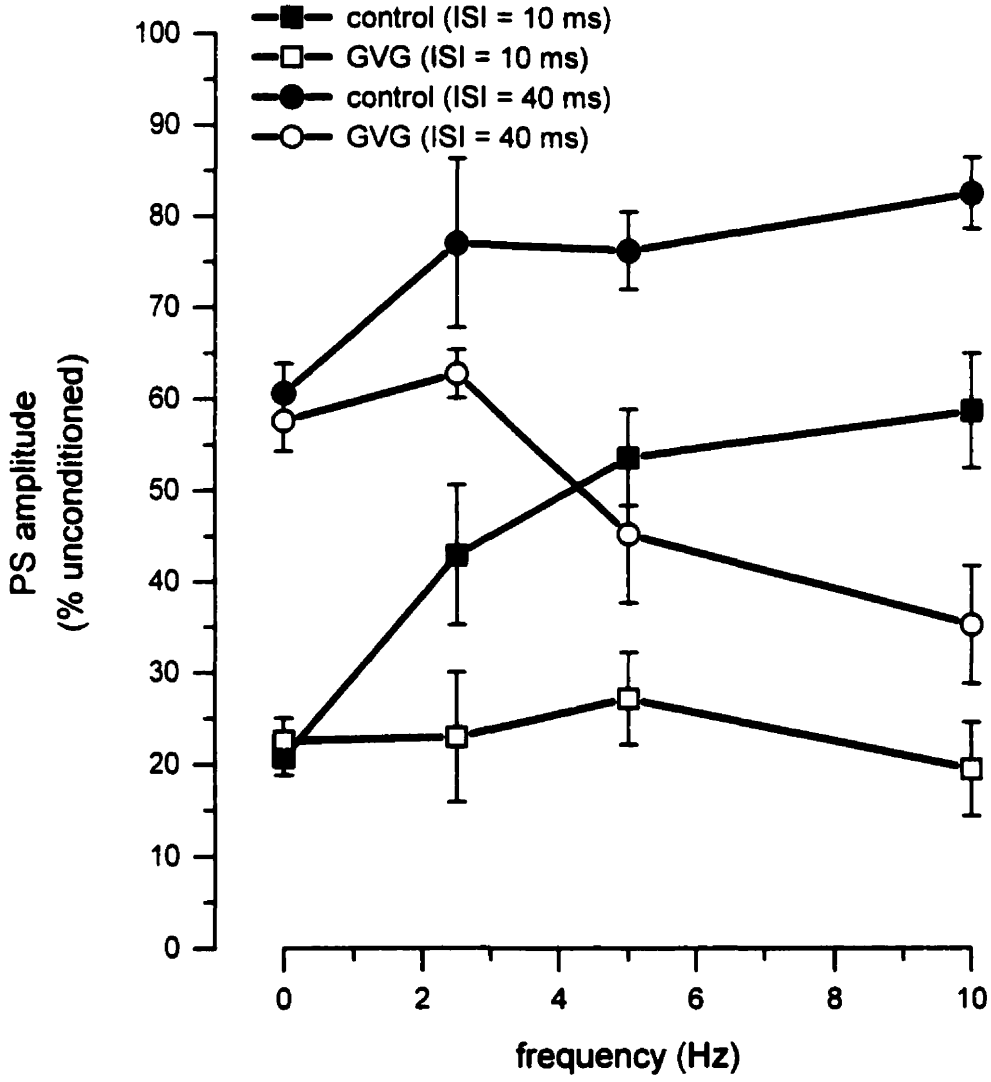


**Fig. 5. GVG prevents the activity-dependent depression of monosynaptically evoked IPSPs.**

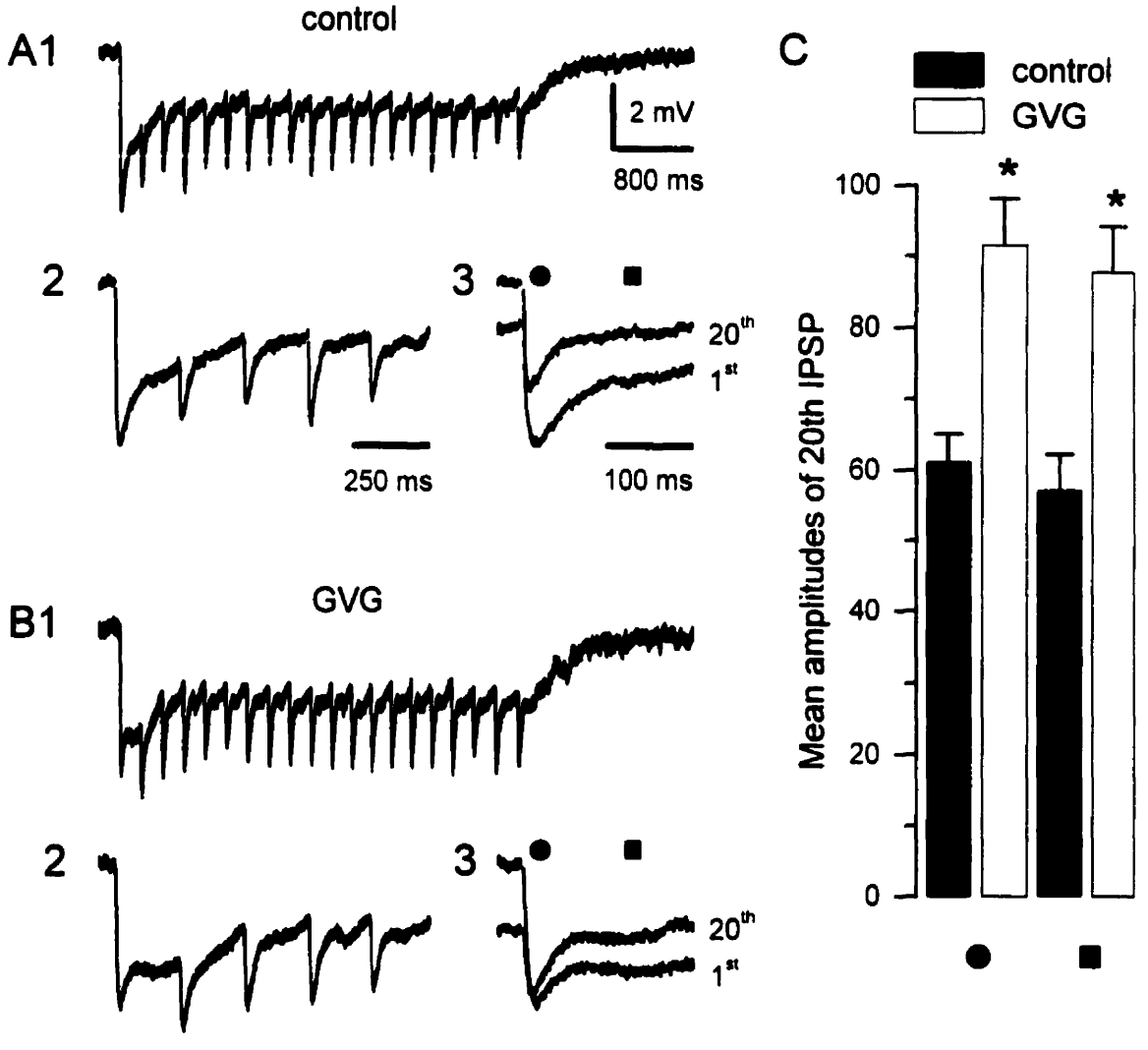
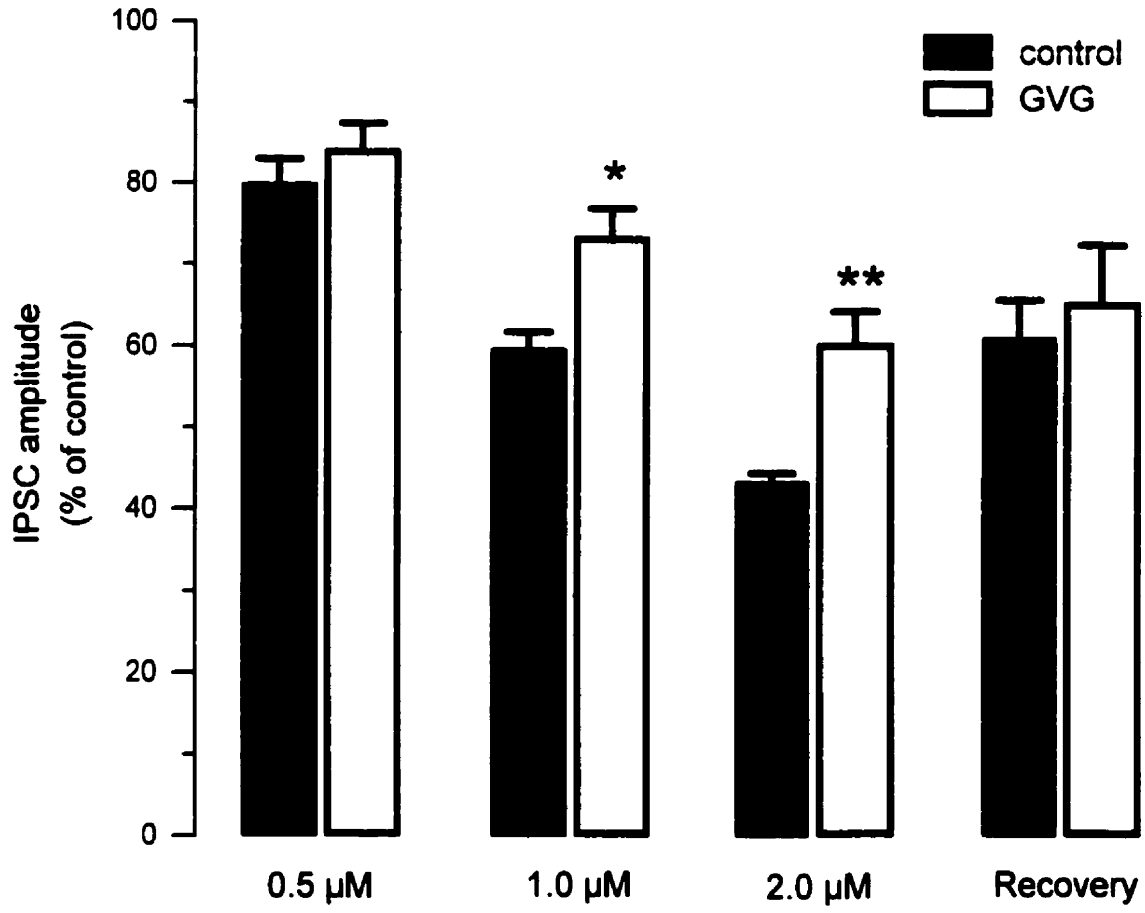


**Fig. 6. GVG reduces the sensitivity of $GABA_B$ autoreceptors to the exogenous agonist, baclofen.**



## Preface to Section 6

In addition to its ability to irreversibly inhibit GABA-T, GVG can reportedly block the reuptake of GABA, an effect which has been suggested to contribute to the anticonvulsant properties of this drug. The inhibition of GABA uptake was determined by examining the effects of GVG on the sodium-dependent binding of [$^3$H]GABA (Löscher, 1980b), which is believed to be associated with the high-affinity GABA transporter, as well as on the extracellular levels of GABA measured by microdialysis (Jolkkonen et al., 1992). Paradoxically, GVG was also demonstrated to inhibit the GABA stimulated uptake of $^{36}Cl^-$ into membrane vesicles which suggests that GVG may act as an antagonist at the $GABA_A$ receptor complex (Suzuki et al., 1991). In contrast to the inhibition of GABA uptake, such an action is expected to result in a proconvulsant effect. The aim of the following study was to determine the outcome of the contrasting neurochemical actions of GVG on GABA-mediated inhibition. In order to separate the actions of GVG on either the GABA transporter or the $GABA_A$ receptor complex from its inhibitory effect on GABA-T, the consequences of which are expected to be more delayed, we examined the effects of GVG on synaptic inhibition during its bath application.

# Acute effects of γ-vinyl GABA (vigabatrin) on hippocampal GABAergic inhibition *in vitro*

Brain Research (1994) **651**, 85-91

Michael Jackson[1], Trevor Dennis[2], Barbara Esplin[1] and Radan Čapek[1]

[1]*Department of Pharmacology and Therapeutics and* [2]*Department of Psychiatry*
*McGill University, Montreal, Que. (Canada)*

*Correspondence*: Dr. Radan Čapek
Department of Pharmacology and Therapeutics
McGill University
3655 Drummond Str.
Montreal, Que. H3G IY6, CANADA
Tel.: (514) 398-3607
FAX: (514) 398-6690

**Abstract**

The acute effects of γ-vinyl-GABA (GVG) on GABAergic inhibition were investigated in the hippocampal slice preparation using the paired-pulse test of inhibition during extracellular recordings. Superfusion of GVG (100-500 μM) for 60 min resulted in a concentration-dependent decrease in GABAergic inhibition. Slices superfused with higher concentrations of GVG (0.5-1 mM) were hyperexcitable as demonstrated by the appearance of multiple spikes. Binding studies showed that GVG (1 mM) had no effect on the binding of [$^3$H]flunitrazepam or [$^3$H]TBOB and displaced no more than 15% of specific [$^3$H]GABA binding, which indicates that GVG-induced disinhibition is not mediated through an action at the $GABA_A$ receptor complex. Consistent with this suggestion is the finding that GVG (500 μM) had little effect on the inhibition of the orthodromically evoked CA1 population spike produced by the $GABA_A$ receptor agonist muscimol (10 μM), whereas this inhibition was considerably attenuated by the $GABA_A$ receptor antagonist, bicuculline methiodide (5 μM). The results of this study suggest that the acute actions of GVG on the GABAergic neurotransmitter system are not involved in its anticonvulsant effect.

*Key words*: Anticonvulsant; GABA; GVG; Hippocampus; Inhibition; Paired-pulse

## Introduction

GVG is a structural analog of GABA designed to act as a suicide inhibitor of GABA-transaminase (GABA-T), the enzyme responsible for the catabolism of GABA. Pretreatment of laboratory animals with GVG has been shown to result in a dramatic increase in brain GABA levels as well as to protect against experimentally induced seizures (Bernasconi et al., 1988; Löscher and Frey, 1987; Schechter et al., 1977). Similarly, clinical trials have demonstrated that GVG elevates cerebrospinal fluid levels of GABA and is an effective antiepileptic in drug resistant epilepsy (Ben-Menachem, 1989; Dam, 1989; Riekkinen et al., 1989a). Increased presynaptic availability and release of GABA following inhibition of GABA-T is generally believed to be the mechanism by which GVG exerts its anticonvulsant action. However, because of its structural similarity with GABA, GVG could conceivably influence GABAergic neurotransmission at other sites. In fact, recent studies on the acute actions of GVG suggest that this compound can inhibit GABA uptake *in vivo* (Jolkkonen et al., 1992) as well as GABA-stimulated chloride flux in brain membrane vesicles (Suzuki et al., 1991), two actions expected to produce opposite effects on GABAergic neurotransmission. Inhibition of GABA uptake would result in an enhancement of GABA-mediated inhibition whereas, in contrast, depression of GABA-stimulated chloride flux would produce its decrease.

In order to determine the functional consequences of these neurochemical effects, the aim of the present study was to investigate the effects of GVG superfusion on GABA-mediated inhibition in the hippocampal slice preparation *in vitro*. The results presented here demonstrate that acute application of GVG produces a concentration-dependent decrease in GABA-mediated inhibition, an effect which does not appear to be mediated through an action at the $GABA_A$ receptor complex. A preliminary account of these results was presented (Jackson et al., 1993b).

## Materials and Methods

*Preparation of slices*

Male Sprague-Dawley rats (100-200 g) were decapitated, the brains quickly removed and placed in cooled, oxygenated artificial cerebrospinal fluid (aCSF) of the following composition (in mM): NaCl, 124; KCl, 3.3; $CaCl_2$, 2.5; $MgSO_4$, 2.4; $NaHCO_3$, 25.6; $KH_2PO_4$, 1.25; glucose, 10. The hippocampus was isolated and transverse hippocampal slices (350-425 mm) were prepared using a McIlwain tissue chopper. Slices were maintained at 35°C and allowed to recover for 1 hour in the recording chamber where they rested on a nylon mesh at the interface between humidified 95% $O_2$ and 5% $CO_2$, and oxygenated aCSF perfused through the chamber at a flow rate of 1.5 ml/min.

*Extracellular Recordings*

Conventional extracellular recording techniques and the paired-pulse test of inhibition used were described previously by us as well as by others (Čapek and Esplin, 1991; Rogers and Hunter, 1992; Xie and Tietz, 1991). Field potentials were recorded in the CA1 pyramidal cell layer with a 3 M NaCl-filled micropipette (5-15 MΩ). Constant current stimuli (100 ms) delivered through bipolar electrodes located in the stratum radiatum and alveus evoked orthodromic and antidromic population spikes (PS), respectively. The paired pulse paradigms were carried out as follows: orthodromic test PSs were inhibited either by orthodromic conditioning stimulation delivered to the stratum radiatum (O/O paired-pulse test) or by antidromic stimulation of the alveus (A/O paired-pulse test). The time interval between the conditioning stimulus and the subsequent orthodromic test stimulus is referred to as the interstimulus interval (ISI) and was in the range of 10 to 320 ms. Orthodromic stimulus intensity was adjusted to produce a PS which was 50% of the maximum. The intensity of the antidromic stimulus was adjusted to inhibit the orthodromic PS to between 10

and 30% of its original amplitude at an ISI of 10 ms. During the O/O paired-pulse stimulation, the orthodromic stimulus intensity producing PSs of 50% of the maximum was used for both the conditioning and test stimulation. The microcomputer based system used for data acquisition and analysis was functionally similar to that previously described (Théorêt et al., 1984). The amplitude of the PS was defined as the average of the absolute potential difference between the first positivity and the maximum negativity, and between the maximum negativity and following positivity. Conditioned PSs were expressed as the percentage of unconditioned ones. Drugs (GVG and nipecotic acid) were dissolved in the aCSF and superfused through the recording chamber for up to 90 min. Paired-pulse responses for each ISI were obtained before and after drug treatment, and were compared using the one-tailed *t*-test for paired samples.

In a separate series of experiments, the effects of GVG on the depression of the PS produced by the $GABA_A$ receptor agonist, muscimol, was studied as described by Kemp et al. (Kemp et al., 1986). Orthodromic CA1 PSs, evoked using the lowest stimulus intensity capable of eliciting a maximal response, were recorded from hippocampal slices superfused with either normal aCSF (naCSF), GVG (500 µM) or bicuculline methiodide (5 µM). Once these initial responses were perfectly stable, muscimol (10 µM) was added to the superfusion and its effect on PS amplitude was compared under each condition.

*Membrane Binding Procedures*

Binding studies were performed on hippocampal tissue obtained from male Sprague-Dawley rats (175-200 g). Hippocampi were rapidly dissected free and stored until needed at -70°C.

*[$^3$H]GABA binding.* [$^3$H]GABA binding to $GABA_A$ receptors was performed according to the method of Hill et al. (Hill et al., 1984). Briefly, hippocampi from

15 rats were pooled and homogenized in 15 volumes of ice-cold Tris-HCl (50 mM pH 7.4) using a Brinkmann Polytron. The homogenate was incubated for 30 min at 37°C, separated into 4 equal volumes and centrifuged for 15 min at 48,000 *g*. The resulting pellets were then stored at -70°C. For the assay, a single pellet was thawed and washed twice in 15 volumes of 50 mM Tris-citrate pH 7.1 containing 0.05% Triton X-100. The final pellet was resuspended in 25 volumes of Tris-citrate buffer. [$^3$H]GABA binding was conducted as follows: incubations were carried out in triplicate at room temperature for 20 min in a final volume of 0.25 ml containing 30 nM [$^3$H]GABA, 100 µM baclofen (in order to mask $GABA_B$ receptors), 300-400 mg of membrane protein, and 12-14 concentrations of either GVG (0.001-1000 µM) or GABA (0.001-100 µM). Bound radioactivity was separated from free by centrifugation at 48,000 *g* for 6 min. The resulting pellet was superficially washed with 1.5 ml distilled water, solubilized in soluene-350 and counted in 4 ml of scintillation cocktail. Non-specific binding was defined by incubation in the presence of 100 µM GABA and represented 55-60% of the total binding.

*[$^3$H]Flunitrazepam binding.* The binding of radiolabeled flunitrazepam to benzodiazepine receptors was performed as described by Suranyi-Cadotte et al. (Suranyi-Cadotte et al., 1984). Briefly, a single hippocampus was homogenized in 10 volumes of ice-cold 50 mM Tris-HCl pH 7.4. The homogenate was centrifuged at 48,000 *g* for 15 min at 4°C. The resulting pellet was resuspended and recentrifuged two more times. The final pellet was resuspended in 150 volumes of the Tris-HCl buffer. Triplicate incubations were carried out at 4°C for 90 min in a final volume of 0.6 ml containing 1 nM [$^3$H]flunitrazepam, 200-250 mg of membrane protein and 12-14 concentrations of either GVG (0.001-1000 µM) or Ro 15-1788 (0.001-1 µM). The incubations were terminated by rapid filtration through Whatman GF/B filters (pre-soaked in Tris-HCl buffer for 1 hr). The filters were rinsed 3 times with 4 ml ice-cold buffer and the radioactivity was determined by liquid scintillation spectroscopy. Ro 15-1788 (1 µM) was used to determine non-specific binding which represented 3-5% of the total binding.

*t-[$^3$H]butylbicycloorthobenzoate ([$^3$H]TBOB) binding.* Hippocampi from 25 rats were pooled and homogenized in 20 volumes of ice-cold 50 mM Tris-citrate buffer pH 7.4. The homogenate was pelleted at 48,000 *g* for 10 min at 4°C. The samples were resuspended, separated into 8 equal volumes and recentrifuged under identical conditions 4 more times. The resulting pellets were stored at -70°C. For the assay, two pellets were thawed, resuspended in 60 volumes of Tris-citrate buffer containing 500 mM NaCl and centrifuged. The final pellets were resuspended in 60 volumes of the Tris-citrate buffer with 500 mM NaCl. [$^3$H]TBOB binding to the picrotoxin binding site was performed according to Tvrdeić et al. (Tvrdeic and Perieic, 1991). Briefly, 12-14 concentrations of either GVG (0.001-1000 µM) or picrotoxin (0.001-1000 µM) and 300-400 mg of membrane protein were preincubated in triplicate at room temperature for 30 min. [$^3$H]TBOB (4 nM) was then added and a second 30 min incubation was carried out (the final incubation volume was 0.5 ml). Incubations were terminated by filtration through Whatman GF/C filters (pre-soaked for 1 hr in Tris-citrate buffer). The filters were rinsed twice with 4 ml ice-cold buffer and the radioactivity was determined by liquid scintillation spectroscopy. Non-specific binding was determined in the presence of 1 µM picrotoxin and represented 10-20% of the total binding.

$IC_{50}$ values, defined as the concentration of drug required to displace 50% of the radiolabeled ligands from the receptor sites, were calculated for each binding experiment using a computer based EBDA-Ligand program (Elsevier-Biosoft, Cambridge, U.K.).

*Compounds*

GVG was generously supplied by Marion Merrell Dow (Cincinnati, Ohio) and nipecotic acid was a gift from Dr. K. Krnjević. Bicuculline methiodide, GABA, muscimol and picrotoxin were purchased from Sigma Chemical Co. (St-Louis, Mo.), baclofen was from Research Biochemicals International (Natick, MA.) and Ro 15-1788 (flumazenil) from Hoffman-LaRoche (Etobicoke, Ont.). [$^3$H]GABA

(specific activity 60 Ci/mmol) and [$^3$H]TBOB (specific activity 25 Ci/mmol) were purchased from Amersham Canada (Oakville, Ont.), and [$^3$H] flunitrazepam (specific activity 74.1 Ci/mmol) from Dupont-NEN (Mississauga, Ont.).

## Results

### *Effects of GVG on GABA-mediated inhibition*

Under control conditions, at an ISI of 10 ms, orthodromic as well as antidromic conditioning stimuli produced strong inhibition which resulted in an almost complete suppression of the subsequent orthodromic PS. Following 60 minutes of superfusion with GVG (100 μM), conditioning orthodromic stimulation no longer inhibited but rather facilitated the test PS. The effectiveness of conditioning antidromic stimulation was reduced as demonstrated by the increase in test PS amplitude following GVG administration (Fig. 1 A and B).

Figures 2 A and B illustrate the effects of GVG (100-500 μM) over the entire range of ISIs tested. In control, conditioning orthodromic stimulation at short ISIs (10-20 ms) inhibited the test PS, while at longer ISIs (40-320 ms) it resulted in a facilitation of the test PS. Meanwhile, conditioning antidromic stimulation resulted in a near complete inhibition of the test PS at an ISI of 10 ms. With longer ISIs, the test PS gradually returned to its original unconditioned amplitude. GVG superfusion resulted in a concentration-dependent decrease in the inhibition. This effect was greatest at ISIs of 10-40 ms while little or no effect was seen at the longer ISIs (80-320 ms).

Superfusion of slices for over 1 hour with a high concentration of GVG (1 mM) resulted in the appearance of multiple spikes following high intensity orthodromic stimulation (Fig. 3), but did not cause a further decrease in inhibition as measured by the paired-pulse test (results not shown). With superfusions lasting more than 2 hrs multiple spikes were also observed with 500 μM GVG (see Fig. 4).

*Mechanisms of GVG-induced disinhibition*

Given the following observations: (1) that during the O/O paired-pulse test GVG only produced significant disinhibition over ISIs which correspond more closely to the time course of $GABA_A$ receptor-mediated inhibitory post-synaptic potentials (IPSP) (Davies et al., 1990), and (2) that GVG reduced the effectiveness of antidromic conditioning stimulation which evokes an early, presumably $GABA_A$ receptor-mediated IPSP but not the late, presumably $GABA_B$ receptor-mediated IPSP (Newberry and Nicoll, 1984), the most straightforward explanation for the disinhibitory effect of GVG would be that it is an antagonist of the $GABA_A$ receptor complex. This was investigated by performing binding studies where we examined GVG's ability to compete with the specific binding of [$^3$H] ligands to the GABA, benzodiazepine and picrotoxin binding sites of the $GABA_A$ receptor complex. The results of these studies are compiled in Table I. When tested for its ability to compete for binding with [$^3$H]GABA, [$^3$H]flunitrazepam or [$^3$H]TBOB, 1mM GVG was able to displace no more than 15 % of [$^3$H]GABA binding while having no effect on the binding of the other two ligands. In these same experiments, the reference compounds, GABA, Ro 15-1788 and picrotoxin, displaced their respective radioligands with $IC_{50}$ values within the range of those reported in the literature (Löscher, 1980b; Massotti et al., 1991; Van Rijn et al., 1990).

The binding data therefore suggest that GVG does not act in a significant way on the $GABA_A$ receptor complex. In order to confirm this in a functional assay, the effects of GVG on the inhibition produced by the $GABA_A$ receptor agonist, muscimol, were compared with those of the competitive $GABA_A$ receptor antagonist, bicuculline methiodide, as described by Kemp et al. (Kemp et al., 1986). The results of these experiments are illustrated in Fig. 4. In normal aCSF, the PS was greatly inhibited (by 83.94% and 84.11% in two separate experiments) after 100 min of superfusion with muscimol 10 µM. Superfusion with GVG, at a concentration (500 µM) which produced a maximal disinhibitory effect (see above), had very little effect on muscimol-induced inhibition. Indeed,

in the presence of GVG, muscimol caused a similar reduction in the initial PS amplitude (69.91% and 73.75%) as in its absence. In contrast to GVG, bicuculline methiodide, at a concentration (5 μM) which mimicked GVG's effect on the PS (compare initial responses in the presence of GVG and bicuculline), was able to partially antagonize muscimol's actions. In its presence, muscimol reduced the PS amplitude by only 24.18% and 41.04%.

Taken together, these results suggest that GVG-induced disinhibition is not mediated through an action at the $GABA_A$ receptor complex. Another possible explanation for GVG's actions would be that, following its uptake into presynaptic terminals, GVG competes for release with GABA and diminishes the effectiveness of the inhibitory drive by acting as a false transmitter. This was indirectly investigated by comparing the effects of GVG on hippocampal GABAergic inhibition with that of nipecotic acid. Evidence has accumulated that this inhibitor of GABA uptake can act as a false transmitter (Lerma et al., 1985; Roepstorff and Lambert, 1992). The effects of nipecotic acid on GABA-mediated inhibition were therefore investigated under identical conditions. In three experiments, nipecotic acid (1000 μM) consistently produced the same effect as GVG, that is, it reduced inhibition of the test PS produced by both orthodromic ($p < 0.05$ at 10 ms ISI) and antidromic ($p < 0.05$ at 20 ms ISI) conditioning stimulation (Fig. 2 A and B).

**Discussion**

In addition to blocking GABA-T, GVG has long been known to inhibit the uptake of GABA, an action expected to result in an enhancement of inhibition and which has been suggested to be partly responsible for the anticonvulsant properties of this compound (Abdul-Ghani et al., 1981; Jolkkonen et al., 1992; Löscher, 1980b). However, the present study, the first to directly assess the functional consequences of acute GVG application on the GABAergic neurotransmitter system, yielded opposite results. Contrary to the anticipated action of a pure uptake blocker, GVG superfusion caused a concentration-

dependent reduction in GABA-mediated inhibition in the CA1 region of the rat hippocampus.

These results are consistent with the recent observation by Suzuki et al. (Suzuki et al., 1991) that GVG caused a concentration-dependent reduction in the net uptake of $^{36}Cl^-$ into membrane vesicles from rat cerebral cortex, an effect which was interpreted as indicating that GVG acts directly at the $GABA_A$ receptor complex as a non-competitive antagonist. Very little is known about GVG's ability to bind to the $GABA_A$ receptor complex. To our knowledge, no information is available regarding the ability of GVG to bind to the benzodiazepine and picrotoxin sites, while in one study, GVG was reported to displace the specific binding of [$^3$H]GABA with an $IC_{50}$ of 4.1 mM (Löscher, 1980b), a value which is more than 8 fold higher than the concentration of GVG needed to produce a maximal disinhibitory effect. The binding assays performed here confirm and extend these findings to the benzodiazepine and picrotoxin binding sites. At a concentration of 1 mM GVG had no influence on the specific binding of [$^3$H]flunitrazepam or [$^3$H]TBOB and displaced no more than 15% of [$^3$H]GABA binding. Although it is possible that GVG acted on a site distinct from those investigated (i.e. neurosteroid site or other as yet unknown sites) or in a manner which does not interfere with the binding of the radioligands used, these results would suggest that GVG-induced disinhibition is not mediated through an action at the $GABA_A$ receptor complex. Such a suggestion is supported by the experiments testing the postsynaptic sensitivity to the $GABA_A$ receptor agonist, muscimol. This drug was chosen because it is specific for the $GABA_A$ receptor and has a very low affinity for the GABA-transporter (Krogsgaard-Larsen, 1980; Johnston et al., 1978). Muscimol retained its postsynaptic inhibitory action even in the presence of GVG, whereas its action was substantially attenuated in the presence of a relatively low concentration of the competitive $GABA_A$ receptor antagonist, bicuculline. The difference between GVG and bicuculline is entirely in line with the receptor binding data indicating the lack of GVG action on the $GABA_A$ receptor complex.

In addition to inhibiting the uptake of GABA, GVG is known to be a substrate for the transporter (Schousboe et al., 1986), and has been reported to have an intrinsic GABA releasing action (Abdul-Ghani et al., 1981). Together with the apparent lack of affinity for the binding sites on the $GABA_A$ receptor complex, the actions of GVG on the GABA system resemble those reported for nipecotic acid. Indeed, nipecotic acid has been shown to inhibit the uptake of GABA (Kemp et al., 1986), to be a substrate for the GABA-transporter (Johnston et al., 1976b), to cause the release of both labelled and endogenous GABA (Abdul-Ghani et al., 1981; Solís and Nicoll, 1992; Szerb, 1982a), and to have no effect at the $GABA_A$ receptor (Roepstorff and Lambert, 1992). When tested for its effects on inhibitory postsynaptic potentials and currents in rat hippocampal CA1 pyramidal cells, nipecotic acid was found to reduce the amplitude of these responses (Dingledine and Korn, 1985; Roepstorff and Lambert, 1992). This effect is thought to be due to nipecotic acid's ability to compete with GABA for release. Having no postsynaptic effects, nipecotic acid would therefore act as a false transmitter and reduce inhibitory responses. If this mechanism is involved in mediating the disinhibitory action of GVG then we predict, based on the comparable neurochemical effects of these two compounds, that nipecotic acid should produce a similar effect when tested under identical conditions, which is exactly what was observed. Based on this indirect evidence, we suggest that GVG is taken up into GABAergic terminals and produces disinhibition by acting as a false transmitter. Another possibility could be that GVG's GABA-releasing action causes an increase in basal GABA release which could lead to either GABA receptor desensitization or to a feedback inhibition of GABA release due to the activation of presynaptic $GABA_B$ autoreceptors.

In summary, the present study demonstrates that the acute application of GVG to rat hippocampal slices caused a concentration-dependent decrease in GABA-mediated inhibition, which further led to the appearance of multiple PSs at the highest concentrations tested . These results suggest that acute actions of GVG are not involved in mediating its anticonvulsant effects, and may help explain the proconvulsant actions of this drug observed in amygdala-kindled rats

(Löscher et al., 1989). During chronic treatment of patients, it is unlikely that the concentrations of GVG needed to produce disinhibition are achieved in the brain. This action of GVG may nevertheless be relevant in cases of acute drug overdose or in susceptible individuals.

**Acknowledgements**

We wish to thank Normand Lavoie for his expert technical assistance in performing the binding assays. The work was supported by the Medical Research Council of Canada. M. Jackson was supported by an FCAR scholarship. T. Dennis is in receipt of an FRSQ scholarship.

**Table 1.**

*Lack of inhibition of specific [$^3$H]GABA, [$^3$H]flunitrazepam and [$^3$H]TBOB binding by GVG compared to specific competing drugs (GABA, Ro 15-1788 and picrotoxin, respectively)*

Binding assays were performed as described in METHODS. $IC_{50}$ values are means ± S.E.M. of 3 separate experiments each performed in triplicate. n.i. indicates no inhibition up to the concentration given in brackets.

| | Competing Drug | GVG |
|---|---|---|
| [$^3$H]GABA | 147±19 nM | >1 mM |
| [$^3$H]flunitrazepam | 1.86±0.05 nM | n.i. (1 mM) |
| [$^3$H]TBOB | 4785±629 nM | n.i. (1 mM) |

**Figure Legends**

**Fig. 1. Effects of GVG on GABA-mediated inhibition.** During control, conditioning orthodromic (A) or antidromic (B) stimulation causes inhibition of the subsequent test PS. Superfusion of GVG (100 μM) for 60 min caused a reduction in this inhibition. The ISI in these traces was 10 msec. Calibration bars: 5 mV, 5 ms.

**Fig 2. Effects of GVG (100-500 μM) on GABAergic inhibition over the entire range of ISIs tested.** GVG superfusion resulted in a concentration-dependent decrease in the inhibition produced by both orthodromic (A) and antidromic (B) conditioning stimulation. At a concentration of 1 mM, nipecotic acid (NA) produced a similar disinhibitory effect. Inhibited or facilitated test PSs are expressed as a % of the unconditioned PS. Each point is the mean ± S.E.M. of values obtained from the number of experiments given in brackets. GVG's effect was significant in (A) at ISIs of 10-40ms for GVG 100-250 μM ($p < 0.05$) and at 10-20 ms for GVG 500 μM ($p < 0.03$), and in (B) at ISIs of 10-40 ms for GVG 100,250 ($p < 0.05$) and 500 μM ($p < 0.02$).

**Fig. 3. Superfusion of GVG (1 mM) resulted in the appearance of multiple PSs.** This effect was reversible. Stimulation was by a single stimulus near maximal intensity. Calibration bars: 10 mV, 5 ms.

**Fig. 4. Comparison of the effects of GVG and bicuculline on the muscimol-induced inhibition of the orthodromically evoked CA1 PS.** Initial responses were recorded from separate slices in the presence of either normal ACSF (nACSF), GVG 500 μM or bicuculline methiodide 5 μM. Then, muscimol (10 μM) was added to the superfusate and its effect on the PS amplitude was determined under each condition. Each trace represents the average of four responses. Calibration bars: 5 mV, 10ms

**Fig. 1. Effects of GVG on GABA-mediated inhibition.**

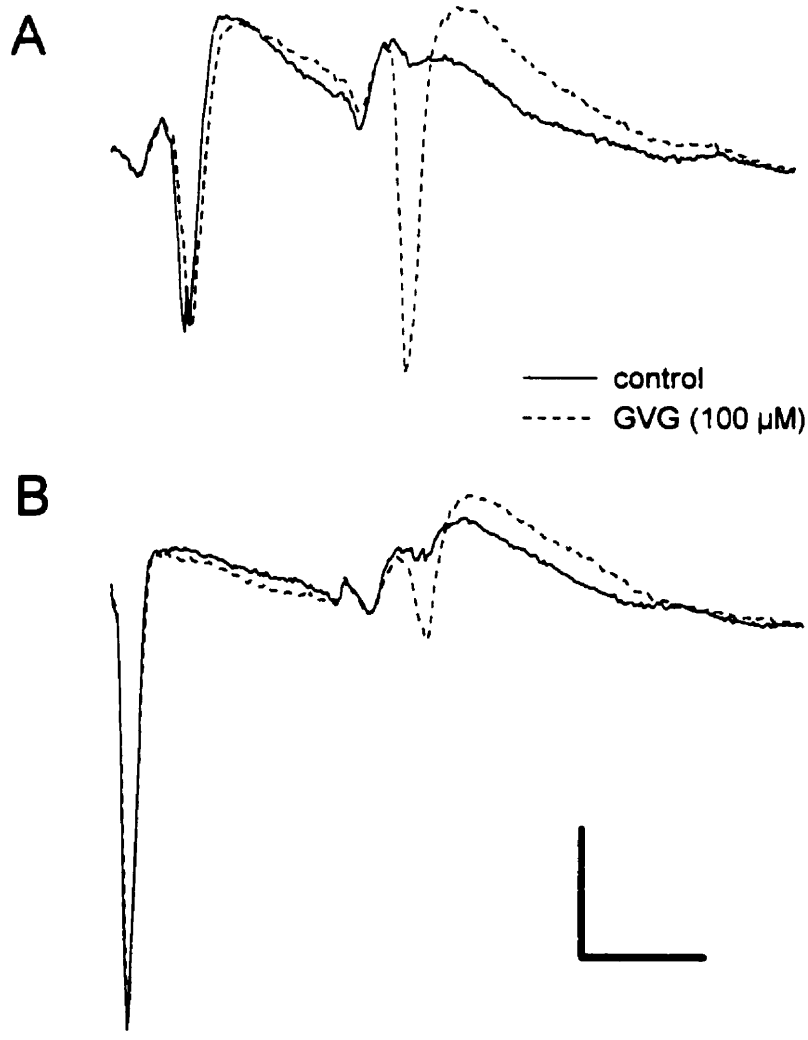


**Fig 2. Effects of GVG (100-500 μM) on GABAergic inhibition over the entire range of ISIs tested.**

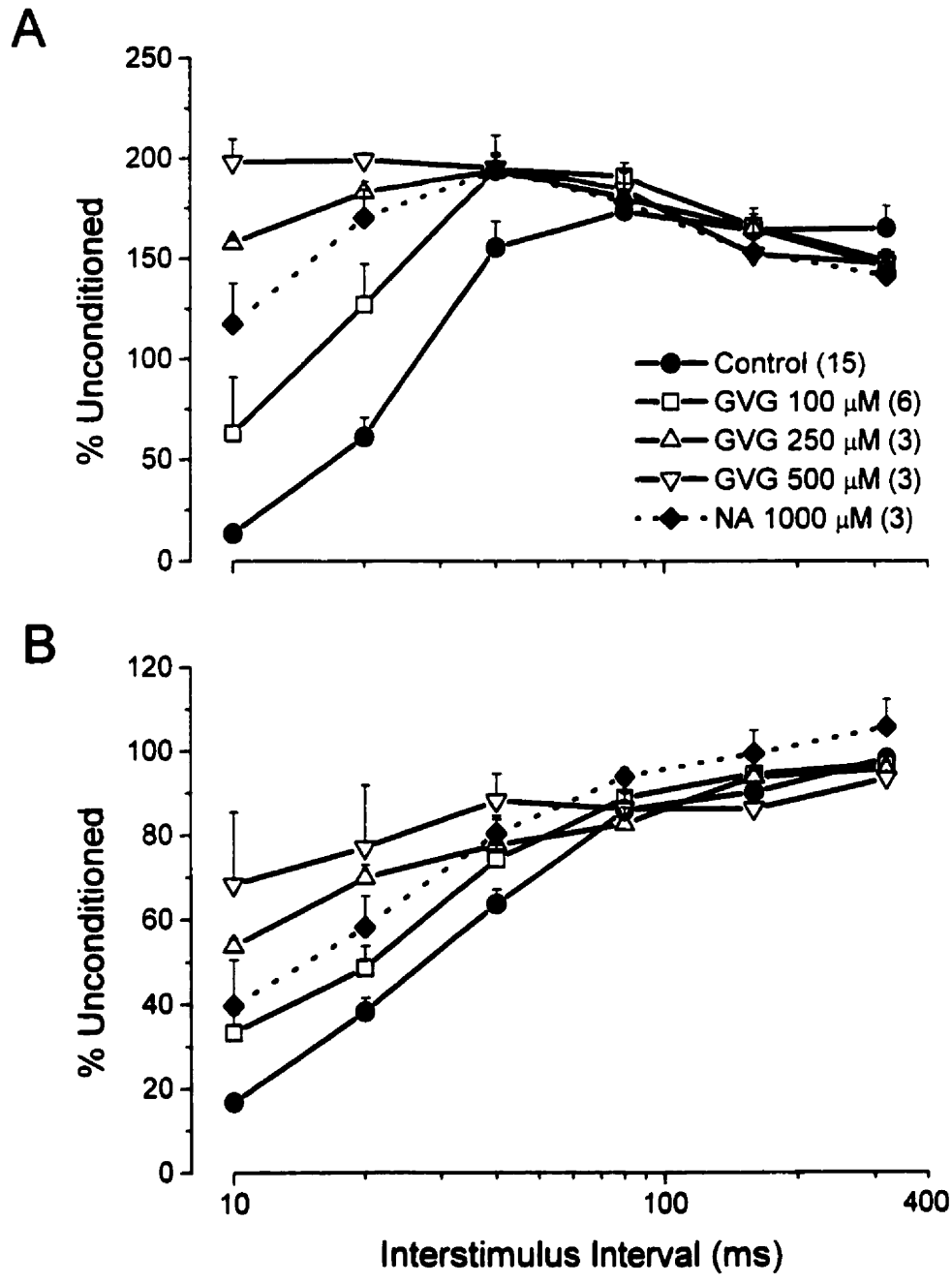


**Fig. 3. Superfusion of GVG (1 mM) resulted in the appearance of multiple PSs.**

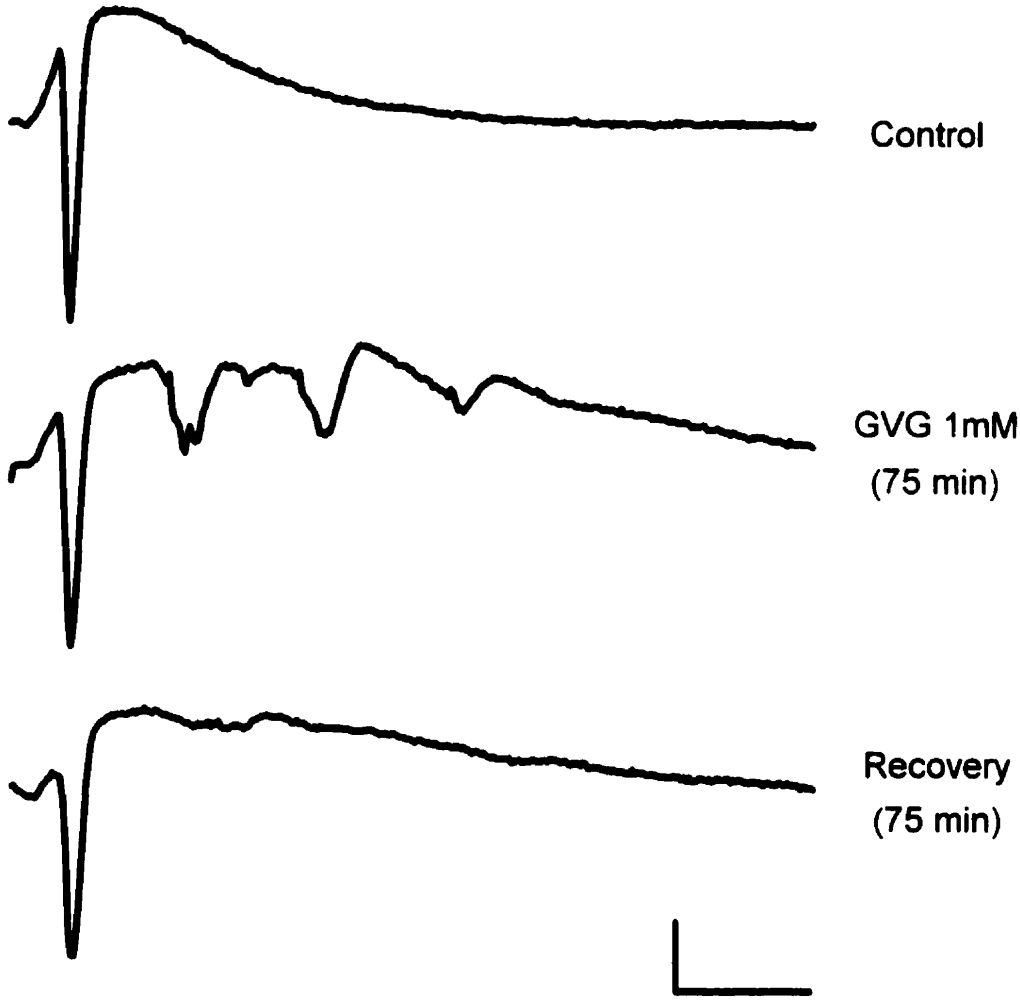
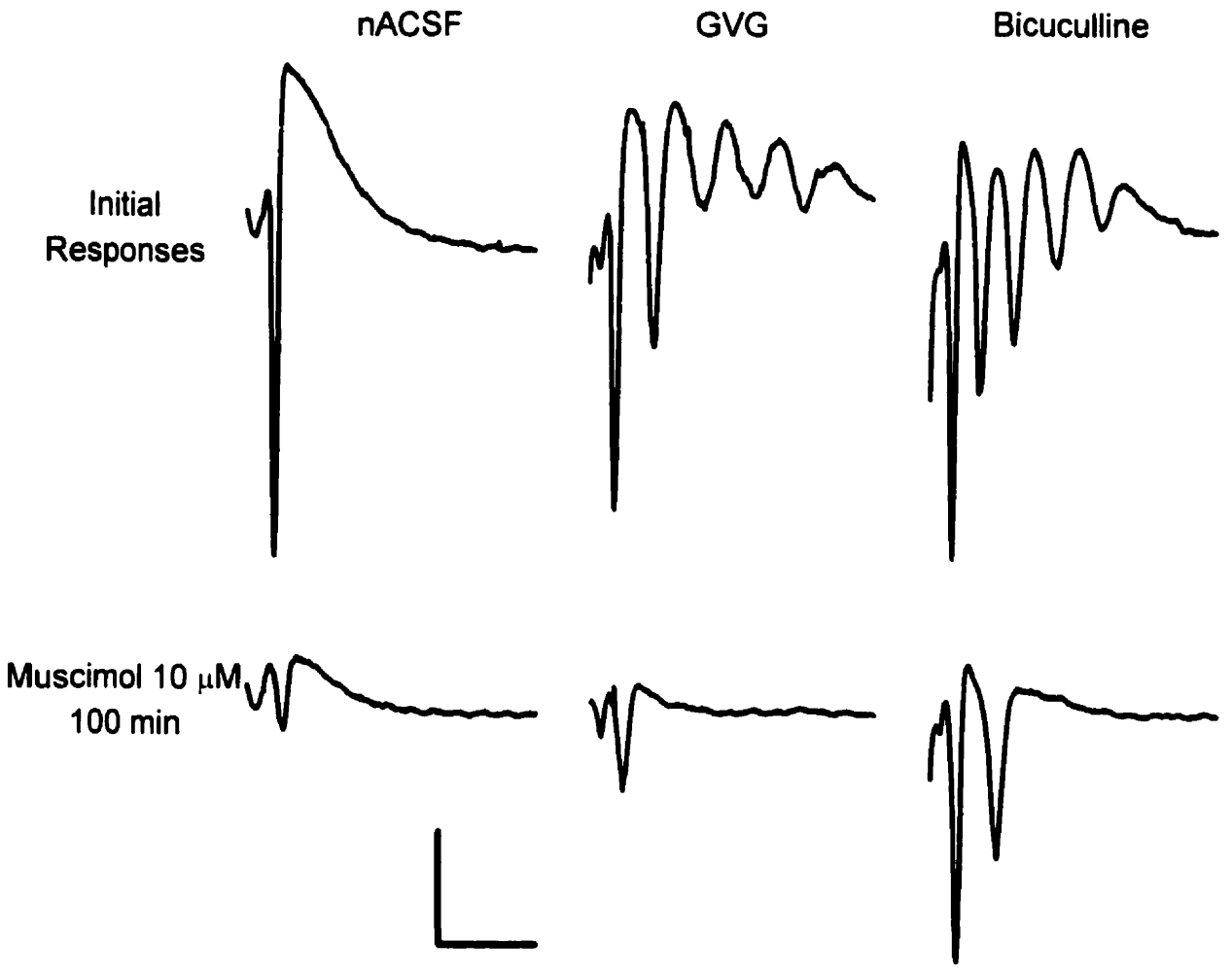


**Fig. 4. Comparison of the effects of GVG and bicuculline on the muscimol-induced inhibition of the orthodromically evoked CA1 PS.**



# Summary and General Discussion

## 7. Summary and General discussion

**1st Study:** Frequency-dependent enhancement of hyperpolarizing and depolarizing GABAergic synaptic responses following inhibition of GABA uptake by tiagabine.

The frequency-dependence of the effects of drugs acting upon the GABA neurotransmitter system was evaluated by Čapek and Esplin in their study of the effects of two novel lipophilic blockers of GABA transport, SKF 89976A and SKF 100330A (Čapek and Esplin, 1993). The impetus for these early studies was provided by the demonstration that while producing relatively modest effects on evoked IPSPs, inhibitors of the uptake of GABA are in contrast capable of producing a robust suppression of epileptiform discharges elicited following the superfusion of hippocampal slices with a solution containing an elevated concentration of $K^+$ (Dingledine and Korn, 1985). An examination of the rate at which the neuronal population was entrained during high $K^+$-induced epileptiform discharges revealed that burst firing could be generated at frequencies of up to 200 Hz. The contrasting effects of GABA uptake blockers on evoked IPSPs and epileptiform bursts might therefore have been due to the ability of these compounds to selectively reinforce inhibition during episodes of hypersynchronous high-frequency activity. This possibility was explored by comparing the effects of uptake inhibitors upon the inhibition produced following the activation of hippocampal recurrent inhibitory pathways by either a single stimulus or by trains of stimuli delivered at low (5 Hz) and high (100 and 500 Hz) frequencies. This study clearly demonstrated the relatively selective ability of GABA uptake blockers to mediate an enhancement of synaptic inhibition during the stimulation of inhibitory pathways at high frequencies. Additionally, this study revealed the contrasting consequences of low versus high frequency stimulation on the effectiveness of GABA-mediated inhibition. Indeed, in contrast to the activity-dependent depression of inhibition which is consistently observed as a result of the low-frequency stimulation of inhibitory pathways, stimulation at high

frequencies was shown to elicit a much stronger inhibitory response than that produced following a single stimulus. Although experiments involving the use of the $GABA_B$ receptor antagonist 2-OH saclofen suggested that an increased activation of $GABA_B$ receptors may have contributed to the observed enhancement of inhibition during high-frequency stimulation, the limitations inherent to the use of extracellular recordings prevented a more accurate assessment of the postsynaptic mechanisms involved. Use of the tight seal whole-cell recording technique under conditions whereby GABA-mediated postsynaptic responses were pharmacologically isolated would certainly allow a more detailed description of the mechanisms through which; (1) high-frequency stimulation of inhibitory interneurons results in an strengthening of synaptic inhibition and, (2) GABA uptake blockers produce a further increase in the effectiveness of GABA-mediated inhibition during high-frequency stimulation.

The first study of this thesis was undertaken with these specific objectives in mind. Our findings can be summarized as follows:

1) With excitatory transmission blocked by the bath application of specific antagonists of both AMPA and NMDA receptors and in comparison to GABA-mediated IPSPs evoked in response to a single stimulus, the direct high-frequency stimulation (HFS) of inhibitory interneurons caused:
    A) An increase in the amplitude and duration of $GABA_A$ and $GABA_B$ receptor-mediated IPSPs.
    B) The appearance of a $GABA_A$ receptor-mediated depolarizing response whose amplitude and duration were dependent upon both the intensity and duration of HFS.

2) Bath application of the GABA uptake blocker tiagabine greatly increased the depolarizing actions of GABA produced in response to HFS, an effect which far exceeded those produced by this drug on the hyperpolarizing IPSPs evoked by a single stimulus.

3) Tiagabine-augmented depolarizing responses evoked more distally by a stimulating electrode located at the border of stratum radiatum/moleculare could trigger the firing of a burst of action potentials, whereas those evoked more proximally by a stimulating electrode located in the alveus elicited fewer if any action potentials despite being of similar amplitude as responses evoked more distally.

Previous studies examining the effects of paired-stimulation on IPSPs revealed that these synaptic events could linearly summate at interstimulus intervals of less than 10 ms (10 ms effectively corresponds to a stimulation frequency of 100 Hz) (Davies et al., 1990). Thus, we suspected that the strong inhibition produced in response to HFS at frequencies greater than 100 Hz resulted from a strong hyperpolarization of pyramidal neurons due to the generation of a large compound hyperpolarizing IPSP. Consistent with this expectation, we observed that HFS using low stimulus intensities provoked a large increase in the amplitude and duration of hyperpolarizing IPSPs. Surprisingly however, as the stimulus intensity and/or duration of HFS was increased, the postsynaptic response was now dominated by a $GABA_A$ receptor-mediated depolarization which was capable of triggering the burst firing of action potentials in hippocampal CA1 pyramidal cells. These results were seemingly incompatible with the strong synaptic inhibition which had previously been shown to occur following the HFS of recurrent inhibitory pathways. In addition, based on these findings alone, the inhibition of GABA uptake produced by tiagabine, which greatly increased the depolarizing actions of GABA, might be expected to produce a proconvulsant rather than anticonvulsant action when administered to epileptic subjects. Yet, as highlighted in the introduction of this thesis, there is little doubt as to the effectiveness of tiagabine as an anticonvulsant.

$GABA_A$ receptor-mediated depolarizing responses in the adult mammalian CNS have been reported to occur under numerous experimental conditions all of which have in common the ability of causing a prolonged and pronounced activation of the postsynaptic $GABA_A$ receptor. More readily elicited in the distal

dendrites of hippocampal pyramidal neurons, these GABA-mediated depolarizations have, in the past, been attributed to the maintenance in these distal compartments of a reversed transmembrane gradient for $Cl^-$ ions (Müller et al., 1989) as well as to the existence of a subtype of $GABA_A$ receptors present on the extrasynaptic dendritic membrane and through which inward (i.e. depolarizing) currents can be generated (Alger and Nicoll, 1982a). A recently proposed model (Staley et al., 1995) suggests that GABA-mediated depolarizations are generated as a result of an increased flux of $HCO_3^-$ ions through the $GABA_A$ receptor-gated channel following a collapse of the transmembrane gradient of $Cl^-$, the normally dominant ionic species whose influx underlies the fast hyperpolarizing component of IPSPs, during prolonged receptor activation. More recently, investigations of the ionic basis of $GABA_A$ receptor-mediated depolarizing responses have suggested that the proposed $Cl^-$ accumulation model may need further refinements in order to fully account for all of the available experimental findings (Perkins and Wong, 1996; Kaila et al., 1997). Nevertheless, the chloride accumulation model is generally consistent with most of the properties and features associated with these depolarizing events, in particular, their dependence on the level of $GABA_A$ receptor activation and their intimate association with a preceding fast $Cl^-$-dependent hyperpolarization.

In considering the possible role, either physiological or pathological, of GABA-mediated depolarizations and the consequence of their augmentation by tiagabine, the conditions under which these responses were observed must be born in mind. Can such a high degree of interneuronal synchronization, as was produced by our use of high intensity electrical stimulation in close proximity to the neuron whose membrane potential was being recorded, be expected to occur through intrinsic synaptic and/or non-synaptic mechanisms? In short, the answer appears to be yes. Anatomical studies have demonstrated that there exists within the hippocampus several distinct populations of interneurons which have been identified on the basis of their neurochemical content and distinctive termination patterns (Somogyi et al., 1983; Gulyás et al., 1993; Buhl et al., 1994;

Sik et al., 1994; Sik et al., 1995; Miles et al., 1996). While certain interneuronal types, such as the basket cell, preferentially target hippocampal pyramidal cells, others, termed interneuron-selective, exclusively innervate other GABAergic cells. The presence of such interconnections and the demonstration of the ability of interneurons to excite one another through $GABA_A$ receptor-mediated depolarizing potentials and possibly gap junctions (Michelson and Wong, 1991; Michelson and Wong, 1994) provides an anatomical and physiological basis for the synchronization of GABAergic interneurons. Furthermore, the observation of spontaneously occurring $GABA_A$ receptor-mediated depolarizing responses in hippocampal slices following the application of the $K^+$ channel blocker and convulsant agent 4-AP (Perreault and Avoli, 1992) suggests that a high degree of interneuronal synchronization can in fact occur under certain circumstances. The persistence of these spontaneously occurring depolarizing events in the absence of glutamatergic excitatory transmission and their simultaneous generation in different hippocampal areas suggests that the mechanisms highlighted above are likely to play a key role in the generation of these GABA-mediated potentials. Recent studies of the ionic mechanisms which underlie the 4-AP-induced GABA-mediated depolarizing responses suggest that, as with those evoked by the monosynaptic high-frequency stimulation of inhibitory interneurons, these events are dependent on the efflux of $HCO^-_3$ through $GABA_A$ receptors (Lamsa and Kaila, 1997).

Given the fact that GABA-mediated depolarizing potentials can be spontaneously generated in an *in vitro* seizure model it is worthwhile considering the possible role that these events may play in the generation of burst discharges. Due to the demonstration of their ability to elicit the firing of bursts of action potentials, several authors have claimed that GABA-mediated depolarizing responses may contribute to the generation of epileptiform discharges. These claims were based solely on findings from studies in which $GABA_A$ receptor-mediated depolarizations were evoked following the high-frequency stimulation of a restricted population of interneurons by an electrode located in stratum radiatum. However, as our own results demonstrate, the ability of $GABA_A$

receptor-mediated depolarizing responses to trigger action potential firing depends on the site from which these depolarizing events are evoked. Thus, in contrast to the relative ease with which bursts of action potentials can be generated by depolarizing responses elicited from the stratum radiatum, those evoked from the alveus trigger very little if any action potential firing. These results are especially interesting in light of the observed paucity of action potential firing observed in response to the spontaneously occurring depolarizing responses that are associated with the application of 4-AP (Perreault and Avoli, 1991; Perreault and Avoli, 1992). Since these spontaneously occurring depolarizing events occur synchronously in different hippocampal areas, the possibility exists that the simultaneous occurrence of a proximally located $GABA_A$ receptor-mediated conductance increase may offset the ability of a distally generated depolarizing response to trigger action potential firing. Taken together, these findings suggest that the functional significance of GABA-mediated depolarizing events and their role in the genesis of epileptic activity is not as clear as had previously been suggested.

Nevertheless, evidence for a role of GABA-mediated potentials in the generation of epileptiform activity has been found in studies of the mechanisms involved in the initiation and propagation of epileptiform activity induced by 4-AP in hippocampal slices from immature rodents (Avoli et al., 1993) as well as in combined hippocampal-entorhinal cortical slices from adults (Avoli et al., 1996; Lopantsev and Avoli, 1998). In these models, 4-AP was shown to induce three types of spontaneously occurring activity which, in addition to the GABA-mediated depolarizing responses, included interictal- and ictal-like events. Interestingly, the ictal-like discharges were typically preceded, and thus appeared to be initiated, by a $GABA_A$ receptor-mediated depolarization of particularly large amplitude. Similarly, the extracellular rise in the concentration of $K^+$, which has been observed to occur in association with GABAergic depolarizing events, was shown to be especially large for those events which appeared to trigger the onset of the ictaform discharge. As mentioned in the introduction to this thesis, an increase in extracellular $K^+$ resulting in an increased neuronal synchronization is

one of the mechanisms which have been implicated in the generation of full blown seizures. Thus, it remains to be determined whether the onset of ictal discharges observed in these experiments is triggered as a result of the direct postsynaptic influence of the $GABA_A$ receptor-mediated depolarizing response or the associated rise in extracellular potassium. Nevertheless, our results with tiagabine could be interpreted as suggesting that the resulting inhibition of GABA uptake will facilitate the onset of ictal discharges and thus of seizures. However, evidence demonstrating that nipecotic acid can suppress the interictal and ictal activity induced by 4-AP in hippocampal slices from immature rodents despite transiently increasing the GABA-mediated potentials suggests that this is unlikely. In the presence of GABA uptake blockers the resulting increased effectiveness of GABA-mediated inhibition may disrupt interneuronal synchronization and prevent the generation of GABA-mediated depolarizations. An examination of the effects of tiagabine on 4-AP-induced epileptiform activity may nevertheless allow for the elucidation of the significance of the seemingly paradoxical increase in the depolarizing actions of GABA produced by this drug.

**2nd Study:** The inhibitory nature of tiagabine-augmented $GABA_A$ receptor -mediated depolarizing responses (DRs) in hippocampal pyramidal cells

As outlined in the preceding section, the ability of $GABA_A$ receptor-mediated responses to depolarize neurons well beyond their threshold for action potential generation has led to the suggestion that under certain circumstances GABA could actually contribute to the generation of epileptiform discharges. In this respect, supplementary mechanisms can be proposed through which, in addition to directly triggering the firing of action potentials, GABA-mediated depolarizations could lead to an increase in excitation. The first is based on the characteristic hyperpolarizing-depolarizing sequence associated with the synchronized monosynaptic GABA-mediated responses elicited following high-frequency stimulation. It was suggested that such a sequential change in

membrane potential would be ideally suited to first deinactivate and then activate low-threshold calcium channels in pyramidal neurons (Lambert and Grover, 1995). As outlined in the introduction of this thesis, GABA-mediated depolarizations could thus activate an intrinsic burst generating mechanism by triggering dendritic $Ca^{2+}$ spikes. The second was suggested following the demonstration of a large increase in NMDA-evoked conductance during GABA-mediated depolarization (Staley et al., 1995), a result which was interpreted as suggesting that GABA-mediated depolarizations may enhance NMDA receptor activation by alleviating the voltage-dependent block of these receptors by $Mg^{2+}$. The implication of this finding is clear given the prominent role that NMDA receptors have been proposed to play in epileptiform activity (Dingledine et al., 1986; Ashwood and Wheal, 1987; Jones, 1988; Hwa and Avoli, 1989; Traub et al., 1993). Although plausible, no evidence has yet been presented implicating any of these potential mechanisms in the generation of epileptiform discharges.

The first study of this thesis confirmed previous observations made by several groups suggesting that high-frequency stimulation of inhibitory interneurons can transform the postsynaptic actions of GABA from mainly hyperpolarizing to predominantly depolarizing. Furthermore, we showed that the inhibition of GABA uptake produced by tiagabine greatly facilitates the depolarizing actions of GABA following high-frequency stimulation. Interestingly, the time course of the GABA-mediated depolarizing response which we recorded closely resembles that of the strong synaptic inhibition which was previously observed to occur following the activation of recurrent inhibitory pathways using stimulation parameters (100 Hz, 200 ms) identical to those with which GABA depolarizations can be evoked (Čapek and Esplin, 1993). Furthermore, if we assume that in this study, the use of GABA uptake blockers, which greatly enhanced the strength and duration of the inhibition resulting from high-frequency stimulation, was associated with an increase in the depolarizing actions of GABA, similar to that which we observed with tiagabine, then the occurrence of $GABA_A$ receptor-mediated depolarizing responses may in fact be associated with a prolonged period of strong inhibition.

The second study of this thesis was thus undertaken with the specific objective of examining the influence of GABA-mediated depolarizing responses, evoked under both control conditions as well as in the presence of tiagabine, upon coinciding excitatory transmission. The main findings of this study can be summarized as follows:

1) As was previously observed with the SKF series of GABA uptake inhibitors, extracellular recordings demonstrated that bath application of tiagabine greatly increases both the strength and duration of hippocampal recurrent inhibition produced following high-frequency stimulation of the alveus.

2) Whole-cell current-clamp recordings revealed that the amplitude of evoked EPSPs superimposed upon tiagabine-enhanced depolarizing responses was reduced and their ability to generate action potentials suppressed.

3) Similarly, in the presence of tiagabine the charge carried to the soma by EPSCs evoked 400 ms following high-frequency stimulation of the alveus was reduced to only ~10% of that carried by responses evoked in the absence of preceding high-frequency stimulation.

Synaptically released GABA acting through postsynaptic $GABA_A$ and $GABA_B$ receptors subtypes produces inhibition by reducing the response of a neuron to its excitatory inputs. The mechanism by which GABA produces robust inhibition in the CNS is customarily assumed to be due to the resulting postsynaptic hyperpolarization which drives the membrane potential further away from the action potential firing threshold. This inhibitory mechanism has generally been referred to as being of the hyperpolarizing type but has also been termed linear inhibition since its effect upon the output of a neuron is expected to depend on the outcome of the linear summation of excitatory and inhibitory inputs. Conversely, in neurons in which the reversal potential for the inhibitory response is near the resting membrane potential, GABA can nevertheless

mediate synaptic inhibition despite producing negligible changes in membrane potential. The resulting inhibition is referred to as nonlinear or shunting inhibition and the key to its effectiveness is the associated increase in membrane conductance which reduces the ability of an excitatory input to depolarize its target neuron. For shunting inhibition to be effective 1) the inhibitory synapse should be located along the path from the excitatory synapse to the cell body, 2) the inhibitory conductance increase should be larger than the excitatory conductance and 3) there must be substantial temporal overlap between the two conductances (Koch et al., 1983; Qian and Sejnowski, 1990).

In granule cells of the dentate gyrus, $GABA_A$ receptor-mediated synaptic responses are depolarizing due to the highly negative membrane potential at which these neurons rest. Despite this, the resulting synaptic responses are considered inhibitory since the reversal potential for the $GABA_A$ receptor-mediated event is below the threshold for action potential generation (Staley and Mody, 1992; Holmes and Levy, 1997). A shunting mechanism was proposed to underlie the ability of GABA to maintain its inhibitory function in these cells (Staley and Mody, 1992). The results of the second study of this thesis suggests that even under circumstances in which GABA clearly depolarizes cells beyond their threshold for action potential generation, strong inhibition can nonetheless be produced as a consequence of the large increase in membrane conductance which is associated with the generation of GABA-mediated depolarizing responses.

It could be argued that the evidence which we presented in favor of such a mechanism is somewhat circumstantial. Indeed, as mentioned in the second manuscript (see Results), the strong inhibition of population spikes resulting from high-frequency stimulation of recurrent inhibitory pathways may have resulted from either strong shunting inhibition or from a reduction in the effectiveness of excitatory transmission due to the activation of $GABA_B$ heteroreceptors located at glutamatergic nerve terminals. Although the latter mechanism was ruled out in our voltage-clamp experiments due to the presence of the $GABA_B$ receptor antagonist, CGP 52432, the effects of shunting inhibition upon evoked excitatory

responses was only examined at a time point (400 ms) corresponding to the peak of the large $GABA_A$ receptor-mediated inward current. Since the membrane conductance increase peaks within the first 100 ms following high-frequency stimulation (see Fig. 2 of Section 3), it is more than likely that a shunting mechanism strongly contributed to the enhanced inhibition of both population spikes and action potentials observed within the first 400 ms following high-frequency stimulation of the alveus, especially following the bath application of tiagabine. However, since both extracellular and current-clamp recordings were performed in the absence of $GABA_B$ receptor antagonists, it is possible that in the presence of tiagabine the prolongation of the inhibitory period to time points extending beyond 1600 ms may have involved an increased activation of the $GABA_B$ heteroreceptors located at glutamatergic terminals. Such a possibility would be consistent with the suggestion, made by Isaacson and colleagues (Isaacson et al., 1993), based on experiments using inhibitors of GABA transport, that GABA uptake plays an important role in limiting the spill-over of GABA to neighboring synapses.

The contribution of a shunting mechanism to the inhibition produced following high-frequency stimulation of recurrent inhibitory pathways could have been more precisely estimated in these experiments by comparing the time course of the inhibition to that of the conductance change resulting from the activation of $GABA_A$ receptors both before and after the inhibition of GABA uptake by tiagabine. The demonstration of a substantial overlap of both the time course of inhibition and the membrane conductance increase would add further support to our suggestion that shunting inhibition strongly contributes to the prolonged period of enhanced inhibition resulting from high-frequency conditioning stimulation in the presence of tiagabine. The additional use of $GABA_B$ receptor antagonists in these experiments would also allow us to examine the contribution of $GABA_B$ receptor-mediated mechanisms. As mentioned earlier, previous evidence had suggested that $GABA_B$ receptors could be involved in the prolonged inhibitory phase observed following HFS (Čapek and Esplin, 1993). These experiments, involving the use of the $GABA_B$ receptor

antagonist 2-OH-saclofen, could not however differentiate between pre- (involving heteroreceptors) versus postsynaptic mechanisms. Despite claims as to the specificity of more recently developed $GABA_B$ receptor antagonists (Olpe et al., 1994; Deisz et al., 1997), it is unlikely that we could precisely differentiate between pre- versus postsynaptic mechanisms based on pharmacological manipulations alone. The postsynaptic $GABA_B$ receptor-mediated response could selectively be blocked by the inclusion of $Cs^+$ and QX-314 in our intracellular recording solutions. Such a procedure has previously been use to abolish postsynaptic $GABA_B$ receptor-mediated responses (Otis and Mody, 1992). Therefore, in both the presence and absence of tiagabine, a comparison of the conductance increase, produced following HFS, between recordings with or without added $Cs^+$ and Qx-314 could allow us to determine the relative contribution of postsynaptic $GABA_B$ receptor activation to the inhibitory response elicited following HFS. Similarly, the use of intracellular $Cs^+$ and QX-314 could also allow us to examine the effect of presynaptic $GABA_B$ heteroreceptor activation on the amplitude of evoked EPSPs. If these receptors underlie the especially prolonged period of inhibition which we observed in the presence of tiagabine, then, under the conditions described above, the bath application of a high-affinity $GABA_B$ receptor antagonist will be expected to diminish the reduction of EPSP amplitude produced following HFS.

Regardless of the specific mechanism involved, our results demonstrate that the occurrence of GABA-mediated depolarizing responses is more likely to be associated with strong inhibition of postsynaptic neurons than with their excitation. This is in contrast to situations where GABA-mediated excitation is generated due to the maintenance in neurons of a $Cl^-$ gradient which is more positive than the threshold for action potential generation. A classical example of such a situation is found in prenatal as well as early postnatal (< P7) mammalian CNS neurons where GABA acts as an excitatory transmitter (Ben-Ari et al., 1989; Mueller et al., 1984; LoTurco et al., 1995; Owens et al., 1996). In these cells GABA can readily mediate excitation since its depolarizing actions do not necessitate a large $GABA_A$ receptor-mediated conductance increase.

Nevertheless, even in cells maintaining a very positive $Cl^-$ gradient, a sufficiently large $GABA_A$ receptor mediated conductance increase can transform the actions of GABA from excitatory to inhibitory. This has been demonstrated in numerous preparations, including frog melanotrophs (Le Foll et al., 1998), rat adrenal chromaffin cells (Busik et al., 1996), as well as rat embryonic cultured hypothalamic neurons (Chen et al., 1996) where exogenously applied GABA initially triggered action potential firing but could then prevent excitation following either an increase in the concentration of GABA or its co-application with a positive allosteric modulator of $GABA_A$ receptor function. Both of these manipulations were shown to be associated with a significant collapse of the input resistance. Interestingly, a similar phenomenon may explain our finding that action potential firing following high-frequency stimulation of stratum radiatum was more likely to occur in slices in which tiagabine was added to the superfusate following a control run in its absence than in slices that were preincubated (> 30 min) with tiagabine (unpublished results). This would indicate that once a steady-state concentration of tiagabine is achieved the conductance increase associated with depolarizing responses is sufficiently large to inhibit action potential firing.

Finally, the possibility nevertheless exists that transient excitation through a $GABA_A$ receptor-mediated mechanism could facilitate the onset of ictal discharges as suggested from recent evidence (Avoli et al., 1996; Lopantsev and Avoli, 1998). Indeed, the strength of shunting inhibition is primarily determined by the size of the conductance increase. However, due to the membrane time constant, the consequences of receptor activation will typically outlast the duration of the associated conductance increase. In this respect it is noteworthy that the peak of the depolarizing response occurs at time points which correspond to the decaying phase of the conductance increase (see Fig. 2 of Section 3). The combined depolarizing influence of the GABA-mediated depolarizing response and a particularly large extracellular accumulation of $K^+$ at a time when the $GABA_A$ receptor-mediated conductance increase is on the decline may participate in triggering ictal events under certain circumstances.

Such a mechanism would be consistent with the slight delay which is observed to occur between the onset of the depolarizing response and that of ictal discharges.

**3rd Study:** Reversal of the activity-dependent suppression of GABA-mediated inhibition in hippocampal slices from γ-vinyl GABA (vigabatrin) -pretreated rats

Gamma-vinyl GABA (GVG) is one of very few examples of the successful utilization of a rational drug design strategy in the development of a novel, clinically effective anticonvulsant (for review see Löscher, 1998). This fact, perhaps more than any other, may have contributed to the widely held belief that GVG is one of the few anticonvulsant compounds whose mechanism of action is precisely known. The anticonvulsant properties of GVG are generally attributed to its ability to enhance synaptic inhibition by elevating the concentration of GABA in the CNS. Although a large number of studies (refer to 2.2.3) have confirmed the ability of GVG to elevate the brain levels of the inhibitory neurotransmitter, these studies could not directly assess the resulting functional consequences. Interestingly, the only previous electrophysiological study which, to our knowledge, investigated the effects of GVG on GABA-mediated synaptic inhibition (Rak and Lothman, 1988) demonstrated that little if any change could be detected following pretreatment of animals with a regimen previously demonstrated to produce an anticonvulsant effect. The aim of the next study of this thesis was therefore to examine the effects of GVG on synaptic inhibition.

Given the likely mechanism of GVG's anticonvulsant actions, namely elevation of brain GABA levels following the irreversible inhibition of GABA-T, it appeared unlikely at the onset of these studies that substantial elevations in GABA levels could be achieved in acutely prepared hippocampal slices superfused with GVG. Indeed, peak brain GABA levels occur approximately 3 hours and are maintained for 24 hours following the pretreatment of animals with GVG (Jung et al., 1977a; Schechter et al., 1977). It is for this reason that we

chose to examine the effects of GVG upon GABA-mediated synaptic responses recorded in hippocampal slices prepared from rats having previously been administered an anticonvulsant dose (1500 mg/kg) of GVG 24 hours before the start of experiments. As a result, the effects of GVG were based on comparisons of the strength of inhibition in slices obtained from separate populations of animals pretreated with either GVG or saline (controls).

The findings from our initial study of the actions of GVG in slices prepared from pretreated animals can be summarized as follows:

1) While producing negligible effects on the inhibition resulting from the activation of inhibitory pathways with a single conditioning stimulation, GVG pretreatment either prevented or reversed the activity-dependent depression of inhibition normally associated with repetitive conditioning stimulation at low-frequencies (2.5-10 Hz).

2) Recordings of low-frequency (5 Hz) train-induced IPSPs revealed that GVG prevented the activity-dependent reduction in the amplitudes of both early, presumably $GABA_A$ receptor-mediated, and late, presumably $GABA_B$ receptor-mediated, hyperpolarizations.

3) GVG pretreatment reduced the sensitivity of evoked IPSCs to the inhibitory effects of baclofen suggesting that a decreased efficacy of the mechanism regulating GABA release via presynaptic $GABA_B$ autoreceptors may underlie the frequency-dependent actions of GVG.

The lability of synaptic inhibition during the repeated activation of the GABA neurotransmitter system has been well recognized since early electrophysiological studies of inhibitory synaptic transmission in motoneurons (Curtis and Eccles, 1960), hippocampal pyramidal cells (Ben-Ari et al., 1979; McCarren and Alger, 1985) and neocortical cells (Connors et al., 1982). Variously referred to as use-, frequency- or activity-dependent depression of

inhibition, this phenomenon was immediately recognized as being a possible contributing factor allowing for the propagation of epileptiform activity into normal brain tissue. In fact, the large number of studies which have investigated the mechanisms involved in the activity-dependent depression of GABA-mediated inhibition attests to the perceived importance of this phenomenon in the control of CNS excitability. The resulting reduction in the effectiveness of GABA-mediated inhibition has most consistently been attributed to a depolarizing shift in the equilibrium potential for $Cl^-$ as well as to a reduction in $GABA_A$ receptor-mediated conductance. The transient depolarizing shift in the $Cl^-$ equilibrium is due to an activity-induced collapse of its transmembrane gradient, normally maintained by the $K^+/Cl^-$ cotransporter. Reestablishment of the $Cl^-$ gradient may be delayed following repetitive stimulation due to the resulting rise in extracellular $K^+$ which reduces the effectiveness of the $K^+/Cl^-$ cotransporter (Thompson and Gähwiler, 1989b). Conversely, although the reduction in $GABA_A$ receptor-mediated conductance has been attributed to both presynaptic and postsynaptic mechanisms, more recent investigations have generally attributed most if not all of the conductance decrease to the activation of presynaptic $GABA_B$ autoreceptors (for review see Deisz, 1997).

The results of our studies would suggest that GVG pretreatment causes a depression of $GABA_B$ autoreceptor-mediated mechanisms regulating the release of GABA. This change is further referred to as downregulation or depression of the $GABA_B$ autoreceptor function while recognizing, as mentioned in the discussion of Section 5 of this thesis, that such a change could have occurred by various mechanisms. Consistent with the prominent role played by $GABA_B$ receptors in the activity-dependent depression of inhibition, low-frequency repetitive stimulation no longer produced a disinhibitory effect in slices from GVG pretreated animals. This conclusion is based predominantly upon the observed reduction of the ability of baclofen to cause a decrease in the amplitude of evoked monosynaptic IPSCs in slices from GVG pretreated rats. Nevertheless, bath application of the $GABA_B$ receptor agonist reduced the amplitude of IPSCs by about 40%. In addition, GVG pretreatment did not affect the facilitation of

population spike amplitude which occurred with paired orthodromic stimulation at ISIs of > 40 ms. As with the activity-dependent depression of inhibition, paired-pulse facilitation has been attributed to a reduction in the strength of inhibition following the activation of presynaptic $GABA_B$ receptors (Nathan et al., 1990). These two observations contrast with those from studies in which a more profound downregulation of $GABA_B$ receptor function was observed. Indeed, in hippocampal slices prepared from animals previously subjected to kainic acid-induced status epilepticus, the downregulation of presynaptic $GABA_B$ receptor function was associated with a complete elimination of the facilitation normally observed during paired perforant path stimulation (Haas et al., 1996). Similarly, in hippocampal slices prepared from animals made epileptic following continuous hippocampal stimulation, a downregulation of $GABA_B$ receptors was noted, as evidenced by the inability of both $GABA_B$ receptor agonists and antagonists to cause a change in the amplitude of monosynaptically evoked IPSPs (Mangan and Lothman, 1996). The observed absence of activity-dependent disinhibition in this latter study could therefore more clearly be attributed to autoreceptor downregulation. Therefore, our results seem to indicate that although reduced, the function of presynaptic $GABA_B$ receptors following the administration of GVG is not abolished.

It is possible nonetheless that the partial reduction in presynaptic $GABA_B$ receptor function that we observed could have accounted for the frequency-dependent actions of GVG on GABA-mediated inhibition. In addition to synaptically located $GABA_B$ receptors, there is increasing evidence supporting the existence of $GABA_B$ receptors of extrasynaptic origin (Pham et al., 1998). As discussed in the introduction to this thesis (refer to 2.2.3) there is evidence suggesting that GVG preferentially inhibits neuronal rather than astrocytic GABA-T. One may therefore expect higher extracellular levels of GABA in the vicinity of inhibitory synapses. As a result, synaptic $GABA_B$ receptors may be downregulated to a greater degree than their extrasynaptic counterparts. This could explain the ability of baclofen (2 μM) to cause a large reduction in the amplitude of monosynaptic IPSCs since the bath application of an exogenous

agonist will cause an activation of $GABA_B$ receptors regardless of whether they are synaptically or extrasynaptically located. However, given a preferential desensitization of synaptic $GABA_B$ receptors, a reduction in paired-pulse facilitation might have been expected to occur following GVG administration.

An explanation for the apparent discrepancy between results from extracellular and intracellular recordings cannot be proposed at the present time. Future experiments involving the use of both $GABA_B$ receptor agonists and antagonists could help strengthen the role of autoreceptor downregulation in the mechanism of action of GVG. If the downregulation of $GABA_B$ autoreceptors underlies the ability of GVG to suppress activity-dependent disinhibition, we would therefore expect that the application of either agonists or antagonists of $GABA_B$ receptors would have little effect upon either the time course of paired-pulse inhibition or the amplitude of monosynaptic IPSPs evoked by low-frequency stimulation in slices from GVG pretreated animals.

Regardless of the exact mechanisms involved, the effects which we have observed confirm the ability of GVG to affect GABA-mediated transmission. However, we found no evidence to support the conventional view whereby GVG enhances synaptic inhibition by facilitating the evoked release of transmitter. Such an action would have been expected to cause an increase in the effectiveness of the conditioning stimulus during paired stimulation, resulting in an increased inhibition of the subsequently evoked population spikes. An example of this can be seen in Fig. 1 of Section 4 of this thesis where the block of GABA uptake by tiagabine was associated with an increase of the strength of inhibition at ISIs of 40-160 ms. However, our tests revealed no such change following GVG pretreatment. Nevertheless, following the initial observation of the frequency-dependent actions of GVG, whole-cell recordings were undertaken with the specific goal of determining the mechanisms contributing to the frequency-dependence of drug action. A more detailed study of GABA-mediated transmission in slices prepared from pretreated animals involving a comparison of evoked monosynaptic IPSC amplitude and kinetics (rate of rise, time to peak and decay time constant) as well as a similar comparison of mIPSCs may have

revealed additional effects of GVG on GABA-mediated transmission. An analysis of the effects of GVG on the frequency of mIPSCs would be of particular interest since it might reveal the source of the increased extracellular GABA needed in order to produce a downregulation of the $GABA_B$ autoreceptor function. The absence of any change in mIPSC frequency following GVG pretreatment might indicate that a reversal of the activity of the GABA transporter due to an increase in the intracellular concentration of GABA may be involved in raising the extracellular levels of transmitter. Although more difficult to address, the effects of GVG pretreatment on the carrier-mediated release of GABA could be examined using an approach similar to that recently used in investigating the functional consequences of the carrier-mediated release of GABA (Gaspary et al., 1998). In this study, the carrier-mediated release of GABA (non-vesicular) could be separated from its vesicular release by pretreating cultured hippocampal neurons with tetanus toxin, a compound which inhibits synaptic vesicle exocytosis (Schiavo et al., 1992). Alternatively, slices could be maintained in aCSF devoid of $Ca^{2+}$ which is necessary for the vesicular release of transmitters. Carrier-mediated release could then be provoked by either high intensity electrical stimulation or bath application of a high concentration of $K^+$.

**4th Study:** Acute effects of γ-vinyl GABA (vigabatrin) on hippocampal GABAergic inhibition in vitro.

Although GVG was designed to selectively inhibit GABA-T it is not surprising, given the fact that GVG is a close structural analog of GABA, that this compound can produce additional effects upon the GABA neurotransmitter system. Indeed, GVG reportedly can inhibit the actions of GABA at the $GABA_A$ receptor complex (Suzuki et al., 1991) and block the reuptake of GABA into neurons and glia (Löscher, 1980b; Jolkkonen et al., 1992). The block of GABA uptake is expected to increase neuronal inhibition by prolonging the postsynaptic actions of synaptically released GABA and could thus be expected to contribute to anticonvulsant actions of GVG. In contrast, block of $GABA_A$ receptors is

expected to result in a decrease in the effectiveness of GABA-mediated synaptic inhibition, which suggests the possibility that GVG could produce proconvulsant actions.

As explained in the preceding section, the rise in brain GABA levels produced as a result of the irreversible inhibition of GABA-T by GVG is expected to occur with a time course of several hours. It is for this reason that in our initial studies of the effects of GVG on GABA-mediated inhibition we pretreated animals with GVG rather than bath applying the drug to acutely prepared hippocampal slices. However, in contrast to the delayed actions of GVG following the irreversible inhibition of GABA-T, it should be possible to observe with minimal delay the consequences of this drug's interaction with either the $GABA_A$ receptor or the GABA transporter. Indeed, the acute effects of GVG on the extracellular levels of GABA were observed within 25 min following its application via a microdialysis probe (Jolkkonen et al., 1992). Similarly, the inhibition of the GABA-stimulated uptake of $^{36}Cl^-$ was observed following a relatively short 10 min preincubation of membrane vesicles in the presence of GVG (Suzuki et al., 1991).

These results suggested to us that the functional consequences of the acute effects of GVG at either the $GABA_A$ receptor or at the GABA transporter could be separated from those occurring due to the inhibition of GABA-T in experiments in which GVG was bath applied to hippocampal slices. Therefore, using paired-pulse tests of inhibition the aim of the final study of this thesis was to determine whether changes in the effectiveness of GABA-mediated transmission, attributable to either of the proposed acute actions of GVG, could be detected following the bath application of this compound.

The main findings of this study can be summarized as follows:

1) Bath application of GVG (100-500 μM) causes a concentration-dependent decrease in the strength of inhibition elicited by both orthodromic (ortho-ortho paired-pulse test) and antidromic (antidromic-orthodromic paired-pulse test) conditioning stimulation.

2) At high concentrations (500-1000 μM), GVG caused hyperexcitability as evidenced by the appearance of multiple population spikes in response to Schaffer-collaterals stimulation.

3) Although these results are consistent with the suggestion that GVG can inhibit $GABA_A$ receptors, binding studies showed that GVG produces negligible or no effects on the binding of tritiated ligands with high affinity for the GABA, picrotoxin and benzodiazepine binding sites. These results were confirmed in a functional assay of $GABA_A$ receptor function in which GVG was unable to prevent the inhibitory effects of the $GABA_A$ receptor agonist, muscimol, on the orthodromically elicited population spike amplitude.

There is little doubt that the development of GVG was a marked improvement over previously introduced GABA-T inhibitors. As previously mentioned, these compounds acted by inactivating pyridoxal phosphate (PLP), a coenzyme required for the enzymatic activity of GABA-T. Since PLP is a required coenzyme for numerous enzymes, including decarboxylases as well as other transaminases, the actions of these drugs showed very little selectivity with respect to the neurotransmitter system upon which they acted. Although neurochemical studies have confirmed the much greater selectivity of GVG for the GABAergic system, there is now ample evidence indicating that within this neurotransmitter system GVG can produce multiple actions.

In contrast to previous suggestions, our demonstration of the disinhibitory effects of bath applied GVG suggests that the acute actions of GVG cause a pro-

rather than anticonvulsant effect. Superficially, our results seemed to support the claim that GVG may act as an antagonist at the $GABA_A$ receptor complex. The ability of GVG to inhibit the association of GABA with its binding site has previously been examined (Löscher, 1980b). In this study, GVG was shown to have a minimal effect on the binding of [$^3$H]GABA with a reported $IC_{50}$ of 4.1 mM. Clearly, such a modest interaction could not account for the disinhibitory action of GVG which could be observed at concentrations of as low as 100 μM. Our own binding studies confirmed the inability of GVG to influence the binding of GABA and demonstrated, for the first time, a similar lack of effect on the binding of tritiated ligands for both the benzodiazepines and picrotoxin binding sites. Further supported by the inability of GVG to reduce the inhibitory actions of muscimol on the amplitude of orthodromically evoked population spikes, these results indicate that the disinhibitory actions of GVG are not mediated through an action at the $GABA_A$ receptor complex.

Although no direct evidence was presented, we proposed that GVG may act to reduce the effectiveness of GABA-mediated inhibition by acting as a false transmitter. For such a mechanism to occur it is clear that GVG must be taken up first into inhibitory terminals and then into synaptic vesicles. Once present within synaptic vesicles GVG could then be released in a $Ca^{2+}$-dependent manner following nerve terminal depolarization. There is little doubt that GVG serves as a substrate for the plasma membrane GABA transporter and can thus be taken up into inhibitory terminals (Schousboe et al., 1986). Previous results have in addition demonstrated the ability of GVG to inhibit the transport of GABA into synaptic vesicles (Christensen et al., 1991). Furthermore, in PC12 cells transfected with VGAT, a recently cloned vesicular GABA transporter, GVG was shown to inhibit the transport of [$^3$H]GABA as potently as unlabelled GABA (McIntire et al., 1997). Although these studies cannot confirm whether GVG serves as a substrate for VGAT, they demonstrate that, if not by acting as a false transmitter, GVG may nevertheless cause disinhibition by reducing the vesicular content of GABA.

In addition to the false transmitter hypothesis we also speculated that the GABA-releasing properties of GVG could promote disinhibition by causing either a desensitization of postsynaptic $GABA_A$ receptors or a reduction in transmitter release due to an increased activation of presynaptic $GABA_B$ receptors. However, in this study we demonstrated that GVG did not reduce the inhibitory effects of muscimol on the PS amplitude, indicating that $GABA_A$ receptor function was largely preserved. Thus, in retrospect, desensitization would not appear to be a plausible mechanism of GVG-induced disinhibition. Another possibility, which was not previously considered, is that the GABA releasing actions of GVG cause a depletion of transmitter stores. Disinhibition through such a mechanism appears unlikely since it would be partially offset by the increasing inhibition of GABA-T over time. Furthermore, the depletion of a cytosolic pool of GABA would not be expected to greatly influence inhibition evoked by a single conditioning stimulus since the resulting inhibitory response is more likely to depend on the $Ca^{2+}$-dependent release of GABA from its vesicular stores. The role of the most plausible of these additional mechanisms, namely the increased activation of presynaptic $GABA_B$ receptors, could be determined in future experiments by evaluating the effects of $GABA_B$ receptor antagonists on the acute actions of bath applied GVG.

It is interesting to note that the previously reported $IC_{50}$ (110 μM) value for the inhibitory effect of GVG on GABA uptake (Löscher, 1980b) suggests that the interaction of this compound with the GABA transporter occurs over a range of concentrations similar to that with which we observed disinhibition (100-500 μM). In addition, the GABA releasing action of GVG, which occurs due to the ability of GVG to act as substrate for the GABA-transporter, also occurs over a similar range of concentrations (250-5000 μM)(Abdul-Ghani et al., 1981). These results would seems to suggest that the acute actions of GVG are most likely attributable to the interaction of this compound with the GABA transporter rather than with the $GABA_A$ receptor complex. However, based on our findings, and contrary to previous suggestions, this additional property of GVG is expected to promote rather than inhibit the generation of seizures.

The GABA-releasing properties can be postulated to have contributed to the results obtained from the previously mentioned studies of the acute effects of GVG. Previous determinations of the effects of high-affinity GABA uptake blockers on the extracellular overflow of GABA have either failed to detect any change (Pittaluga et al., 1987) or have reported a 4.5-fold maximal increase (Fink-Jensen et al., 1992; Inglefield et al., 1995). In contrast, GVG was reported to cause an approximate 10-fold increase in the basal concentration of GABA (Jolkkonen et al., 1992). Since GVG is a relatively poor GABA uptake blocker, it seems possible that its ability to cause a larger increase in the resting extracellular levels of GABA was partly due to its GABA-releasing effects. In keeping with our own results, it is interesting that in this study the $K^+$-stimulated release of GABA was decreased by 33% in the presence of GVG.

The results suggesting that GVG can act as an antagonist of the $GABA_A$ receptor (Suzuki et al., 1991) could also be explained if we consider how the GABA-releasing properties of GVG may have influenced the results obtained. In this study, $GABA_A$ receptor function was evaluated in membrane vesicles by measuring the intracellular accumulation of $^{36}Cl^-$ following the GABA-simulated activation of receptors. The maximal extent of the stimulated intracellular accumulation of $^{36}Cl^-$ through the $GABA_A$ receptor gated ionophore will clearly depend on the maintenance within these vesicles of a transmembrane gradient allowing for the inward movement of $Cl^-$. The key to the proposed involvement of the GABA-releasing properties of GVG in causing a reduction of $^{36}Cl^-$ uptake resides in the fact that no effect was observed unless membrane vesicles were preincubated in the presence of GVG (100-500 μM). It is therefore possible that during the preincubation period GVG stimulated the release of GABA from membrane vesicles which then activated the $GABA_A$ receptors resulting in a partial collapse of the transmembrane $Cl^-$ gradient. Under these conditions, influx will consequently be reduced during the subsequent coapplication of $^{36}Cl^-$ and GABA.

Although our results suggest that the acute actions of GVG are proconvulsant, it is unlikely that a sufficiently high drug concentration will be

reached in the CNS during chronic treatment in order to elicit the disinhibitory actions of GVG. The disinhibitory effects associated with high concentrations of GVG may nevertheless explain the observation that, contrary to most others anticonvulsants, a reduction in the anticonvulsant efficacy of GVG occurs following the administration of higher drug doses (McKee et al., 1993; Harden, 1994; Ben-Menachem, 1995b). Finally, with respect to our previous study it would appear that the GABA-releasing actions of GVG were likely to have been elicited by our pretreatment of rats with GVG at a dose of 1500 mg/kg. Indeed, the GABA-releasing actions of GVG have been reported *in vivo* (Abdul-Ghani et al., 1980) following the i.p. injection of GVG at a dose (1500 mg/kg) identical to that which we used. However, given the short elimination half-life of GVG (~4 hrs) it is unlikely that this action contributed significantly to our results which were obtained from slices prepared 24 hour after treatment. Nevertheless, as previously discussed, the GABA-releasing properties of GVG could have contributed to the elevated extracellular levels of GABA which likely caused the reduced $GABA_B$ autoreceptor function.

**Implications**

Epileptic seizures can be simplistically viewed as being the result of either an excess of excitatory or a deficiency of inhibitory neurotransmission. Based on the recognized importance of GABA-mediated synaptic inhibition in the control of neuronal excitability and the ease with which seizures can be induced following the administration of pharmacological agents which reduce the effectiveness of GABAergic transmission, the "GABA hypothesis" of epilepsy was proposed. In support of this hypothesis, studies of brain tissue removed during the neurosurgical resection of epileptogenic areas have provided evidence which suggests that in as many as 60-70% of epileptic patients deficits in GABAergic transmission consisting predominantly of a reduction in the synthetic activity of GAD and/or of a reduction of $GABA_A$ receptor binding sites can be identified (for review see Lloyd et al., 1986). Similarly, reductions in inhibitory function have

been reported in animal models of seizures and epilepsy (Kapur et al., 1989; Kapur et al., 1994; Luhmann et al., 1995). Still, other studies have either failed to reveal any abnormality in GABAergic transmission or have paradoxically observed an increase in its function (Kostopoulos and Antoniadis, 1991; Troyer et al., 1992; Prince et al., 1997) suggesting that the GABA hypothesis cannot fully account for all forms of epilepsy.

Nevertheless, regardless of the underlying pathological mechanism which is responsible for the maintenance of the epileptic state, it is clear that pharmacological interventions which produce an enhancement of GABA-mediated inhibition can effectively limit the ability of neuronal networks to support epileptiform activity. However, in the past, therapies aimed at facilitating synaptic inhibition have been associated with numerous problems. As a result, the clinical use of drugs such as the benzodiazepines and barbiturates has become severely restricted due to the pronounced development of tolerance and physical dependence which can be associated with the prolonged use of these drugs.

While the anticonvulsant actions of the barbiturates were fortuitously discovered in psychiatric patients, those of the benzodiazepines were revealed serendipitously during the screening of compounds in animal seizure models. Even though drug screening and structural variations of known anticonvulsant drugs remains one of the predominant strategies through which many novel compounds are being developed (Löscher and Schmidt, 1994b), a more rational approach based on a greater understanding of the mechanisms involved in the initiation and propagation of epileptiform activity has been attempted in recent years (Porter and Rogawski, 1992). Although the design of drugs aimed at reducing the level of excitability in CNS through a decrease in glutamatergic neurotransmission has so far proven to be disappointing from a therapeutic standpoint, the development of compounds through a strategy based on the GABA hypothesis has proven to be more fruitful. Developed in such a manner, tiagabine, a potent inhibitor of the uptake of GABA, and GVG, an irreversible inhibitor of the breakdown of GABA, are two novel anticonvulsant drugs recently introduced into clinical practice which are effective in the treatment of patients

suffering from previously drug resistant epilepsy. In contrast to the benzodiazepines and barbiturates, the chronic use of these novel GABA enhancing agents is generally better tolerated and their effectiveness is well maintained over prolonged periods. Important differences in the manner in which these drugs interact with the GABA transmitter system can be postulated to partially account for the differences in the degree to which the various GABA enhancing anticonvulsants are tolerated.

Compared with an indiscriminate sustained activation of the GABA receptors produced by a direct agonist action, an allosteric enhancement of $GABA_A$ receptor function, such as that produced by the benzodiazepines and barbiturates, is clearly a more desirable mechanism of action for an anticonvulsant drug since the consequences of the resulting augmented inhibition should predominantly be felt during the activation of the postsynaptic receptors following the synaptic release of GABA. Thus, the temporal and spatial characteristics of GABA-mediated synaptic inhibition are expected to be preserved under these conditions. However, drugs acting through such a mechanism are not expected to selectively act upon the neurons involved in the generation and/or propagation of epileptiform activity. As a result, the effectiveness of $GABA_A$ receptor-mediated synaptic inhibition will be potentiated throughout the CNS. This may partially explain the relatively poor therapeutic profile of existing benzodiazepines and barbiturates.

Despite being mechanistically preferable to a direct agonist action, the allosteric enhancement of $GABA_A$ receptor function produced by the benzodiazepines and barbiturates can nevertheless be expected to cause a qualitatively similar (i.e. irrespective of synaptic activity) indiscriminate increase in the resting inhibitory tone. Such an effect could occur as a result of the enhancement, by each of these compounds, of the current generated through the activation of $GABA_A$ receptors by ambient transmitter levels present in the extracellular space. The barbiturates may, in addition, increase tonic inhibition as a result of their ability to mimic GABA and directly cause $GABA_A$ receptor channel openings. In contrast, due to the mechanisms by which they are

believed to produce their anticonvulsant actions, both GVG, which elevates the presynaptic stores of GABA following the inhibition of GABA-T, and tiagabine, which reduces the transporter-mediated reuptake of GABA from the synaptic cleft, will selectively enhance the postsynaptic activation of GABA receptors by transiently increasing the synaptic concentration of neurotransmitter, above that which would normally be achieved in the absence of these drugs. The facilitation of GABA-mediated inhibition by these drugs should therefore be truly contingent on synaptic activity. Nevertheless, based on the widespread CNS distribution of both the GABA transporter and GABA-T, neither of these drugs can be expected to selectively act upon the synchronously discharging neurons involved in the generation of seizure activity. In support of this, the *in vivo* binding of [$H^3$]tiagabine has been shown to be widely distributed throughout the CNS (Suzdak et al., 1992b; Suzdak et al., 1994). Similarly, elevated GABA levels have been reported following the administration of GVG in several brain regions (Löscher and Frey, 1987; Löscher and Hörstermann, 1994a). Therefore, the resulting increase in the effectiveness of synaptic inhibition produced by these two compounds could interfere not only with neuronal populations involved in epileptiform activity but with those involved in normal CNS functions as well. Thus, based solely on a consideration of their targets and mechanisms of action, the more favorable therapeutic profile of these novel GABA-enhancing drugs remains unclear.

Due to the necessity in epileptic patients of maintaining anticonvulsant therapy for prolonged periods, an ideal anticonvulsant should completely suppress seizure activity while having no effect on the ability of neurons to carry out their normal physiological functions. Established and novel anticonvulsant drugs produce their therapeutic actions by acting upon excitatory and inhibitory neurotransmitter systems, as well as on voltage-dependent ion channels. The difficulty in achieving such a therapeutic ideal is therefore due to the involvement of the targets of anticonvulsant drugs in normal synaptic transmission. How can an anticonvulsant drug produce a selective inhibition of seizure discharges while preserving normal neuronal activities? One possibility can be proposed based

on a consideration of the most striking difference between populations of neurons involved in a seizure and those serving a normal physiological function, namely their level of activity. Indeed, during a seizure the firing patterns in numerous regions of the CNS become synchronized such that large populations of neurons experience prolonged depolarization upon which high-frequency bursts of action potentials are superimposed (Matsumoto and Ajmone Marsan, 1964a; Matsumoto and Ajmone Marsan, 1964b). Consequently, an anticonvulsant can be expected to produce a relatively more selective action if it specifically targets the rapidly discharging neurons participating in the seizure activity. Such an effect can be achieved if the actions of a drug are use- or frequency-dependent, that is, contingent on or increasing sharply with the level of neuronal activity.

The anticonvulsants phenytoin and carbamazepine, both of which produce a voltage-dependent block of voltage-gated $Na^+$ channels, are classic examples of anticonvulsant drugs which possess such a property. These compounds are considered drugs of choice in the treatment of partial epilepsy (Mattson et al., 1985) due to the protection against seizures which they afford and the lack of sedative side-effects associated with their use. The favorable therapeutic profile of these drugs has generally been attributed to their ability to selectively inhibit the high-frequency firing of action potentials while producing little or no effect on the firing of single action potentials or those elicited at low frequencies. Such frequency-dependent action occurs because openings of the gates during the action potential provide ready access of the drug to the binding site within the channels and because the channels in the active and inactivated state have much higher affinity for the drug than the channels at rest. Thus, both of these anticonvulsants, as well as others possessing a similar mechanism of action, cause the $Na^+$ channels to accumulate in an inactivated state following their transient activation during membrane depolarization. Over time, as the channels recover from inactivation, the blocking actions of phenytoin and carbamazepine are relieved and action potentials can again be generated despite the continued presence of the drug.

Although use-dependence is well recognized as being an important property of $Na^+$ channel blockers, its contribution to the actions of drugs acting upon the GABAergic neurotransmitter system to produce enhanced synaptic inhibition has not previously been considered. In the past, the effects of anticonvulsant drugs proposed to act through a GABAergic mechanism have solely been investigated on postsynaptic inhibitory responses generated in response to GABA released either spontaneously (spontaneous and miniature IPSP/Cs) or following the single as well as paired stimulation of inhibitory interneurons. While these studies allowed the identification of compounds capable of facilitating GABAergic transmission their experimental design did not permit a characterization of the effects of these drugs under stimulation conditions which more accurately resemble the pattern of interneuronal activation expected to occur during seizure activity. Thus, our results suggest that additional properties which may importantly contribute to the anticonvulsant actions of GABA enhancing anticonvulsant drugs were overlooked in these previous studies.

## Contribution to original knowledge

## 8. Contribution to original knowledge

1) High-frequency conditioning stimulation (HFS), previously demonstrated to evoke a strong inhibitory response from hippocampal recurrent pathways, can evoke large depolarizing responses capable of triggering action potential firing. These depolarizing responses occurred despite the presence of the excitatory amino acid antagonists, CNQX and CPP, and could be blocked by the $GABA_A$ receptor antagonist, bicuculline methiodide, suggesting that they are $GABA_A$ receptor-mediated.

2) The GABA uptake blocker, tiagabine, can facilitate the monosynaptic GABA-mediated depolarizing responses (DRs) evoked by HFS of inhibitory interneurons.

3) Differences in the functional consequences of tiagabine-augmented GABA-mediated depolarizing responses were demonstrated depending on the hippocampal region from which they are evoked: DRs evoked from the stratum radiatum were able to fire action potentials whereas those evoked from the alveus were not, despite being of the same magnitude.

4) Tiagabine was demonstrated to cause a greater increase in the effectiveness of synaptic inhibition elicited by HFS than on the inhibition evoked by a single stimulus.

5) The functional consequences of tiagabine-augmented depolarizing responses are largely inhibitory due to the associated large $GABA_A$ receptor-mediated conductance increase which greatly reduces the depolarizing influence of excitatory transmission through a shunting mechanism.

6) The irreversible blocker of GABA transaminase, γ-vinyl GABA (GVG, vigabatrin) was demonstrated to cause a frequency-dependent enhancement

of GABA-mediated inhibition due to its ability to prevent or reverse the activity-dependent depression of inhibition normally associated with low-frequency (2.5-5 Hz) repetitive stimulation.

7) The frequency-dependent actions of GVG could be attributed in part to a lower efficacy of the negative feedback mechanism regulating GABA release from interneurons via presynaptic $GABA_B$ autoreceptors.

8) The acute actions of GVG at the GABA neurotransmitter system are associated with a concentration-dependent disinhibitory effect.

9) I have shown that GVG does not interact with either the benzodiazepine- or convulsant-binding sites of the $GABA_A$ receptor complex.

# References

## 9. References


Abdul-Ghani, A., Norris, P. H., Smith, C. C. T., and Bradford, H. F. (1981) Effect of γ-acetylenic GABA and γ-vinyl GABA on synaptosomal release and uptake of GABA. *Biochem. Pharmacol.* 30: 1203-1209.

Abdul-Ghani, A. S., Coutinho-Netto, J., and Bradford, H. F. (1980) The action of gamma-vinyl-GABA and gamma-acetylenic-GABA on the resting and stimulated release of GABA in vivo. *Brain Res.* 191: 471-481.

Abe, M. and Matsuda, M. (1983) On the existence of two GABA pools associated with newly synthesized GABA and with newly taken up GABA in nerve terminals. *Neurochem. Res.* 8: 563-573.

Aicardi, J., Mumford, J. P., Dumas, C., and Wood, S. (1996) Vigabatrin as initial therapy for infantile spasms: a European retrospective survey. Sabril IS Investigator and Peer Review Groups. *Epilepsia* 37: 638-642.

Aizawa, M., Ito, Y., and Fukuda, H. (1997) Pharmacological profiles of generalized absence seizures in lethargic, stargazer and gamma-hydroxybutyrate-treated model mice. *Neurosci. Res.* 29: 17-25.

Akaike, N., Inomata, N., and Yakushiji, T. (1989) Differential effects of extra- and intracellular anions on GABA-activated currents in bullfrog sensory neurons. *J. Neurophysiol.* 62: 1388-1398.

Alger, B. E. Hippocampus. Electrophysiological studies of epileptiform activity in vitro. In: *Brain Slices*, edited by R. Dingledine. New York: Plenum Press, 1984, p. 155-199.

Alger, B. E. and Nicoll, R. A. (1980) Epileptiform burst afterhyperpolarization: calcium-dependent potassium potential in hippocampal CA1 pyramidal cells. *Science* 210: 1122-1124.

Alger, B. E. and Nicoll, R. A. (1982a) Feed-forward dendritic inhibition in rat hippocampal pyramidal cells studied in vitro. *J. Physiol.* 328: 105-123.

Alger, B. E. and Nicoll, R. A. (1982b) Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied in vitro. *J. Physiol.* 328: 125-141.

Ali, F. E., Bondinell, W. E., Dandridge, P. A., Frazee, J. S., Garvey, E., Girard, G. R., Kaiser, C., Ku, T. W., Lafferty, J. J., and Moonsammy, G. I. (1985) Orally active and potent inhibitors of gamma-aminobutyric acid uptake. *J. Med. Chem.* 28: 653-660.

Alonso-DeFlorida, F. and Delgado, J. M. R. (1958) Lasting behavioral and EEG changes in cats induced by prolonged stimulation of the amygdala. *Am. J. Physiol.* 193: 223-229.

Andersen, K. E., Braestrup, C., Gronwald, F. C., Jorgensen, A. S., Nielsen, E. B., Sonnewald, U., Sorensen, P. O., Suzdak, P. D., and Knutsen, L. J. (1993) The synthesis of novel GABA uptake inhibitors. 1. Elucidation of the structure-activity studies leading to the choice of (R)-1-[4, 4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid (tiagabine) as an anticonvulsant drug candidate. *J. Med. Chem.* 36: 1716-1725.

Andersen, P., Dingledine, R., Gjerstad, L., Langmoen, I. A., and Mosfeldt Laursen, A. (1980) Two different responses of hippocampal pyramidal cells to application of gamma-amino butyric acid. *J. Physiol.* 305: 279-296.

Andrade, R., Malenka, R. C., and Nicoll, R. A. (1986) A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. *Science* 234: 1261-1265.

Arzimanoglou, A. A., Dumas, C., and Ghirardi, L. (1997) Multicentre clinical evaluation of vigabatrin (Sabril) in mild to moderate partial epilepsies. French Neurologists Sabril Study Group. *Seizure.* 6: 225-231.

Asada, H., Kawamura, Y., Maruyama, K., Kume, H., Ding, R., Ji, F. Y., Kanbara, N., Kuzume, H., Sanbo, M., Yagi, T., and Obata, K. (1996) Mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65) maintain normal levels of GAD67 and GABA in their brains but are susceptible to seizures. *Biochem. Biophys. Res. Commun.* 229: 891-895.

Ashwood, T. J. and Wheal, H. V. (1987) The expression of N-methyl-D-aspartate-receptor-mediated component during epileptiform synaptic activity in hippocampus. *Br. J. Pharmacol.* 91: 815-822.

Attwell, D., Barbour, B., and Szatkowski, M. (1993) Nonvesicular release of neurotransmitter. *Neuron* 11: 401-407.

Avoli, M., Psarropoulou, C., Tancredi, V., and Fueta, Y. (1993) On the synchronous activity induced by 4-aminopyridine in the CA3 subfield of juvenile rat hippocampus. *J. Neurophysiol.* 70: 1018-1029.

Avoli, M., Barbarosie, M., Lücke, A., Nagao, T., Lopantsev, V., and Köhling, R. (1996) Synchronous GABA-mediated potentials and epileptiform discharges in the rat limbic system *in vitro*. *J. Neurosci.* 16: 3912-3924.

Ayala, G. F., Matsumoto, H., and Gumnit, R. J. (1970) Excitability changes and inhibitory mechanisms in neocortical neurons during seizures. *J. Neurophysiol.* 33: 73-85.

Ayala, G. F., Dichter, M., Gumnit, R. J., Matsumoto, H., and Spencer, W. A. (1973) Genesis of epileptic interictal spikes: new knowledge of cortical feedback systems suggests a neurophysiological explanation of brief paroxysms. *Brain Res.* 52: 1-17.

Barker, J. L., McBurney, R. N., and MacDonald, J. F. (1982) Fluctuation analysis of neutral amino acid responses in cultured mouse spinal neurones. *J. Physiol. (Lond)* 322: 365-387.

Barker, J. L. and Mathers, D. A. (1981) GABA analogues activate channels of different duration on cultured mouse spinal neurons. *Science* 212: 358-361.

Barker, J. L. and McBurney, R. N. (1979) Phenobarbitone modulation of postsynaptic GABA receptor function on cultured mammalian neurons. *Proc. R. Soc. Lond. B. Biol. Sci.* 206: 319-327.

Bateson, A. N., Lasham, A., and Darlison, M. G. (1991) γ-Aminobutyric acid A receptor heterogeneity is increased by alternative splicing of a novel beta-subunit gene transcript. *J. Neurochem.* 56: 1437-1440.

Bauer, G. (1994) Seizure types and epileptic syndromes in adults. *Eur. Neurol.* 34 Suppl. 1: 13-17.

Baxter, C. F. and Roberts, E. (1961) Elevation of γ-aminobutyric acid in brain: selective inhibition of γ-aminobutyric acid-α-ketoglutaric acid transminase. *J. Biol. Chem.* 236: 3287-3294.

Ben-Ari, Y., Krnjević, K., and Reinhardt, W. (1979) Hippocampal seizures and failure of inhibition. *Can. J. Physiol. Pharmacol.* 57: 1462-1466.

Ben-Ari, Y., Krnjević, K., and Reinhardt, W. (1980) Lability of synaptic inhibition of hippocampal pyramidal cells. *J. Physiol.* 298: 36P-37P.

Ben-Ari, Y., Cherubini, E., Corradetti, R., and Gaiarsa, J.-L. (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol.* 416: 303-325.

Ben-Menachem, E. (1989) Pharmacokinetic effects of vigabatrin on cerebrospinal fluid amino acids in humans. *Epilepsia* 30(S3): S12-S14.

Ben-Menachem, E., Persson, L., and Mumford, J. (1990) Long-term evaluation of once daily vigabatrin in drug-resistant partial epilepsy. *Epilepsy Res.* 5: 240-246.

Ben-Menachem, E., Persson, L. I., Mumford, J., Haegele, K. D., and Huebert, N. (1991) Effect of long-term vigabatrin therapy on selected neurotransmitter concentrations in cerebrospinal fluid. *J. Child Neurol.* Suppl 2: S11-S16.

Ben-Menachem, E. (1995a) International experience with tiagabine add-on therapy. *Epilepsia* 36: S14-S21.

Ben-Menachem, E. (1995b) Vigabatrin. *Epilepsia* 36 Suppl 2: S95-S104.

Bernasconi, R., Klein, M., Martin, P., Christen, P., Hafner, T., Portet, C., and Schmutz, M. (1988) γ-Vinyl GABA: comparison of neurochemical and anticonvulsant effects in mice. *J. Neural Transm.* 72: 213-233.

Bernath, S. (1992) Calcium-independent release of amino acid neurotransmitters: Fact or artifact. *Prog. Neurobiol.* 38: 57-91.

Bernath, S. and Zigmond, M. J. (1988) Characterization of [3H]GABA release from striatal slices: evidence for a calcium-independent process via the GABA uptake system. *Neuroscience* 27: 563-570.

Biton, V., Boellner, S., Mercante, D., Phillips, H., Alto, G., and Sommerville, K. (1996) Monotherapy with tiagabine in an open-label study of partial seizures. *Epilepsia* 37: 40

Bjorge, S., Black, A., Bockbrader, H., Chang, T., Gregor, V. E., Lobbestael, S. J., Nugiel, D., Pavia, M. R., Radulovic, L., and Woolf, T. (1990) Synthesis and metabolic profile of CI-966: a potent, orally-active inhibitor of GABA uptake. *Drug Dev. Res.* 21: 189-193.

Blanton, M. G., Lo Turco, J. J., and Kriegstein, A. R. (1989) Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *J. Neurosci. Methods* 30: 203-210.

Bloom, F. E. and Iversen, L. L. (1971) Localizing $^{3}$H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. *Nature* 229: 628-630.

Blum, B. and Liban, E. (1960) Experimental baso-temporal epilepsy in the cat. Discrete epileptogenic lesions produced in the hippocampus or amygdaloid nucleus by tungstic acid. *Neurology* 10: 546-554.

Bon, C. and Galvan, M. (1996) Electrophysiological actions of GABAB agonists and antagonists in rat dorso-lateral septal neurones in vitro. *Br. J. Pharmacol.* 118: 961-967.

Borden, L. A., Smith, K. E., Hartig, P. R., Branchek, T. A., and Weinshank, R. L. (1992) Molecular heterogeneity of the Y-aminobutyric acid (GABA) transport system. Cloning of two novel high affinity GABA transporters from rat brain. *J. Biol. Chem.* 267: 21098-21104.

Borden, L. A., Dhar, T. G. M., Smith, K. E., Weinshank, R. L., Branchek, T. A., and Gluchowski, C. (1994) Tiagabine, SK&F 89976-A, CI-966, and NNC-711 are selective for the cloned GABA transporter GAT-1. *Eur. J. Pharmacol. Mol. Pharmacol.* 269: 219-224.

Borden, L. A., Smith, K. E., Gustafson, E. L., Branchek, T. A., and Weinshank, R. L. (1995) Cloning and expression of a betaine/GABA transporter from human brain. *J. Neurochem.* 64: 977-984.

Borden, L. A. (1996) GABA transporter heterogeneity: Pharmacology and cellular localization. *Neurochem. Int.* 29: 335-356.

Bormann, J., Hamill, O. P., and Sakmann, B. (1987) Mechanisms of anion permeation through channels gated by glycine and gamma-aminobutyric acid in mouse cultured spinal neurons. *J. Physiol. (Lond. )* 385: 243-286.

Bormann, J. and Clapham, D. E. (1985) gamma-Aminobutyric acid receptor channels in adrenal chromaffin cells: a patch-clamp study. *Proc. Natl. Acad. Sci. U. S. A.* 82: 2168-2172.

Braestrup, C., Nielsen, E. B., Sonnewald, U., Knutsen, L. J. S., Andersen, K. E., Jansen, J. A., Frederiksen, K., Andersen, P. H., Mortensen, A., and Suzdak, P. D. (1990) (R)-N-[4,4-bis(3-methyl-2-thienyl)but-3-en-1-yl]nipecotic acid binds with high affinity to the brain γ-aminobutyric acid uptake carrier. *J. Neurochem.* 54: 639-647.

Brodie, M. J. and Dichter, M. A. (1996) Drug therapy - Antiepileptic drugs. *N. Engl. J. Med.* 334: 168-175.

Browne, T. R., Mattson, R. H., Penry, J. K., Smith, D. B., Treiman, D. M., Wilder, B. J., Ben-Menachem, E., Napoliello, M. J., Sherry, K. M., and Szabo, G. K.

(1987) Vigabatrin for refractory complex partial seizures: multicenter single- blind study with long-term follow-up. *Neurology* 37: 184-189.

Browne, T. R., Mattson, R. H., Penry, J. K., Smith, D. B., Treiman, D. M., Wilder, B. J., Ben-Menachem, E., McBride, R. G., and Sherry, K. M. (1991) Multicenter long-term safety and efficacy study of vigabatrin for refractory complex partial seizures: An update. *Neurology* 41: 363-364.

Buhl, E. H., Halasy, K., and Somogyi, P. (1994) Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature* 368: 823-828.

Buhl, E. H., Otis, T. S., and Mody, I. (1996) Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. *Science* 271: 369-373.

Busik, J., Nakamura, M., Abe, Y., Shibuya, I., and Kanno, T. (1996) Effects of GABA on spontaneous [Ca2+]c dynamics and electrical properties of rat adrenal chromaffin cells. *Brain Res.* 739: 97-103.

Buu, N. T. and van Gelder, N. M. (1974) Differences in biochemical properties of gamma-aminobutyric acid aminotransferase from synaptosome-enriched and cytoplasmic mitochondria- enriched subcellular fractions of mouse brain. *Can. J. Physiol. Pharmacol.* 52: 674-680.

Cain, C. R. and Simmonds, M. A. (1982) Effects of baclofen on the olfactory cortex slice preparation. *Neuropharmacology* 21: 371-373.

Cammack, J. N., Rakhilin, S. V., and Schwartz, E. A. (1994) A GABA transporter operates asymmetrically and with variable stoichiometry. *Neuron* 13: 949-960.

Čapek, R. Multiple targets of antiepileptic drugs at GABAergic synapses. In: *Molecular and Cellular Targets for Antiepileptic Drugs*, edited by G. Avanzini, G. Regesta, P. Tanganelli and M. Avoli. London: John Libbey & Co, 1997, p. 163-182.

Čapek, R. and Esplin, B. (1991) Attenuation of hippocampal inhibition by a NMDA (*N*-methyl-D-aspartate) receptor antagonist. *Neurosci. Lett.* 129: 145-148.

Čapek, R. and Esplin, B. (1993) Frequency-dependent enhancement of hippocampal recurrent inhibition by GABA uptake blockers. *Epilepsy Res.* 16: 123-130.

Cash, C., Maître, M., Ciesielski, L., and Mandel, P. (1974) Purification and partial characterisation of 4-aminobutyrate 2-ketoglutarate transaminase from human brain. *FEBS Lett.* 47: 199-203.

Chang, Y., Wang, R., Barot, S., and Weiss, D. S. (1996) Stoichiometry of a recombinant $GABA_A$ receptor. *J. Neurosci.* 16: 5415-5424.

Chen, G., Trombley, P. Q., and Van den Pol, A. N. (1996) Excitatory actions of GABA in developing rat hypothalamic neurones. *J. Physiol. (Lond)* 494: 451-464.

Chen, G. and Van den Pol, A. N. (1998) Presynaptic $GABA_B$ autoreceptor modulation of P/Q-type calcium channels and GABA release in rat suprachiasmatic nucleus neurons. *J. Neurosci.* 18: 1913-1922.

Chen, J. C. T. and Chesler, M. (1992) Modulation of extracellular pH by glutamate and GABA in rat hippocampal slices. *J. Neurophysiol.* 67: 29-36.

Cherubini, E., Rovira, C., Gaiarsa, J. L., Corradetti, R., and Ben Ari, Y. (1990) GABA mediated excitation in immature rat CA3 hippocampal neurons. *Int. J. Dev. Neurosci.* 8: 481-490.

Chiron, C., Dulac, O., Beaumont, D., Palacios, L., Pajot, N., and Mumford, J. (1991) Therapeutic trial of vigabatrin in refractory infantile spasms. *J. Child Neurol.* Suppl 2: S52-S59.

Chiron, C., Dumas, C., Jambaque, I., Mumford, J., and Dulac, O. (1997) Randomized trial comparing vigabatrin and hydrocortisone in infantile spasms due to tuberous sclerosis. *Epilepsy Res.* 26: 389-395.

Christensen, H., Fykse, E. M., and Fonnum, F. (1991) Inhibition of gamma-aminobutyrate and glycine uptake into synaptic vesicles. *Eur. J. Pharmacol.* 207: 73-79.

Clark, J. A., Deutch, A. Y., Gallipoli, P. Z., and Amara, S. G. (1992) Functional expression and CNS distribution of a beta-alanine-sensitive neuronal GABA transporter. *Neuron* 9: 337-348.

Clark, J. A. (1997) Analysis of the transmembrane topology and membrane assembly of the GAT- 1 gamma-aminobutyric acid transporter. *J. Biol. Chem.* 272: 14695-14704.

Clark, J. A. and Amara, S. G. (1994) Stable expression of a neuronal gamma-aminobutyric acid transporter, GAT-3, in mammalian cells demonstrates unique pharmacological properties and ion dependence. *Mol. Pharmacol.* 46: 550-557.

Cocito, L., Maffini, M., Perfumo, P., Roncallo, F., and Loeb, C. (1989) Vigabatrin in complex partial seizures: a long-term study. *Epilepsy Res.* 3: 160-166.

Cocito, L., Maffini, M., and Loeb, C. (1993) Vigabatrin in chronic epilepsy: a 7-year follow-up study of responder patients. *Seizure.* 2: 301-307.

Colmers, W. F. and Williams, J. T. (1988) Pertussis toxin pretreatment discriminates between pre- and postsynaptic actions of baclofen in rat dorsal raphe nucleus in vitro. *Neurosci. Lett.* 93: 300-306.

Commission on Classification and Terminology of the ILAE (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. *Epilepsia* 22: 489-501.

Commission on Classification and Terminology of the ILAE (1989) Proposal for revised classification of epilepsies and epileptic syndromes. *Epilepsia* 30: 389-399.

Connors, B. W., Gutnick, M. J., and Prince, D. A. (1982) Electrophysiological properties of neocortical neurons *in vitro*. *J. Neurophysiol.* 48: 1302-1320.

Crawford, M. L. and Young, J. M. (1988) $GABA_B$ receptor-mediated inhibition of histamine H1-receptor-induced inositol phosphate formation in slices of rat cerebral cortex. *J. Neurochem.* 51: 1441-1447.

Croucher, M. J., Meldrum, B. S., and Krogsgaard-Larsen, P. (1983) Anticonvulsant activity of GABA uptake inhibitors and their prodrugs following central or systemic administration. *Eur. J. Pharmacol.* 89: 217-228.

Cull-Candy, S. G. and Usowicz, M. M. (1989) Whole-cell current noise produced by excitatory and inhibitory amino acids in large cerebellar neurones of the rat. *J. Physiol. (Lond)* 415: 533-553.

Curtis, D. R., Game, C. J. A., and Lodge, D. (1976) The in vivo inactivation of GABA and other inhibitory amino acids in the cat nervous system. *Exp. Brain Res.* 25: 413-428.

Curtis, D. R., Gynther, B. D., Beattie, D. T., Kerr, D. I. B., and Prager, R. H. (1988) Baclofen antagonism by 2-hydroxy-saclofen in the cat spinal cord. *Neurosci. Lett.* 92: 97-101.

Curtis, D. R. and Eccles, J. C. (1960) Synaptic action during and after repetitive stimulation. *J. Physiol. (Lond)* 150: 374-398.

Cutting, G. R., Lu, L., O'Hara, B. F., Kasch, L. M., Montrose-Rafizadeh, C., Donovan, D. M., Shimada, S., Antonarakis, S. E., Guggino, W. B., and Uhl, G. R. (1991) Cloning of the gamma-aminobutyric acid (GABA) rho 1 cDNA: a GABA receptor subunit highly expressed in the retina. *Proc. Natl. Acad. Sci. U. S. A.* 88: 2673-2677.

Dalby, N. O. and Nielsen, E. B. (1997) Tiagabine exerts an anti-epileptogenic effect in amygdala kindling epileptogenesis in the rat. *Neurosci. Lett.* 229: 135-137.

Dam, M. (1989) Long-term evaluation of vigabatrin (gamma vinyl GABA) in epilepsy. *Epilepsia* 30(S3): S26-S30.

Davenport, J., Schwindt, P. C., and Crill, W. E. (1978) Presynaptic and long-lasting postsynaptic inhibition during penicillin- induced spinal seizures. *Neurology* 28: 592-597.

Davenport, J., Schwindt, P. C., and Crill, W. E. (1979) Epileptogenic doses of penicillin do not reduce a monosynaptic GABA- mediated postsynaptic inhibition in the intact anesthetized cat. *Exp. Neurol.* 65: 552-572.

Davies, C. H., Davies, S. N., and Collingridge, G. L. (1990) Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. *J. Physiol.* 424: 513-531.

Davies, C. H., Pozza, M. F., and Collingridge, G. L. (1993a) CGP 55845A: A potent antagonist of $GABA_B$ receptors in the CA1 region of rat hippocampus. *Neuropharmacology* 32: 1071-1073.

Davies, C. H. and Collingridge, G. L. (1993b) The physiological regulation of synaptic inhibition by $GABA_B$ autoreceptors in rat hippocampus. *J. Physiol. (Lond. )* 472: 245-265.

Davies, P. A., Hanna, M. C., Hales, T. G., and Kirkness, E. F. (1997) Insensitivity to anaesthetic agents conferred by a class of $GABA_A$ receptor subunit. *Nature* 385: 820-823.

De Koninck, Y. and Mody, I. (1994) Noise analysis of miniature IPSCs in adult rat brain slices: Properties and modulation of synaptic $GABA_A$ receptor channels. *J. Neurophysiol.* 71: 1318-1335.

De Koninck, Y. and Mody, I. (1997) Endogenous GABA activates small-conductance $K^+$ channels underlying slow IPSCs in rat hippocampal neurons. *J. Neurophysiol.* 77: 2202-2208.

Decavel, C. and Van den Pol, A. N. (1990) GABA: a dominant neurotransmitter in the hypothalamus. *J. Comp. Neurol.* 302: 1019-1037.

Deisz, R. A. Electrophysiology of $GABA_B$ receptors. In: *The GABA Receptors*, edited by S. J. Enna and N. G. Bowery. Totowa, NJ: Humana Press Inc. 1997, p. 157-207.

Deisz, R. A., Billard, J.-M., and Zieglgänsberger, W. (1997) Presynaptic and postsynaptic $GABA_B$ receptors of neocortical neurons of the rat in vitro: differences in pharmacology and ionic mechanisms. *Synapse* 25: 62-72.

Deisz, R. A. and Prince, D. A. (1989) Frequency-dependent depression of inhibition in guinea pig neocortex in vitro by $GABA_B$ receptor feedback on GABA release. *J. Physiol.* 412: 513-542.

Devillers-Thiéry, A., Galzi, J. L., Eiselé, J. L., Bertrand, S., Bertrand, D., and Changeux, J. P. (1993) Functional architecture of the nicotinic acetylcholine receptor: A prototype of ligand-gated ion channels. *J. Membr. Biol.* 136: 97-112.

Dichter, M. and Spencer, W. A. (1969a) Penicillin-induced interictal discharges from the cat hippocampus. II. Mechanisms underlying origin and restrictions. *J. Neurophysiol.* 32: 663-687.

Dichter, M. and Spencer, W. A. (1969b) Penicillin-induced interictal discharges from the cat hippocampus. I. Characteristics and topographical features. *J. Neurophysiol.* 32: 649-662.

Dichter, M. A. and Ayala, G. F. (1987) Cellular mechanisms of epilepsy: a status report. *Science* 237: 157-164.

Dingledine, R., Hynes, M. A., and King, G. L. (1986) Involvement of N-methyl-D-aspartate receptors in epileptiform bursting in the rat hippocampal slice. *J. Physiol.* 380: 175-189.

Dingledine, R. and Korn, S. J. (1985) γ-Aminobutyric acid uptake and the termination of inhibitory synaptic potentials in the rat hippocampal slice. *J. Physiol.* 366: 387-409.

Dow, R. S., Fernandez-Guardiola, A., and Manni, E. (1962) The production of experimental cobalt epilepsy in the rat. *Electroencephalogr. Clin. Neurophysiol.* 14: 399-407.

Doze, V. A., Cohen, G. A., and Madison, D. V. (1995) Calcium channel involvement in $GABA_B$ receptor-mediated inhibition of GABA release in area CA1 of the rat hippocampus. *J. Neurophysiol.* 74: 43-53.

Duman, R. S., Karbon, E. W., Harrington, C., and Enna, S. J. (1986) An examination of the involvement of phospholipases A2 and C in the alpha-adrenergic and gamma-aminobutyric acid receptor modulation of cyclic AMP accumulation in rat brain slices. *J. Neurochem.* 47: 800-810.

During, M. J., Ryder, K. M., and Spencer, D. D. (1995) Hippocampal GABA transporter function in temporal-lobe epilepsy. *Nature* 376: 174-177.

Durkin, M. M., Smith, K. E., Borden, L. A., Weinshank, R. L., Branchek, T. A., and Gustafson, E. L. (1995) Localization of messenger RNAs encoding three GABA transporters in rat brain: An in situ hybridization study. *Mol. Brain Res.* 33: 7-21.

Dutar, P. and Nicoll, R. A. (1988) A physiological role for $GABA_B$ receptors in the central nervous system. *Nature* 332: 156-158.

Eghbali, M., Curmi, J. P., Birnir, B., and Gage, P. W. (1997) Hippocampal $GABA_A$ channel conductance increased by diazepam. *Nature* 388: 71-75.

Enz, R., Brandstatter, J. H., Hartveit, E., Wassle, H., and Bormann, J. (1995) Expression of GABA receptor rho 1 and rho 2 subunits in the retina and brain of the rat. *Eur. J. Neurosci.* 7: 1495-1501.

Erlander, M. G., Tillakaratne, N. J., Feldblum, S., Patel, N., and Tobin, A. J. (1991) Two genes encode distinct glutamate decarboxylases. *Neuron* 7: 91-100.

Esplin, B. and Čapek, R. (1990) Frequency-dependent changes in efficacy of the hippocampal recurrent inhibition. *Soc. Neurosci. Abstr.* 16: 60, Abstr. # 31.8

Faingold, C. L., Randall, M. E., and Anderson, C. A. B. (1994) Blockade of GABA uptake with tiagabine inhibits audiogenic seizures and reduces neuronal firing in the inferior colliculus of the genetically epilepsy-prone rat. *Exp. Neurol.* 126: 225-232.

Feigenspan, A. and Bormann, J. (1994) Differential pharmacology of $GABA_A$ and $GABA_C$ receptors on rat retinal bipolar cells. *Eur. J. Pharmacol. Mol. Pharmacol.* 288: 97-104.

Feria-Velasco, A., Olivares, N., Rivas, F., Velasco, M., and Velasco, F. (1980) Alumina cream-induced focal motor epilepsy in cats. IV. Thickness and cellularity of layers in the perilesional motor cortex. *Arch. Neurol.* 37: 287-290.

Fink-Jensen, A., Suzdak, P. D., Swedberg, M. D. B., Judge, M. E., Hansen, L., and Nielsen, P. G. (1992) The γ-aminobutyric acid (GABA) uptake inhibitor, tiagabine, increases extracellular brain levels of GABA in awake rats. *Eur. J. Pharmacol.* 220: 197-201.

Fowler, L. J. (1973) Analysis of the major amino acids of rat brain after in vivo inhibition of GABA transaminase by ethanolamine O-sulphate. *J. Neurochem.* 21: 437-440.

Fowler, L. J. and John, R. A. (1972) Active-site-directed irreversible inhibition of rat brain 4-aminobutyrate aminotransferase by ethanolamine O-sulphate in vitro and in vivo. *Biochem. J.* 130: 569-573.

French, J. A., Mosier, M., Walker, S., Sommerville, K., Sussman, N., Barry, E., Bell, W., Bergen, D., Browne, T. ,III, Ferrendelli, J., Fisher, R., Fromm, G., Homan, R., Krauss, G., Lai, C. W., Leppik, I., Leroy, R., Pellock, J., Penovich, P., Ramsay, R. E., and Shinnar, S. (1996) A double-blind, placebo-controlled study of vigabatrin three g/day in patients with uncontrolled complex partial seizures. *Neurology* 46: 54-61.

Frey, H.-H., Popp, C., and Loscher, W. (1979) Influence of inhibitors of the high affinity GABA uptake and seizure thresholds in mice. *Neuropharmacology* 18: 581-590.

Frisk-Holmberg, M., Kerth, P., and Meyer, P. (1989) Effect of food on the absorption of vigabatrin. *Br. J. Clin. Pharmacol.* 27 Suppl. 1: 23S-25S.

Froestl, W., Mickel, S. J., Hall, R. G., Von Sprecher, G., Strub, D., Baumann, P. A., Brugger, F., Gentsch, C., Jaekel, J., Olpe, H. R., Rihs, G., Vassout, A.,

Waldmeier, P. C., and Bittiger, H. (1995) Phosphinic acid analogues of GABA. 1. New potent and selective $GABA_B$ agonists. *J. Med. Chem.* 38: 3297-3312.

Gale, K. (1992) Role of GABA in the genesis of chemoconvulsant seizures. *Toxicol. Lett.* 64-65: 417-428.

Gaspary, H. L., Wang, W., and Richerson, G. B. (1998) Carrier-mediated GABA release activates GABA receptors on hippocampal neurons. *J. Neurophysiol.* 80: 270-281.

Gastaut, H., Gastaut, J. L., Goncalves e Silva, G. E., and Fernandez Sanchez, G. R. (1975) Relative frequency of different types of epilepsy: a study employing the classification of the International League Against Epilepsy. *Epilepsia* 16: 457-461.

Gibbs, J. W.,III, Shumate, M. D., and Coulter, D. A. (1997) Differential epilepsy-associated alterations in postsynaptic $GABA_A$ receptor function in dentate granule and CA1 neurons. *J. Neurophysiol.* 77: 1924-1938.

Giusti, P., Ducic, I., Puia, G., Arban, R., Walser, A., Guidotti, A., and Costa, E. (1993) Imidazenil: a new partial positive allosteric modulator of γ-aminobutyric acid (GABA) action at the $GABA_A$ receptors. *J. Pharmacol. Exp. Ther.* 266: 1018-1028.

Goddard, G. V. (1967) Development of epileptic seizures through brain stimulation at low intensity. *Nature* 214: 1020-1021.

Gram, L., Lyon, B. B., and Dam, M. (1983) Gamma-vinyl-GABA: a single-blind trial in patients with epilepsy. *Acta Neurol.Scand.* 68: 34-39.

Gram, L., Klosterskov, P., and Dam, M. (1985) gamma-Vinyl GABA: a double-blind placebo-controlled trial in partial epilepsy. *Ann. Neurol.* 17: 262-266.

Gram, L., Larsson, O. M., Johnsen, A., and Schousboe, A. (1989) Experimental studies of the influence of vigabatrin on the GABA system. *Br. J. Clin. Pharmacol.* 27 Suppl. 1: 13S-17S.

Grant, S. M. and Heel, R. C. (1991) Vigabatrin: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in epilepsy and disorders of motor control. *Drugs* 41: 889-926.

Grove, J., Alken, R. G., and Schechter, P. J. (1984) Assay of gamma-vinyl-gamma-aminobutyric acid (4-amino-hex-5-enoic acid) in plasma and urine by automatic amino acid analysis. Application to human pharmacokinetics. *J. Chromatogr.* 306:383-7: 383-387.

Grover, L. M., Lambert, N. A., Schwartzkroin, P. A., and Teyler, T. J. (1993) Role of $HCO_3^-$ ions in depolarizing $GABA_A$ receptor-mediated responses in pyramidal cells of rat hippocampus. *J. Neurophysiol.* 69: 1541-1555.

Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M. C., Davidson, N., Lester, H. A., and Kanner, B. I. (1990) Cloning and expression of a rat brain GABA transporter. *Science* 249: 1303-1306.

Guberman, A. (1996) Vigabatrin. *Can. J. Neurol. Sci.* 23: S13-S17.

Gulyás, A. I., Miles, R., Sík, A., Tóth, K., Tamamaki, N., and Freund, T. F. (1993) Hippocampal pyramidal cells excite inhibitory neurons through a single release site. *Nature* 366: 683-687.

Gutnick, M. J. and Prince, D. A. (1981) Dye coupling and possible electrotonic coupling in the guinea pig neocortical slice. *Science* 211: 67-70.

Haas, H. L. and Jefferys, J. G. R. (1984) Low-calcium field burst discharges of CA1 pyramidal neurones in rat hippocampal slices. *J. Physiol. (Lond)* 354: 185-201.

Haas, K. Z., Sperber, E. F., Moshé, S. L., and Stanton, P. K. (1996) Kainic acid-induced seizures enhance dentate gyrus inhibition by downregulation of $GABA_B$ receptors. *J. Neurosci.* 16: 4250-4260.

Hablitz, J. J. (1981) Effects of intracellular injections of chloride and EGTA on postepileptiform-burst hyperpolarizations in hippocampal neurons. *Neurosci. Lett.* 22: 159-163.

Hablitz, J. J. and Heinemann, U. (1987) Extracellular $K^+$ and $Ca^{2+}$ changes during epileptiform discharges in the immature rat neocortex. *Brain Res.* 433: 299-303.

Hackam, A. S., Wang, T. L., Guggino, W. B., and Cutting, G. R. (1997) The N-terminal domain of human GABA receptor rho1 subunits contains signals for homooligomeric and heterooligomeric interaction. *J. Biol. Chem.* 272: 13750-13757.

Hackam, A. S., Wang, T. L., Guggino, W. B., and Cutting, G. R. (1998) Sequences in the amino termini of GABA rho and $GABA_A$ subunits specify their selective interaction in vitro. *J. Neurochem.* 70: 40-46.

Haegele, K. D. and Schechter, P. J. (1986) Kinetics of the enantiomers of vigabatrin after an oral dose of the racemate or the active S-enantiomer. *Clin. Pharmacol. Ther.* 40: 581-586.

Haglund, M. M. and Schwartzkroin, P. A. (1990) Role of Na-K pump potassium regulation and IPSPs in seizures and spreading depression in immature rabbit hippocampal slices. *J. Neurophysiol.* 63: 225-239.

Halonen, T., Nissinen, J., Jansen, J. A., and Pitkänen, A. (1996) Tiagabine prevents seizures, neuronal damage and memory impairment in experimental status epilepticus. *Eur. J. Pharmacol.* 299: 69-81.

Hamill, O. P., Bormann, J. P., and Sakmann, B. (1983) Activation of the multiple conductance state chloride channels in spinal cord neurons by glycine and GABA. *Nature* 305: 805-808.

Hanna, G. R. and Stalmaster, R. M. (1973) Cortical epileptic lesions produced by freezing. *Neurology* 23: 918-925.

Harden, C. L. (1994) New antiepileptic drugs. *Neurology* 44: 787-795.

Hauptmann, A. (1912) Luminal bei epilepsie. *Munch. Med. Wochenschr.* 59: 1907-1909

Hauser, W. A., Annegers, J. F., and Kurland, L. T. (1993) Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935-1984. *Epilepsia* 34: 453-468.

Haycock, J. W., Levy, W. B., Denner, L. A., and Cotman, C. W. (1978) Effects of elevated $[K^+]_0$ on the release of neurotransmitters from cortical synaptosomes: efflux or secretion? *J. Neurochem.* 30: 1113-1125.

Heinemann, U., Lux, H. D., and Gutnick, M. J. (1977) Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the cat. *Exp. Brain Res.* 27: 237-243.

Heinemann, U. and Gutnick, M. J. (1979) Relation between extracellular potassium concentration and neuronal activities in cat thalamus (VPL) during projection of cortical epileptiform discharge. *Electroencephalography & Clinical Neurophysiology* 47: 345-347.

Higashima, M., Kinoshita, H., Yamaguchi, N., and Koshino, Y. (1996) Activation of GABAergic function necessary for afterdischarge generation in rat hippocampal slices. *Neurosci. Lett.* 207: 101-104.

Hill, D. R., Bowery, N. G., and Hudson, A. L. (1984) Inhibition of $GABA_B$ receptor binding by guanyl nucleotides. *J. Neurochem.* 42: 652-657.

Hills, J. M., Dingsdale, R. A., Parsons, M. E., Dolle, R. E., and Howson, W. (1989) 3-Aminopropylphosphinic acid--a potent, selective $GABA_B$ receptor agonist in the guinea-pig ileum and rat anococcygeus muscle. *Br. J. Pharmacol.* 97: 1292-1296.

Hoke, J. F., Yuh, L., Antony, K. K., Okerholm, R. A., Elberfeld, J. M., and Sussman, N. M. (1993) Pharmacokinetics of vigabatrin following single and multiple oral doses in normal volunteers. *J. Clin. Pharmacol.* 33: 458-462.

Holmes, W. R. and Levy, W. B. (1997) Quantifying the role of inhibition in associative long-term potentiation in dentate granule cells with computational models. *J. Neurophysiol.* 78: 103-116.

Hosford, D. A., Clark, S., Cao, Z., Wilson, W. A.,Jr., Lin, F., Morrisett, R. A., and Huin, A. (1992) The role of $GABA_B$ receptor activation in absence seizures of lethargic (*lh/lh*) mice. *Science* 257: 398-401.

Hotson, J. R. and Prince, D. A. (1980) A calcium-activated hyperpolarization follows repetitive firing in hippocampal neurons. *J. Neurophysiol.* 43: 409-419.

Howson, W., Mistry, J., Broekman, M., and Hills, J. M. (1993) Biological activity of 3-aminopropyl (methyl) phosphinic acid, a potent and selective $GABA_B$ agonist with CNS activity. *Bioorg. Med. Chem. Lett.* 3: 515-518.

Hu, R. Q. and Davies, J. A. (1997) Tiagabine hydrochloride, an inhibitor of gamma-aminobutyric acid (GABA) uptake, induces cortical depolarizations in vitro. *Brain Res.* 753: 260-268.

Hwa, G. G. and Avoli, M. (1989) NMDA receptor antagonists CPP and MK-801 partially suppress the epileptiform discharges induced by the convulsant drug bicuculline in the rat neocortex. *Neurosci. Lett.* 98: 189-193.

Inglefield, J. R., Perry, J. M., and Schwartz, R. D. (1995) Postischemic inhibition of GABA reuptake by tiagabine slows neuronal death in the gerbil hippocampus. *Hippocampus* 5: 460-468.

Isaacson, J. S., Solís, J. M., and Nicoll, R. A. (1993) Local and diffuse synaptic actions of GABA in the hippocampus. *Neuron* 10: 165-175.

Itouji, A., Sakai, N., Tanaka, C., and Saito, N. (1996) Neuronal and glial localization of two GABA transporters (GAT1 and GAT3) in the rat cerebellum. *Mol. Brain Res.* 37: 309-316.

Jackson, J. H. (1870) A study of convulsions. *Trans. St. And. Med. Grad. Assoc.* 3: 162-204

Jackson, M. F., Esplin, B., and Čapek, R. (1993a) Gamma-vinyl GABA (GVG) reverses activity-dependent disinhibition in the rat hippocampus in vitro. *Soc. Neurosci. Abstr.* 19: 1632, # 668.14 (Abstract).

Jackson, M. F., Esplin, B., and Čapek, R. (1993b) γ-Vinyl GABA (GVG) causes disinhibition in area CA1 of the rat hippocampus. *Proc. Can. Fed. Biol. Soc.* 36: 123, # 450 (Abstract).

Jackson, M. F., Dennis, T., Esplin, B., and Čapek, R. (1994) Acute effects of gamma-vinyl GABA (vigabatrin) on hippocampal GABAergic inhibition in vitro. *Brain Res.* 651: 85-91.

Jackson, M. F., Esplin, B., and Čapek, R. (1995) High-frequency synchronous firing of interneurons increases the effectiveness of GABAergic transmission. *Soc. Neurosci. Abstr.* 21: 983, # 388.15 (Abstract).

Jackson, M. F., Esplin, B., and Čapek, R. (1996) Activity-dependent enhancement of inhibitory postsynaptic potentials (IPSPs) by the GABA-uptake inhibitor tiagabine in hippocampal slices. *Soc. Neurosci. Abstr.* 22: 2104, # 824.1 (Abstract).

Jackson, M. F., Esplin, B., and Čapek, R. (1997) Functional consequences of depolarizing GABA(A) receptor-mediated synaptic potentials evoked by different populations of interneurons in the hippocampus. *Soc. Neurosci. Abstr.* 23: 115, # 53.4 (Abstract).

Jarolimek, W., Demmelhuber, J., Bijak, M., and Misgeld, U. (1993) CGP 55845A blocks baclofen, γ-aminobutyric acid and inhibitory postsynaptic potassium currents in guinea pig CA3 neurons. *Neurosci. Lett.* 154: 31-34.

Jarolimek, W. and Misgeld, U. (1997) $GABA_B$ receptor-mediated inhibition of tetrodotoxin-resistant GABA release in rodent hippocampal CA1 pyramidal cells. *J. Neurosci.* 17: 1025-1032.

John, R. A. and Fowler, L. J. (1976) Kinetic and spectral properties of rabbit brain 4-aminobutyrate aminotransferase. *Biochem. J.* 155: 645-651.

Johnston, G. A. (1996) GABAc receptors: relatively simple transmitter-gated ion channels? *Trends. Pharmacol. Sci.* 17: 319-323.

Johnston, G. A. R., Krogsgaard-Larsen, P., and Stephanson, A. (1975) Betel nut constituents as inhibitors of gamma-aminobutyric acid uptake. *Nature* 258: 627-628.

Johnston, G. A. R., Krogsgaard-Larsen, P., Stephanson, A. L., and Twitchin, B. (1976a) Inhibition of the uptake of GABA and related amino acids in rat brain slices by the optical isomers of nipecotic acid. *J. Neurochem.* 26: 1029-1032.

Johnston, G. A. R., Stephanson, A. L., and Twitchin, B. (1976b) Uptake and release of nipecotic acid by rat brain slices. *J. Neurochem.* 26: 83-87.

Johnston, G. A. R., Kennedy, M. E., and Lodge, D. (1978) Muscimol uptake release and binding in rat brain slices. *J. Neurochem.* 31: 1519-1523.

Jolkkonen, J., Mazurkiewicz, M., Lahtinen, H., and Riekkinen, P. (1992) Acute effects of γ-vinyl GABA on the GABAergic system in rats as studied by microdialysis. *Eur. J. Pharmacol.* 229: 269-272.

Jones, R. S. (1988) Epileptiform events induced by GABA-antagonists in entorhinal cortical cells in vitro are partly mediated by N-methyl-D-aspartate receptors. *Brain Res.* 457: 113-121.

Jung, M. J., Lippert, B., Metcalf, B. W., Böhlen, P., and Schechter, P. J. (1977a) γ-vinyl GABA (4-amino-hex-5-enoic acid), a new selective irreversible inhibitor of GABA-T: effects on brain GABA metabolism in mice. *J. Neurochem.* 29: 797-802.

Jung, M. J., Lippert, B., Metcalf, B. W., Schechter, P. J., Bohlen, P., and Sjoerdsma, A. (1977b) The effect of 4-amino hex-5-ynoic acid (gamma-acetylenic GABA, gammma-ethynyl GABA) a catalytic inhibitor of GABA transaminase, on brain GABA metabolism in vivo. *J. Neurochem.* 28: 717-723.

Jung, M. J. and Metcalf, B. W. (1975) Catalytic inhibition of gamma-aminobutyric acid - alpha-ketoglutarate transaminase of bacterial origin by 4-aminohex-5-ynoic acid, a substrate analog. *Biochem. Biophys. Res. Commun.* 67: 301-306.

Kaila, K., Pasternack, M., Saarikoski, J., and Voipio, J. (1989) Influence of GABA-gated bicarbonate conductance on potential, current and intracellular chloride in crayfish muscle fibres. *J. Physiol.* 416: 161-181.

Kaila, K., Voipio, J., Paalasmaa, P., Pasternack, M., and Deisz, R. A. (1993) The role of bicarbonate in $GABA_A$ receptor-mediated IPSPs of rat neocortical neurones. *J. Physiol.* 464: 273-289.

Kaila, K. (1994) Ionic basis of $GABA_A$ receptor channel function in the nervous system. *Prog. Neurobiol.* 42: 489-537.

Kaila, K., Lamsa, K., Smirnov, S., Taira, T., and Voipio, J. (1997) Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent $K^+$ transient. *J. Neurosci.* 17: 7662-7672.

Kaneda, M., Farrant, M., and Cull-Candy, S. G. (1995) Whole-cell and single-channel currents activated by GABA and glycine in granule cells of the rat cerebellum. *J. Physiol. (Lond)* 485: 419-435.

Kapur, A., Pearce, R. A., Lytton, W. W., and Haberly, L. B. (1997) $GABA_A$-mediated IPSCs in piriform cortex have fast and slow components with different properties and locations on pyramidal cells. *J. Neurophysiol.* 78: 2531-2545.

Kapur, J., Stringer, J. L., and Lothman, E. W. (1989) Evidence that repetitive seizures in the hippocampus cause a lasting reduction of GABAergic inhibition. *J. Neurophysiol.* 61: 417-426.

Kapur, J., Lothman, E. W., and DeLorenzo, R. J. (1994) Loss of $GABA_A$ receptors during partial status epilepticus. *Neurology* 44: 2407-2408.

Karlsson, G., Klebs, K., Hafner, T., Schmutz, M., and Olpe, H. R. (1992) Blockade of $GABA_B$ receptors accelerates amygdala kindling development. *Experientia* 48: 748-751.

Kaufman, D. L., Houser, C. R., and Tobin, A. J. (1991) Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. *J. Neurochem.* 56: 720-723.

Kaupmann, K., Huggel, K., Heid, J., Flor, P. J., Bischoff, S., Mickel, S. J., McMaster, G., Angst, C., Bittiger, H., Froestl, W., and Bettler, B. (1997) Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. *Nature* 386: 239-246.

Kemp, J. A., Marshall, G. R., and Woodruff, G. N. (1986) Quantitative evaluation of the potencies of GABA-receptor agonists and antagonists using the rat hippocampal slice preparation. *Br. J. Pharmacol.* 87: 677-684.

Kerr, D. I., Ong, J., Prager, R. H., Gynther, B. D., and Curtis, D. R. (1987) Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.* 405: 150-154.

Knight, A. R. and Bowery, N. G. (1996) The pharmacology of adenylyl cyclase modulation by $GABA_B$ receptors in rat brain slices. *Neuropharmacology* 35: 703-712.

Knowles, W. D. and Schwartzkroin, P. A. (1981) Local circuit synaptic interactions in hippocampal brain slices. *J. Neurosci.* 1: 318-322.

Koch, C., Poggio, T., and Torre, V. (1983) Nonlinear interactions in a dendritic tree: localization, timing, and role in information processing. *Proc. Natl. Acad. Sci. USA* 80: 2799-2802.

Konnerth, A., Heinemann, U., and Yaari, Y. (1986) Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. I. Development of seizure-like activity in low extracellular calcium. *J. Neurophysiol.* 56: 409-423.

Kopeloff, L. M., Chusid, J. G., and Kopeloff, N. (1955) Epilepsy in *Macacca mulatta* after cortical or intracerebral alumina. *Arch. Neurol. Psychiatry* 74: 523-526.

Kosaka, T. and Hama, K. (1985) Gap junctions between non-pyramidal cell dendrites in the rat hippocampus (CA1 and CA3 regions): a combined Golgi-electron microscopy study. *J. Comp. Neurol.* 231: 150-161.

Kostopoulos, G. and Antoniadis, G. (1991) A comparison of recurrent inhibition and of paired-pulse facilitation in hippocampal slices from normal and genetically epileptic mice. *Epilepsy Res.* 9: 184-194.

Krauss, G. L., Johnson, M. A., and Miller, N. R. (1998) Vigabatrin-associated retinal cone system dysfunction: electroretinogram and ophthalmologic findings. *Neurology* 50: 614-618.

Krogsgaard-Larsen, P. (1980) Inhibitors of the GABA uptake systems. *Mol.Cell.Biochem.* 31: 105-121.

Krogsgaard-Larsen, P., Falch, E. and Hjeds, H. (1985) Heterocyclic analogues of GABA: chemistry, molecular pharmacology and therapeutics aspects. *Prog. Med. Chem.* 22: 67-120.

Kumar, N. M. and Gilula, N. B. (1996) The gap junction communication channel. *Cell* 84: 381-388.

Kuriyama, K., Roberts, E., and Rubinstein, M. K. (1966) Elevation of gamma-aminobutyric acid in brain with amino-oxyacetic acid and susceptibility to convulsive seizures in mice: a quantitative re-evaluation. *Biochem. Pharmacol.* 15: 221-236.

Kwon, O. S., Park, J., and Churchich, J. E. (1992) Brain 4-aminobutyrate aminotransferase. Isolation and sequence of a cDNA encoding the enzyme. *J. Biol. Chem.* 267: 7215-7216.

Lacaille, J.-C., Mueller, A. L., Kunkel, D. D., and Schwartzkroin, P. A. (1987) Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: Electrophysiology and morphology. *J. Neurosci.* 7: 1979-1993.

Lader, M. (1995) Clinical pharmacology of anxiolytic drugs: past, present and future. *Adv. Biochem. Pharmacol.* 48: 135-152.

Lambert, N. and Grover, L. (1995) The mechanism of biphasic GABA responses. *Science* 269: 928-929.

Lambert, N. A., Harrison, N. L., Kerr, D. I. B., Ong, J., Prager, R. H., and Teyler, T. J. (1989) Blockade of the late IPSP in rat CA1 hippocampal neurons by 2-hydroxy-saclofen. *Neurosci. Lett.* 107: 125-128.
Lambert, N. A., Harrison, N. L., and Teyler, T. J. (1991) Baclofen-induced disinhibition in area CA1 of rat hippocampus is resistant to extracellular $Ba^{2+}$. *Brain Res.* 547: 349-352.

Lambert, N. A. and Wilson, W. A. (1996) High-threshold Ca2+ currents in rat hippocampal interneurones and their selective inhibition by activation of $GABA_B$ receptors. *J. Physiol. (Lond)* 492: 115-127.

Lamsa, K. and Kaila, K. (1997) Ionic mechanisms of spontaneous GABAergic events in rat hippocampal slices exposed to 4-aminopyridine. *J. Neurophysiol.* 78: 2582-2591.

Lancaster, B. and Wheal, H. V. (1984) The synaptically evoked late hyperpolarisation in hippocampal CA1 pyramidal cells is resistant to intracellular EGTA. *Neuroscience* 12: 267-275.

Lange, S. C., Neafsey, E. J., and Wyler, A. R. (1980) Neuronal activity in chronic ferric chloride epileptic foci in cats and monkey. *Epilepsia* 21: 251-254.

Larsson, O. M., Thorbek, P., Krogsgaard-Larsen, P., and Schousboe, A. (1981) Effect of homo-beta-proline and other heterocyclic GABA analogues on GABA uptake in neurons and astroglial cells and on GABA receptor binding. *J. Neurochem.* 37: 1509-1516.

Larsson, O. M., Johnston, G. A., and Schousboe, A. (1983) Differences in uptake kinetics of cis-3-aminocyclohexane carboxylic acid into neurons and astrocytes in primary cultures. *Brain Res.* 260: 279-285.

Larsson, O. M., Gram, L., Schousboe, I., and Schousboe, A. (1986) Differential effect of gamma-vinyl GABA and valproate on GABA-transaminase from cultured neurones and astrocytes. *Neuropharmacology* 25: 617-625.

Laurie, D. J., Seeburg, P. H., and Wisden, W. (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J. Neurosci.* 12: 1063-1076.

Le Foll, F., Castel, H., Soriani, O., Vaudry, H., and Cazin, L. (1998) Gramicidin-perforated patch revealed depolarizing effect of GABA in cultured frog melanotrophs. *J. Physiol. (Lond)* 507: 55-69.

Leppik, I. E. (1995) Tiagabine: The safety landscape. *Epilepsia* 36: S10-S13.

Lerma, J., Herreras, O., and Del Rio, R. M. (1985) Electrophysiological evidence that nipecotic acid can be used in vivo as a false transmitter. *Brain Res.* 335: 377-380.

Li, X.-G., Somogyi, P., Tepper, J. M., and Buzsáki, G. (1992) Axonal and dendritic arborization of an intracellularly labeled chandelier cell in the CA1 region of the rat hippocampus. *Exp. Brain Res.* 90: 519-525.

Lippert, B., Metcalf, B. W., Jung, M. J., and Casara, P. (1977) 4-Amino-hex-5-enoic acid, a selective catalytic inhibitor of 4-aminobutyric-acid aminotransferase in mammalian brain. *Eur. J. Biochem.* 74: 441-445.

Livingston, J. H., Beaumont, D., Arzimanoglou, A., and Aicardi, J. (1989) Vigabatrin in the treatment of epilepsy in children. *Br. J. Clin. Pharmacol.* 27 Suppl. 1: 109S-112S.

Llinás, R. and Yarom, Y. (1981) Electrophysiology of mammalian inferior olivary neurones in vitro. Different types of voltage-dependent ionic conductances. *J. Physiol. (Lond)* 315: 549-567.

Lloyd, K. G., Bossi, L., Morselli, P. L., Munari, C., Rougier, M., and Loiseau, H. (1986) Alterations in GABA-mediated synaptic transmission in human epilepsy.

In: *Advances in Neurology*, edited by A. V. Delgado-Escueta, A. A. Ward, D. M. Woodbury and R. J. Porter. New Yok: Raven Press, p. 1033-1044.

Loiseau, H., Averet, N., Arrigoni, E., and Cohadon, F. (1987) The early phase of cryogenic lesions: an experimental model of seizures updated. *Epilepsia* 28: 251-258.

Lopantsev, V. and Avoli, M. (1998) Participation of $GABA_A$-mediated inhibition in ictalike discharges in the rat entorhinal cortex. *J. Neurophysiol.* 79: 352-360.
LoTurco, J. J., Owens, D. F., Heath, M. J. S., Davis, M. B. E., and Kriegstein, A. R. (1995) GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 15: 1287-1298.

Löscher, W. (1980a) A comparative study of the pharmacology of inhibitors of GABA-metabolism. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 315: 119-128.

Löscher, W. (1980b) Effect of inhibitors of GABA transaminase on the synthesis, binding, uptake, and metabolism of GABA. *J. Neurochem.* 34: 1603-1608.

Löscher, W. (1981) Effect of inhibitors of GABA aminotransferase on the metabolism of GABA in brain tissue and synaptosomal fractions. *J. Neurochem.* 36: 1521-1527.

Löscher, W. (1982) Anticonvulsant and biochemical effects of inhibitors of GABA aminotransferase and valproic acid during subchronic treatment in mice. *Biochem. Pharmacol.* 31: 837-842.

Löscher, W., Jäckel, R., and Müller, F. (1989) Anticonvulsant and proconvulsant effects of inhibitors of GABA degradation in the amygdala-kindling model. *Eur. J. Pharmacol.* 163: 1-14.

Löscher, W. (1998) New visions in the pharmacology of anticonvulsion. *Eur. J. Pharmacol.* 342: 1-13.

Löscher, W. and Frey, H.-H. (1987) One to three day dose intervals during subchronic treatment of epileptic gerbils with γ-vinyl GABA: anticonvulsant

efficacy and alterations in regional brain GABA levels. *Eur. J. Pharmacol.* 143: 335-342.

Löscher, W. and Hörstermann, D. (1994a) Differential effects of vigabatrin, gamma-acetylenic GABA, aminooxyacetic acid, and valproate on levels of various amino acids in rat brain regions and plasma. *Naunyn Schmiedebergs Arch. Pharmacol.* 349: 270-278.

Löscher, W. and Schmidt, D. (1994b) Strategies in antiepileptic drug development: Is rational drug design superior to random screening and structural variation. *Epilepsy Res.* 17: 95-134.

Löscher, W. and Rundfeldt, C. (1991) Kindling as a model of drug-resistant partial epilepsy: Selection of phenytoin-resistant and nonresistant rats. *J. Pharmacol. Exp. Ther.* 258: 483-489.

Luhmann, H. J., Mittmann, T., Van Luijtelaar, G., and Heinemann, U. (1995) Impairment of intracortical GABAergic inhibition in a rat model of absence epilepsy. *Epilepsy Res.* 22: 43-51.

Macdonald, R. L., Rogers, C. J., and Twyman, R. E. (1989) Kinetic properties of the $GABA_A$ receptor main conductance state of mouse spinal cord neurones in culture. *J. Physiol. (Lond)* 410: 479-499.

Macdonald, R. L. and Olsen, R. W. (1994) $GABA_A$ receptor channels. *Annu. Rev. Neurosci.* 17: 569-602.

MacVicar, B. A. and Dudek, F. E. (1982) Electrotonic coupling between granule cells of rat dentate gyrus: physiological and anatomical evidence. *J. Neurophysiol.* 47: 579-592.

Mager, S., Kleinberger-Doron, N., Keshet, G. I., Davidson, N., Kanner, B. I., and Lester, H. A. (1996) Ion binding and permeation at the GABA transporter GAT1. *J. Neurosci.* 16: 5405-5414.

Maître, M., Ciesielski, L., Cash, C., and Mandel, P. (1975) Purification and studies on some properties of the 4-aminobutyrate: 2-oxoglutarate transaminase from rat brain. *Eur. J. Biochem.* 52: 157-169.

Malcangio, M., Libri, V., Teoh, H., Constanti, A., and Bowery, N. G. (1995) Chronic (-)baclofen or CGP 36742 alters $GABA_B$ receptor sensitivity in rat brain and spinal cord. *NeuroReport* 6: 399-403.

Mangan, P. S. and Lothman, E. W. (1996) Profound disturbances of pre- and postsynaptic $GABA_B$-receptor-mediated processes in region CA1 in a chronic model of temporal lobe epilepsy. *J. Neurophysiol.* 76: 1282-1296.

Martin, D. L. and Rimvall, K. (1993) Regulation of gamma-aminobutyric acid synthesis in the brain. *J. Neurochem.* 60: 395-407.

Mason, A., Nicoll, A., and Stratford, K. (1991) Synaptic transmission between individual pyramidal neurons of the rat visual cortex in vitro. *J. Neurosci.* 11: 72-84.

Mason, M.J., Mattsson, K., Pasternack, M., Voipio, J., and Kaila, K. (1990) Postsynaptic fall in intracellular pH and increase in surface pH caused by efflux of formate and acetate anions through GABA-gated channels in crayfish muscle fibres. *Neuroscience* 34: 359-368.

Massotti, M., Schlichting, J. L., Antonacci, M. D., Giusti, P., Memo, M., Costa, E., and Guidotti, A. (1991) Gamma aminobutyric acid A receptor heterogeneity in rat central nervous system: studies with clonazepam and other benzodiazepine ligands. *J. Pharmacol. Exp. Ther.* 256: 1154-1160.

Matsumoto, H. and Ajmone Marsan, C. (1964a) Cortical cellular phenomena in experimental epilepsy: Interictal manifestations. *Exp. Neurol.* 9: 286-304.

Matsumoto, H. and Ajmone Marsan, C. (1964b) Cortical cellular phenomena in experimental epilepsy: Ictal manifestations. *Exp. Neurol.* 9: 305-326.

Matthews, W. D., McCafferty, G. P., and Setler, P. E. (1981) An electrophysiological model of GABA-mediated neurotransmission. *Neuropharmacology* 20: 561-565.

Mattson, R. H., Cramer, J. A., Collins, J. F., Smith, D. B., Delgado-Escueta, A. V., Browne, T. R., Williamson, P. D., Treiman, D. M., McNamara, J. O., and McCutchen, C. B. (1985) Comparison of carbamazepine, phenobarbital, phenytoin, and primidone in partial and secondarily generalized tonic-clonic seizures. *N. Engl. J. Med.* 313: 145-151.

Mattson, R. H., Petroff, O., Rothman, D., and Behar, K. (1994) Vigabatrin: Effects on human brain GABA levels by nuclear magnetic resonance spectroscopy. *Epilepsia* 35 Suppl. 5: S29-S32.

Mayer, M. L. (1985) A calcium-activated chloride current generates the afterdepolarization of rat sensory neurones in culture. *J. Physiol.* 364: 217-239.

McCarren, M. and Alger, B. E. (1985) Use-dependent depression of IPSPs in rat hippocampal pyramidal cells in vitro. *J. Neurophysiol.* 53: 557-571.

McIntire, S. L., Reimer, R. J., Schuske, K., Edwards, R. H., and Jorgensen, E. M. (1997) Identification and characterization of the vesicular GABA transporter. *Nature* 389: 870-876.

McKee, P. J. W., Blacklaw, J., Friel, E., Thompson, G. G., Gillham, R. A., and Brodie, M. J. (1993) Adjuvant vigabatrin in refractory epilepsy: A ceiling to effective dosage in individual patients. *Epilepsia* 34: 937-943.

McKernan, R. M. and Whiting, P. J. (1996) Which $GABA_A$-receptor subtypes really occur in the brain? *Trends Neurosci.* 19: 139-143.

Medina, M. A. (1963) The *in vivo* effects of hydrazides and vitamin $B_6$ on the metabolism of gamma-aminobutyric acid. *J. Pharmacol. Exp. Ther.* 140: 133-137.

Medina-Kauwe, L. K., Tillakaratne, N. J. K., Wu, J.-Y., and Tobin, A. J. (1994) A rat cDNA encodes enzymatically active GABA transaminase and provides a molecular probe for GABA-catabolizing cells. *J. Neurochem.* 62: 1267-1275.

Mellanby, J., Hawkins, C., Mellanby, H., Rawlins, J. N., and Impey, M. E. (1984) Tetanus toxin as a tool for studying epilepsy. *J. Physiol. (Paris)* 79: 207-215.

Metcalf, B. W. (1979) Inhibitors of GABA metabolism. *Biochem. Pharmacol.* 28: 1705-1712.

Michelson, H. B. and Wong, R. K. S. (1991) Excitatory synaptic responses mediated by $GABA_A$ receptors in the hippocampus. *Science* 253: 1420-1423.

Michelson, H. B. and Wong, R. K. S. (1994) Synchronization of inhibitory neurones in the guinea-pig hippocampus *in vitro*. *J. Physiol. (Lond. )* 477: 35-45.

Michelucci, R. and Tassinari, C. A. (1989) Response to vigabatrin in relation to seizure type. *Br. J. Clin. Pharmacol.* 27(S1): 119S-124S.

Miles, R., Tóth, K., Gulyás, A. I., Hájos, N., and Freund, T. F. (1996) Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* 16: 815-823.

Miles, R. and Wong, R. K. (1983) Single neurones can initiate synchronized population discharge in the hippocampus. *Nature* 306: 371-373.

Miles, R. and Wong, R. K. (1986) Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. *J. Physiol. (Lond. )* 373: 397-418.

Miles, R. and Wong, R. K. S. (1987) Inhibitory control of local excitatory circuits in the guinea-pig hippocampus. *J. Physiol.* 388: 611-629.

Misgeld, U., Klee, M. R., and Zeise, M. L. (1984) Differences in baclofen-sensitivity between CA3 neurons and granule cells of the guinea pig hippocampus in vitro. *Neurosci. Lett.* 47: 307-311.

Misgeld, U., Deisz, R. A., Dodt, H. U., and Lux, H. D. (1986) The role of chloride transport in postsynaptic inhibition of hippocampal neurons. *Science* 232: 1413-1415.

Misgeld, U., Müller, W., and Brunner, H. (1989) Effects of (-)baclofen on inhibitory neurons in the guinea pig hippocampal slice. *Pflügers Arch.* 414: 139-144.

Morimoto, K., Sato, H., Yamamoto, Y., Watanabe, T., and Suwaki, H. (1997) Antiepileptic effects of tiagabine, a selective GABA uptake inhibitor, in the rat kindling model of temporal epilepsy. *Epilepsia* 38: 966-974.

Morrisett, R. A., Mott, D. D., Lewis, D. V., Swartzwelder, H. S., and Wilson, W. A. (1991) $GABA_B$-receptor-mediated inhibition of the *N*-methyl-D-aspartate component of synaptic transmission in the rat hippocampus. *J. Neurosci.* 11: 203-209.

Mouginot, D., Kombian, S. B., and Pittman, Q. J. (1998) Activation of presynaptic $GABA_B$ receptors inhibits evoked IPSCs in rat magnocellular neurons in vitro. *J. Neurophysiol.* 79: 1508-1517.

Mueller, A. L., Taube, J. S., and Schwartzkroin, P. A. (1984) Development of hyperpolarizing inhibitory postsynaptic potentials and hyperpolarizing response to gamma-aminobutyric acid in rabbit hippocampus studied in vitro. *J. Neurosci.* 4: 860-867.

Mumford, J. P. (1988) A profile of vigabatrin. *Br. J. Clin. Pract. Suppl.* 61:7-9: 7-9.

Müller, W., Misgeld, U., and Lux, H. D. (1989) γ-Aminobutyric acid-induced ion movements in the guinea pig hippocampal slice. *Brain Res.* 484: 184-191.

Nathan, T., Jensen, M. S., and Lambert, J. D. C. (1990) $GABA_B$ receptors play a major role in paired-pulse facilitation in area CA1 of the rat hippocampus. *Brain Res.* 531: 55-65.

Nathan, T. and Lambert, J. D. C. (1991) Depression of the fast IPSP underlies paired-pulse facilitation in area CA1 of the rat hippocampus. *J. Neurophysiol.* 66: 1704-1715.

Nayeem, N., Green, T. P., Martin, I. L., and Barnard, E. A. (1994) Quaternary structure of the native $GABA_A$ receptor determined by electron microscopic image analysis. *J. Neurochem.* 62: 815-818.

Nelson, M. T. and Blaustein, M. P. (1982) GABA efflux from synaptosomes: effects of membrane potential, and external GABA and cations. *J. Membr. Biol.* 69: 213-223.

Newberry, N. R. and Nicoll, R. A. (1984) A bicuculline-resistant inhibitory post-synaptic potential in rat hippocampal pyramidal cells in vitro. *J. Physiol.* 348: 239-254.

Newberry, N. R. and Nicoll, R. A. (1985) Comparison of the action of baclofen with gamma-aminobutyric acid on rat hippocampal pyramidal cells in vitro. *J. Physiol. (Lond)* 360: 161-185.

Newland, C. F., Colquhoun, D., and Cull-Candy, S. G. (1991) Single channels activated by high concentrations of GABA in superior cervical ganglion neurones of the rat. *J. Physiol. (Lond)* 432: 203-233.

Nicoll, R. A. and Alger, B. E. (1981) Synaptic excitation may activate a calcium-dependent potassium conductance in hippocampal pyramidal cells. *Science* 212: 957-959.

Nielsen, E. B., Suzdak, P. D., Andersen, K. E., Knutsen, L. J. S., Sonnewald, U., and Braestrup, C. (1991) Characterization of tiagabine (NO-328), a new potent and selective GABA uptake inhibitor. *Eur. J. Pharmacol.* 196: 257-266.

O'Beirne, M., Bulloch, A. G., and MacVicar, B. A. (1987) Dye and electrotonic coupling between cultured hippocampal neurons. *Neurosci. Lett.* 78: 265-270.

Ogurusu, T., Eguchi, G., and Shingai, R. (1997) Localization of gamma-aminobutyric acid (GABA) receptor rho 3 subunit in rat retina. *Neuroreport.* 8: 925-927.

Ogurusu, T. and Shingai, R. (1996) Cloning of a putative gamma-aminobutyric acid (GABA) receptor subunit *rho*3 cDNA. *Biochim. Biophys. Acta Gene Struct. Expression* 1305: 15-18.

Oh, D. J. and Dichter, M. A. (1994) Effect of a gamma-aminobutyric acid uptake inhibitor, NNC-711, on spontaneous postsynaptic currents in cultured rat hippocampal neurons: Implications for antiepileptic drug development. *Epilepsia* 35: 426-430.

Olbrich, H.-G. and Braak, H. (1985) Ratio of pyramidal cells versus non-pyramidal cells in sector CA1 of the human Ammon's horn. *Anat. Embryol.* 173: 105-110.

Olpe, H.-R., Steinmann, M. W., Ferrat, T., Pozza, M. F., Greiner, K., Brugger, F., Froestl, W., Mickel, S. J., and Bittiger, H. (1993) The actions of orally active $GABA_B$ receptor antagonists on GABAergic transmission in vivo and in vitro. *Eur. J. Pharmacol.* 233: 179-186.

Olpe, H.-R., Steinmann, M. W., Greiner, K., and Pozza, M. F. (1994) Contribution of presynaptic GABA-B receptors to paired-pulse depression of GABA-responses in the hippocampus. *Naunyn Schmiedebergs Arch. Pharmacol.* 349: 473-477.

Olpe, H.-R. and Karlsson, G. (1990) The effects of baclofen and two $GABA_B$-receptor antagonists on long-term potentiation. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 342: 194-197.

Olpe, H. R., Karlsson, G., Pozza, M. F., Brugger, F., Steinmann, M., Van Riezen, H., Fagg, G., Hall, R. G., Froestl, W., and Bittiger, H. (1990) CGP 35348: a centrally active blocker of $GABA_B$ receptors. *Eur. J. Pharmacol.* 187: 27-38.

Olsen, R. W. and Tobin, A. J. (1990) Molecular biology of $GABA_A$ receptors. *FASEB J.* 4: 1469-1480.

Otis, T. S., Staley, K. J., and Mody, I. (1991) Perpetual inhibitory activity in mammalian brain slices generated by spontaneous GABA release. *Brain Res.* 545: 142-150.

Otis, T. S. and Mody, I. (1992) Differential activation of $GABA_A$ and $GABA_B$ receptors by spontaneously released transmitter. *J. Neurophysiol.* 67: 227-234.

Owen, D. G., Segal, M., and Barker, J. L. (1984) A Ca-dependent Cl-conductance in cultured mouse spinal neurones. *Nature* 311: 567-570.

Owens, D. F., Boyce, L. H., Davis, M. B. E., and Kriegstein, A. R. (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. *J. Neurosci.* 16: 6414-6423.

Paalasmaa, P. and Kaila, K. (1996) Role of voltage-gated calcium channels in the generation of activity-induced extracellular pH transients in the rat hippocampal slice. *J. Neurophysiol.* 75: 2354-2360.

Pearce, R. A. (1993) Physiological evidence for two distinct $GABA_A$ responses in rat hippocampus. *Neuron* 10: 189-200.

Pedersen, S. A., Klosterskov, P., Gram, L., and Dam, M. (1985) Long-term study of gamma-vinyl GABA in the treatment of epilepsy. *Acta Neurol.Scand.* 72: 295-298.

Pei, Y., Zhao, D., Huang, J., and Cao, L. (1983) Zinc-induced seizures: a new experimental model of epilepsy. *Epilepsia* 24: 169-176.

Perez-Velazquez, J. L., Valiante, T. A., and Carlen, P. L. (1994) Modulation of gap junctional mechanisms during calcium-free induced field burst activity: A possible role for electrotonic coupling in epileptogenesis. *J. Neurosci.* 14: 4308-4317.

Perkins, K. L. and Wong, R. K. S. (1996) Ionic basis of the postsynaptic depolarizing GABA response in hippocampal pyramidal cells. *J. Neurophysiol.* 76: 3886-3894.

Perreault, P. and Avoli, M. (1991) Physiology and pharmacology of epileptiform activity induced by 4-aminopyridine in rat hippocampal slices. *J. Neurophysiol.* 65: 771-785.

Perreault, P. and Avoli, M. (1992) 4-Aminopyridine-induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. *J. Neurosci.* 12: 104-115.

Persohn, E., Malherbe, P., and Richards, J. G. (1992) Comparative molecular neuroanatomy of cloned $GABA_A$ receptor subunits in the rat CNS. *J. Comp. Neurol.* 326: 193-216.

Petroff, O. A. and Rothman, D. L. (1998) Measuring human brain GABA in vivo: effects of GABA-transaminase inhibition with vigabatrin. *Mol. Neurobiol.* 16: 97-121.

Petroff, O. A. C., Rothman, D. L., Behar, K. L., and Mattson, R. H. (1996) Human brain GABA levels rise after initiation of vigabatrin therapy but fail to rise further with increasing dose. *Neurology* 46: 1459-1463.

Pfeiffer, M., Draguhn, A., Meierkord, H., and Heinemann, U. (1996) Effects of gamma-aminobutyric acid (GABA) agonists and GABA uptake inhibitors on pharmacosensitive and pharmacoresistant epileptiform activity *in vitro*. *Br. J. Pharmacol.* 119: 569-577.

Pham, T. M., Nurse, S., and Lacaille, J. C. (1998) Distinct $GABA_B$ actions via synaptic and extrasynaptic receptors in rat hippocampus In vitro. *J. Neurophysiol.* 80: 297-308.

Pitkänen, A., Halonen, T., Ylinen, A., and Riekkinen, P. (1987) Somatostatin, beta-endorphin, and prolactin levels in human cerebrospinal fluid during the gamma-vinyl-GABA treatment of patients with complex partial epilepsy. *Neuropeptides* 9: 185-195.

Pitler, T. A. and Alger, B. E. (1994) Differences between presynaptic and postsynaptic $GABA_B$ mechanisms in rat hippocampal pyramidal cells. *J. Neurophysiol.* 72: 2317-2327.

Pittaluga, A., Asaro, D., Pellegrini, G., and Raiteri, M. (1987) Studies on [$^3$H]GABA and endogenous GABA release in rat cerebral cortex suggest the presence of autoreceptors of the $GABA_B$ type. *Eur. J. Pharmacol.* 144: 45-52.

Porter, R. J. and Rogawski, M. A. (1992) New antiepileptic drugs: From serendipity to rational discovery. *Epilepsia* 33 Suppl. 1: S1-S6.

Potier, B. and Dutar, P. (1993) Presynaptic inhibitory effect of baclofen on hippocampal inhibitory synaptic transmission involves a pertussis toxin-sensitive G-protein. *Eur. J. Pharmacol.* 231: 427-433.

Premkumar, L. S., Chung, S. H., and Gage, P. W. (1990) GABA-induced potassium channels in cultured neurons. *Proc. R. Soc. Lond. B. Biol. Sci.* 241: 153-158.

Prince, D. A. (1968a) The depolarization shift in "epileptic" neurons. *Exp. Neurol.* 467-485.

Prince, D. A. (1968b) Inhibition in "epileptic" neurons. *Exp. Neurol.* 21: 307-321.

Prince, D. A., Jacobs, K. M., Salin, P. A., Hoffman, S., and Parada, I. (1997) Chronic focal neocortical epileptogenesis: does disinhibition play a role? *Canadian Journal of Physiology & Pharmacology* 75: 500-507.

Prince, D. A. and Connors, B. W. (1986) Mechanisms of interictal epileptogenesis. In: *Advances in Neurology, Vol. 44*, edited by A. V. Delgado-Escueta, A. A. Ward,Jr., D. M. Woodbury and R. J. Porter. New York: Raven Press, p. 275-299.

Prince, D. A. and Wilder, B. J. (1967) Control mechanisms in cortical epileptogenic foci. *Arch. Neurol.* 16: 194-202.

Pritchett, D. B., Sontheimer, H., Shivers, B. D., Ymer, S., Kettenmann, H., Schofield, P. R., and Seeburg, P. H. (1989) Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. *Nature* 338: 582-585.

Qian, H. and Dowling, J. E. (1993) Novel GABA responses from rod-driven retinal horizontal cells. *Nature* 361: 162-164.

Qian, N. and Sejnowski, T. J. (1990) When is an inhibitory synapse effective? *Proc. Natl. Acad. Sci. USA* 87: 8145-8149.

Quirk, K., Whiting, P. J., Ragan, C. I., and McKernan, R. M. (1995) Characterisation of delta-subunit containing $GABA_A$ receptors from rat brain. *Eur. J. Pharmacol.* 290: 175-181.

Qume, M. and Fowler, L. J. (1996) Effects of chronic oral treatment with GABA-transaminase inhibitors on the GABA system in brain, liver, kidney, and plasma of the rat. *Biochem. Pharmacol.* 52: 1355-1363.

Rak, I. W. and Lothman, E. W. (1988) Electrophysiologic effects of acute and chronic gamma-vinyl-GABA on GABA-mediated inhibition in the rat hippocampus. *Neurology* 38 (Suppl. 1): 240, Abstr. # PP371-240, (Abstract).

Reid, S. A., Sypert, G. W., Boggs, W. M., and Willmore, L. J. (1979) Histopathology of the ferric-induced chronic epileptic focus in cat: a Golgi study. *Exp. Neurol.* 66: 205-219.

Rekling, J. C., Jahnsen, H., and Laursen, A. M. (1990) The effect of two lipophilic gamma-aminobutyric acid uptake blockers in CA1 of the rat hippocampal slice. *Br. J. Pharmacol.* 99: 103-106.

Ribak, C. E., Tong, W. M. Y., and Brecha, N. C. (1996) GABA plasma membrane transporters, GAT-1 and GAT-3, display different distributions in the rat hippocampus. *J. Comp. Neurol.* 367: 595-606.

Richens, A. Potential antiepileptic drugs. Vigabatrin. In: *Antiepileptic Drugs, Third Edition*, edited by R. Levy, R. Mattson, B. Meldrum, J. K. Penry and F. E. Dreifuss. New York: Raven Press, 1989, p. 937-946.

Riekkinen, P. J., Pitkanen, A., Ylinen, A., Sivenius, J., and Halonen, T. (1989a) Specificity of vigabatrin for the GABAergic system in human epilepsy. *Epilepsia* 30(S3): S18-S22.

Riekkinen, P. J., Ylinen, A., Halonen, T., Sivenius, J., and Pitkanen, A. (1989b) Cerebrospinal fluid GABA and seizure control with vigabatrin. *Br. J. Clin. Pharmacol.* 27(S1): 87S-94S.

Rimmer, E. M. and Richens, A. (1989) Interaction between vigabatrin and phenytoin. *Br. J. Clin. Pharmacol.* 27 Suppl. 1: 27S-33S.

Rimvall, K., Sheikh, S. N., and Martin, D. L. (1993) Effects of increased gamma-aminobutyric acid levels on GAD67 protein and mRNA levels in rat cerebral cortex. *J. Neurochem.* 60: 714-720.

Rimvall, K. and Martin, D. L. (1994) The level of $GAD_{67}$ protein is highly sensitive to small increases in intraneuronal γ-aminobutyric acid levels. *J. Neurochem.* 62: 1375-1381.

Roepstorff, A. and Lambert, J. D. C. (1992) Comparison of the effect of the GABA uptake blockers, tiagabine and nipecotic acid, on inhibitory synaptic efficacy in hippocampal CA1 neurones. *Neurosci. Lett.* 146: 131-134.

Rogawski, M. A. and Porter, R. J. (1990) Antiepileptic drugs: Pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. *Pharmacol. Rev.* 42: 223-270.

Rogers, C. J. and Hunter, B. E. (1992) Chronic ethanol treatment reduces inhibition in CA1 of the rat hippocampus. *Brain Res. Bull.* 28: 587-592.

Rudy, B. (1988) Diversity and ubiquity of K channels. *Neuroscience* 25: 729-749.

Rundfeldt, C. and Löscher, W. (1992) Development of tolerance to the anticonvulsant effect of vigabatrin in amygdala-kindled rats. *Eur. J. Pharmacol.* 213: 351-366.

Sachdeo, R. C., Leroy, R. F., Krauss, G. L., Drake, M. E.,Jr., Green, P. M., Leppik, I. E., Shu, V. S., Ringham, G. L., and Sommerville, K. W. (1997) Tiagabine therapy for complex partial seizures - A dose-frequency study. *Arch. Neurol.* 54: 595-601.

Sakmann, B., Hamill, O. P., and Bormann, J. (1983) Patch-clamp measurements of elementary chloride currents activated by the putative inhibitory transmitter GABA and glycine in mammalian spinal neurons. *J. Neural Transm. Suppl.* 18: 83-95.

Saletu, B., Grunberger, J., Linzmayer, L., Schwartz, J. J., Haegele, K. D., and Schechter, P. J. (1986) Psychophysiological and psychometric studies after manipulating the GABA system by vigabatrin, a GABA-transaminase inhibitor. *Int. J. Psychophysiol.* 4: 63-80.

Sander, J. W. A. S., Trevisol-Bittencourt, P. C., Hart, Y. M., and Shorvon, S. D. (1990) Evaluation of vigabatrin as an add-on drug in the management of severe epilepsy. *J. Neurol. Neurosurg. Psychiatry* 53: 1008-1010.

Sander, J. W. A. S. and Shorvon, S. D. (1996) Epidemiology of the epilepsies. *J. Neurol. Neurosurg. Psychiatry* 433-444.

Santos, A. E., Carvalho, C. M., Macedo, T. A., and Carvalho, A. P. (1995) Regulation of intracellular [$Ca^{2+}$] and GABA release by presynaptic $GABA_B$ receptors in rat cerebrocortical synaptosomes. *Neurochem. Int.* 27: 397-406.

Santos, M. S., Rodriguez, R., and Carvalho, A. P. (1992) Effect of depolarizing agents on the $Ca^{2+}$-independent and $Ca^{2+}$-dependent release of [3H]GABA from sheep brain synaptosomes. *Biochem. Pharmacol.* 44: 301-308.

Sarhan, S. and Seiler, N. (1979) Metabolic inhibitors and subcellular distribution of GABA. *J. Neurosci. Res.* 4: 399-421.

Scanziani, M., Gähwiler, B. H., and Thompson, S. M. (1991) Paroxysmal inhibitory potentials mediated by $GABA_B$ receptors in partially disinhibited rat hippocampal slice cultures. *J. Physiol. (Lond. )* 444: 375-396.

Schachter, S. C. (1995) Tiagabine monotherapy in the treatment of partial epilepsy. *Epilepsia* 36: S2-S6.

Schechter, P. J., Tranier, Y., Jung, M. J., and Böhlen, P. (1977) Audiogenic seizure protection by elevated brain GABA concentration in mice: effects of γ-acetylenic GABA and γ-vinyl GABA, two irreversible GABA-T inhibitors. *Eur. J. Pharmacol.* 45: 319-328.

Schechter, P. J., Hanke, N. F. J., Grove, J., Huebert, N., and Sjoerdsma, A. (1984) Biochemical and clinical effects of γ-vinyl GABA in patients with epilepsy. *Neurology* 34: 182-186.

Schechter, P. J. (1989) Clinical pharmacology of vigabatrin. *Br. J. Clin. Pharmacol.* 27(S1): 19S-22S.

Schiavo, G., Benfenati, F., Poulain, B., Rossetto, O., Polverino de Laureto, P., DasGupta, B. R., and Montecucco, C. (1992) Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin [see comments]. *Nature* 359: 832-835.

Schmidt, M. F. and Perkel, D. J. (1998) Slow synaptic inhibition in nucleus HVc of the adult zebra finch. *J. Neurosci.* 18: 895-904.

Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., and Glencorse, T. A. (1987) Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. *Nature* 328: 221-227.

Schousboe, A., Wu, J. Y., and Roberts, E. (1973) Purification and characterization of the 4-aminobutyrate-2-ketoglutarate transaminase from mouse brain. *Biochemistry* 12: 2868-2873.

Schousboe, A., Thorbek, P., Hertz, L., and Krogsgaard-Larsen, P. (1979) Effects of GABA analogues of restricted conformation on GABA transport in astrocytes and brain cortex slices and on GABA receptor binding. *J. Neurochem.* 33: 181-189.

Schousboe, A., Larsson, O. M., and Seiler, N. (1986) Stereoselective uptake of the GABA-transaminase inhibitors gamma-vinyl GABA and gamma-acetylenic GABA into neurons and astrocytes. *Neurochem. Res.* 11: 1497-1505.

Schousboe, I., Bro, B., and Schousboe, A. (1977) Intramitochondrial localization of the 4-aminobutyrate-2-oxoglutarate transaminase from ox brain. *Biochem. J.* 162: 303-307.

Schönrock, B. and Bormann, J. (1993) Functional heterogeneity of hippocampal $GABA_A$ receptors. *Eur. J. Neurosci.* 5: 1042-1049.

Schwartzkroin, P. A. and Mathers, L. H. (1978) Physiological and morphological identification of a nonpyramidal hippocampal cell type. *Brain Res.* 157: 1-10.

Schwartzkroin, P. A. and Prince, D. A. (1980) Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity. *Brain Res.* 183: 61-76.

Schwartzkroin, P. A. and Stafstrom, C. E. (1980) Effects of EGTA on the calcium-activated afterhyperpolarization in hippocampal CA3 pyramidal cells. *Science* 210: 1125-1126.

Schwarzer, C., Tsunashima, K., Wanzenbock, C., Fuchs, K., Sieghart, W., and Sperk, G. (1997) $GABA_A$ receptor subunits in the rat hippocampus II: altered distribution in kainic acid-induced temporal lobe epilepsy. *Neuroscience* 80: 1001-1017.

Sedman, A. J., Gilmet, G. P., Sayed, A. J., and Posvar, E. L. (1990) Initial human safety and tolerance study of a GABA uptake inhibitor, CI-966: potential role of GABA as a mediator in the pathogenesis of schizophrenia and mania. *Drug Dev. Res.* 21: 235-242.

Segal, M. and Barker, J. L. (1984) Rat hippocampal neurons in culture: voltage-clamp analysis of inhibitory synaptic connections. *J. Neurophysiol.* 52: 469-487.

Shimada, S., Cutting, G., and Uhl, G. R. (1992) γ-Aminobutyric acid A or C receptor? γ-Aminobutyric acid rho 1 receptor RNA induces bicuculline-,

barbiturate-, and benzodiazepine- insensitive gamma-aminobutyric acid responses in Xenopus oocytes. *Mol. Pharmacol.* 41: 683-687.

Shin, C., Rigsbee, L. C., and McNamara, J. O. (1986) Anti-seizure and anti-epileptogenic effect of γ-vinyl γ-aminobutyric acid in amygdaloid kindling. *Brain Res.* 398: 370-374.

Shinnar, S. (1997) Tiagabine. [Review]. *Seminars in Pediatric Neurology* 4: 24-33.

Shiosaka, S., Yamamoto, T., Hertzberg, E. L., and Nagy, J. I. (1989) Gap junction protein in rat hippocampus: Correlative light and electron microscope immunohistochemical localization. *J. Comp. Neurol.* 281: 282-297.

Shivers, B. D., Killisch, I., Sprengel, R., Sontheimer, H., Köhler, M., Schofield, P. R., and Seeburg, P. H. (1989) Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. *Neuron* 3: 327-337.

Sihra, T. S. and Nicholls, D. G. (1987) 4-Aminobutyrate can be released exocytotically from guinea-pig cerebral cortical synaptosomes. *J. Neurochem.* 49: 261-267.

Sik, A., Ylinen, A., Penttonen, M., and Buzsáki, G. (1994) Inhibitory CA1-CA3-hilar region feedback in the hippocampus. *Science* 265: 1722-1724.

Sik, A., Penttonen, M., Ylinen, A., and Buzsáki, G. (1995) Hippocampal CA1 interneurons: An *in vivo* intracellular labeling study. *J. Neurosci.* 15: 6651-6665.

Silverman, R. B., Andruszkiewicz, R., Nanavati, S. M., Taylor, C. P., and Vartanian, M. G. (1991) 3-Alkyl-4-aminobutyric acids: The first class of anticonvulsant agents that activates L-glutamic acid decarboxylase. *J. Med. Chem.* 34: 2295-2298.

Sitges, M., Chiu, L. M., and Gonzalez, L. (1993) Vesicular and carrier-mediated depolarization-induced release of [3H]GABA: inhibition by amiloride and verapamil. *Neurochem. Res.* 18: 1081-1087.

Sivenius, M. R., Ylinen, A., Murros, K., Matilainen, R., and Riekkinen, P. (1987) Double-blind dose reduction study of vigabatrin in complex partial epilepsy. *Epilepsia* 28: 688-692.

Smith, K. A. and Fisher, R. S. (1996) The selective $GABA_B$ antagonist CGP-35348 blocks spike-wave bursts in the cholesterol synthesis rat absence epilepsy model. *Brain Res.* 729: 147-150.

Smith, S. S. and Li, J. (1991) $GABA_B$ receptor stimulation by baclofen and taurine enhances excitatory amino acid induced phosphatidylinositol turnover in neonatal rat cerebellum. *Neurosci. Lett.* 132: 59-64.

Snead, O. C.,III (1996) Antiabsence seizure activity of specific $GABA_B$ and gamma-hydroxybutyric acid receptor antagonists. *Pharmacol. Biochem. Behav.* 53: 73-79.

Solís, J. M. and Nicoll, R. A. (1992) Postsynaptic action of endogenous GABA released by nipecotic acid in the hippocampus. *Neurosci. Lett.* 147: 16-20.

Soltesz, I., Haby, M., Leresche, N., and Crunelli, V. (1988) The $GABA_B$ antagonist phaclofen inhibits the late $K^+$-dependent IPSP in cat and rat thalamic and hippocampal neurones. *Brain Res.* 448: 351-354.

Somjen, G. G. (1984) Acidification of interstitial fluid in hippocampal formation caused by seizures and by spreading depression. *Brain Res.* 311: 186-188.

Somjen, G. G. and Giacchino, J. L. (1985) Potassium and calcium concentrations in interstitial fluid of hippocampal formation during paroxysmal responses. *J. Neurophysiol.* 53: 1098-1108.

Somogyi, P., Nunzi, M. G., Gorio, A., and Smith, A. D. (1983) A new type of specific interneuron in the monkey hippocampus forming synapses exclusively with the axon initial segments of pyramidal cells. *Brain Res.* 259: 137-142.

Sperk, G., Schwarzer, C., Tsunashima, K., Fuchs, K., and Sieghart, W. (1997) $GABA_A$ receptor subunits in the rat hippocampus I: immunocytochemical distribution of 13 subunits. *Neuroscience* 80: 987-1000.

Staley, K. J., Soldo, B. L., and Proctor, W. R. (1995) Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science* 269: 977-981.

Staley, K. J. and Mody, I. (1992) Shunting of excitatory input to dentate gyrus granule cells by a depolarizing $GABA_A$ receptor-mediated postsynaptic conductance. *J. Neurophysiol.* 68: 197-212.

Storm, J. F. Potassium currents in hippocampal pyramidal cells. In: *Progress in Brain Research*, edited by J. Storm-Mathisen, J. Zimmer and O. P. Ottersen. Elsevier Science Publishers B.V. 1990, p. 161-187.

Su, A., Mager, S., Mayo, S. L., and Lester, H. A. (1996) A multi-substrate single-file model for ion-coupled transporters. *Biophys. J.* 70: 762-777.

Suranyi-Cadotte, B. E., Dam, T. V., and Quirion, R. (1984) Antidepressant-anxiolytic interaction: decreased density of benzodiazepine receptors in rat brain following chronic administration of antidepressants. *Eur. J. Pharmacol.* 106: 673-675.

Suzdak, P. D., Frederiksen, K., Andersen, K. E., Sorensen, P. O., Knutsen, L. J. S., and Nielsen, E. B. (1992a) NNC-711, a novel potent and selective gamma-aminobutyric acid uptake inhibitor: pharmacological characterization. *Eur. J. Pharmacol.* 224: 189-198.

Suzdak, P. D., Swedberg, M. D. B., Andersen, K. E., Knutsen, L. J. S., and Braestrup, C. (1992b) In vivo labeling of the central GABA uptake carrier with $^3$H-Tiagabine. *Life Sci.* 51: 1857-1868.

Suzdak, P. D. (1994) Lack of tolerance to the anticonvulsant effects of tiagabine following chronic (21 day) treatment. *Epilepsy Res.* 19: 205-213.

Suzdak, P. D., Foged, C., and Andersen, K. E. (1994) Quantitative autoradiographic characterization of the binding of [$^3$H]tiagabine (NNC 05-328) to the GABA uptake carrier. *Brain Res.* 647: 231-241.

Suzdak, P. D. and Jansen, J. A. (1995) A review of the preclinical pharmacology of tiagabine: a potent and selective anticonvulsant GABA uptake inhibitor. *Epilepsia* 36: 612-626.

Suzuki, Y., Mimaki, T., Arai, H., Okada, S., and Kuriyama, K. (1991) Effect of γ-vinyl γ-aminobutyric acid on the γ-aminobutyric acid receptor-coupled chloride ion channel in vesicles from the brain of the rat. *Neuropharmacology* 30: 423-427.

Szerb, J. C. (1982a) Effect of nipecotic acid, a γ-aminobutyric acid transport inhibitor, on the turnover and release of γ-aminobutyric acid in the cortical slices. *J. Neurochem.* 39: 850-858.

Szerb, J. C. (1982b) Turnover and release of GABA in rat cortical slices: effect of a GABA-T inhibitor, gabaculine. *Neurochem. Res.* 7: 191-204.

Taira, T., Paalasmaa, P., Voipio, J., and Kaila, K. (1995) Relative contributions of excitatory and inhibitory neuronal activity to alkaline transients evoked by stimulation of Schaffer collaterals in the rat hippocampal slice. *J. Neurophysiol.* 74: 643-649.

Tartara, A., Manni, R., Galimberti, C. A., Hardenberg, J., Orwin, J., and Perucca, E. (1986) Vigabatrin in the treatment of epilepsy: a double-blind placebo-controlled study. *Epilepsia* 27: 717-723.

Tartara, A., Manni, R., Galimberti, C. A., Mumford, J. P., Iudice, A., and Perucca, E. (1989) Vigabatrin in the treatment of epilepsy: a long-term follow-up study. *J. Neurol. Neurosurg. Psychiatry* 52: 467-471.

Tartara, A., Manni, R., Galimberti, C. A., Morini, R., Mumford, J. P., Iudice, A., and Perucca, E. (1992) Six-year follow-up study on the efficacy and safety of vigabatrin in patients with epilepsy. *Acta Neurol.Scand.* 86: 247-251.

Tassinari, C. A., Michelucci, R., Ambrosetto, G., and Salvi, F. (1987) Double-blind study of vigabatrin in the treatment of drug-resistant epilepsy. *Arch. Neurol.* 44: 907-910.

Taylor, C. P., Vartanian, M. G., Andruszkiewicz, R., and Silverman, R. B. (1992) 3-Alkyl GABA and 3-alkylglutamic acid analogues: Two new classes of anticonvulsant agents. *Epilepsy Res.* 11: 103-110.

Taylor, C. P. and Dudek, F. E. (1982) Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. *Science* 218: 810-812.

Thalmann, R. H., Peck, E. J., and Ayala, G. F. (1981) Biphasic response of hippocampal pyramidal neurons to GABA. *Neurosci. Lett.* 21: 319-324.
Thalmann, R. H. (1988) Evidence that guanosine triphosphate (GTP)-binding proteins control a synaptic response in brain: effect of pertussis toxin and GTPγS on the late inhibitory postsynaptic potential of hippocampal CA3 neurons. *J. Neurosci.* 8: 4589-4602.

Thalmann, R. H. and Ayala, G. F. (1982) A late increase in potassium conductance follows synaptic stimulation of granule neurons of the dentate gyrus. *Neurosci. Lett.* 29: 243-248.

Théorêt, Y., Brown, A., Fleming, S. P., and Čapek, R. (1984) Hippocampal field potential: a microcomputer aided comparison of amplitude and integral. *Brain Res. Bull.* 12: 589-595.

Thompson, S. M. and Gähwiler, B. H. (1989a) Activity-dependent disinhibition. I. Repetitive stimulation reduces IPSP driving force and conductance in the hippocampus in vitro. *J. Neurophysiol.* 61: 501-511.

Thompson, S. M. and Gähwiler, B. H. (1989b) Activity-dependent disinhibition. III. Desensitization and $GABA_B$ receptor-mediated presynaptic inhibition in the hippocampus in vitro. *J. Neurophysiol.* 61: 524-533.

Thompson, S. M. and Gähwiler, B. H. (1989c) Activity-dependent disinhibition. II. Effects of extracellular potassium, furosemide, and membrane potential on $E_{Cl^-}$ in hippocampal CA3 neurons. *J. Neurophysiol.* 61: 512-523.

Thompson, S. M. and Gähwiler, B. H. (1992a) Effects of the GABA uptake inhibitor tiagabine on inhibitory synaptic potentials in rat hippocampal slice cultures. *J. Neurophysiol.* 67: 1698-1701.

Thompson, S. M. and Gähwiler, B. H. (1992b) Comparison of the actions of baclofen at pre- and postsynaptic receptors in the rat hippocampus *in vitro*. *J. Physiol. (Lond. )* 451: 329-345.

Traub, R. D., Miles, R., and Wong, R. K. S. (1987a) Models of synchronized hippocampal bursts in the presence of inhibition. I. Single population events. *J. Neurophysiol.* 58: 739-751.

Traub, R. D., Miles, R., Wong, R. K. S., Schulman, L. S., and Schneiderman, J. H. (1987b) Models of synchronized hippocampal bursts in the presence of inhibition. II. Ongoing spontaneous population events. *J. Neurophysiol.* 58: 752-764.

Traub, R. D., Miles, R., and Jefferys, J. G. (1993) Synaptic and intrinsic conductances shape picrotoxin-induced synchronized after-discharges in the guinea-pig hippocampal slice. *J. Physiol. (Lond)* 461: 525-547.

Traub, R. D., Colling, S. B., and Jefferys, J. G. R. (1995) Cellular mechanisms of 4-aminopyridine-induced synchronized after-discharges in the rat hippocampal slice. *J. Physiol. (Lond. )* 489: 127-140.

Traub, R. D. and Wong, R. K. (1983) Synchronized burst discharge in disinhibited hippocampal slice. II. Model of cellular mechanism. *J. Neurophysiol.* 49: 459-471.

Tretter, V., Ehya, N., Fuchs, K., and Sieghart, W. (1997) Stoichiometry and assembly of a recombinant $GABA_A$ receptor subtype. *J. Neurosci.* 17: 2728-2737.

Troyer, M. D., Blanton, M. G., and Kriegstein, A. R. (1992) Abnormal action-potential bursts and synchronized, GABA-mediated inhibitory potentials in an in vitro model of focal epilepsy. *Epilepsia* 33: 199-212.

Tunnicliff, G., Ngo, T. T., Rojo-Ortega, J. M., and Barbeau, A. (1977) The inhibition by substrate analogues of gamma-aminobutyrate aminotransferase from mitochondria of different subcellular fractions of rat brain. *Can. J. Biochem.* 55: 479-484.

Turski, W. A., Czuczwar, S. J., Kleinrok, Z., and Turski, L. (1983) Cholinomimetics produce seizures and brain damage in rats. *Experientia* 39: 1408-1411.

Turski, W. A., Cavalheiro, E. A., Bortolotto, Z. A., Mello, L. M., Schwarz, M., and Turski, L. (1984) Seizures produced by pilocarpine in mice: a behavioral, electroencephalographic and morphological analysis. *Brain Res.* 321: 237-253.

Tvrdeić, A. and Perieic, D. (1991) Dihydrogenated ergot compounds bind with high affinity to $GABA_A$ receptor-associated $Cl^-$ ionophore. *Eur. J. Pharmacol.* 202: 109-111.

Twyman, R. E., Rogers, C. J., and Macdonald, R. L. (1990) Intraburst kinetic properties of the GABAA receptor main conductance state of mouse spinal cord neurones in culture. *J. Physiol. (Lond)* 423: 193-220.

Upton, N. (1994) Mechanisms of action of new antiepileptic drugs: Rational design and serendipitous findings. *Trends Pharmacol. Sci.* 15: 456-463.

Valdizán, E. M. and Armijo, J. A. (1991) Relationship between platelet and brain GABA transaminase inhibition by single and multiple doses of vigabatrin in rats. *Epilepsia* 32: 735-742.

Valdizán, E. M. and Armijo, J. A. (1992) Effects of single and multiple increasing doses of vigabatrin on brain GABA metabolism and correlation with vigabatrin plasma concentration. *Biochem. Pharmacol.* 43: 2143-2150.

Van Rijn, C. M., Willems-van Bree, E., Van der Velden, T. J. A. M., and Rodrigues de Miranda, J. F. (1990) Binding of the cage convulsant [3H]TBOB, to sites linked to the $GABA_A$ receptor complex. *Eur. J. Pharmacol.* 179: 419-425.

Vergnes, M., Boehrer, A., Simler, S., Bernasconi, R., and Marescaux, C. (1997) Opposite effects of $GABA_B$ receptor antagonists on absences and convulsive seizures. *Eur. J. Pharmacol.* 332: 245-255.

Vu, E. T. and Krasne, F. B. (1992) Evidence for a computational distinction between proximal and distal neuronal inhibition. *Science* 255: 1710-1712.

Waksman, A., Rubinstein, M. K., Kuriyama, K., and Roberts, E. (1968) Localization of gamma-aminobutyric-alpha-oxoglutaric acid transaminase in mouse brain. *J. Neurochem.* 15: 351-357.

Waldmeier, P. C., Wicki, P., Feldtrauer, J. J., Mickel, S. J., Bittiger, H., Baumann, and PA. (1994) GABA and glutamate release affected by $GABA_B$ receptor antagonists with similar potency: no evidence for pharmacologically different presynaptic receptors. *Br. J. Pharmacol.* 113: 1515-1521.

Walker, A. E. and Johnson, H. C. (1945) Convulsive factor in commercial penicillin. *Arch Surg* 50: 69-73.

Walz, W. (1989) pH shifts evoked by neuronal stimulation in slices of rat hippocampus. *Can. J. Physiol. Pharmacol.* 67: 577-581.

Wang, T. L., Guggino, W. B., and Cutting, G. R. (1994) A novel gamma-aminobutyric acid receptor subunit (rho 2) cloned from human retina forms bicuculline-insensitive homooligomeric receptors in Xenopus oocytes. *J. Neurosci.* 14: 6524-6531.

Weiss, D. S. and Hablitz, J. J. (1984) Interaction of penicillin and pentobarbital with inhibitory synaptic mechanisms in neocortex. *Cell Mol. Neurobiol.* 4: 301-317.

White, G. (1992) Heterogeneity in EC50 and nH of $GABA_A$ receptors on dorsal root ganglion neurons freshly isolated from adult rats. *Brain Res.* 585: 56-62.

Wilder, B. J. (1996) Antiepileptic drugs - Current use. *Can. J. Neurol. Sci.* 23: S18-S23.

Williams, S., Samulack, D. D., Beaulieu, C., and Lacaille, J.-C. (1994) Membrane properties and synaptic responses of interneurons located near the stratum lacunosum-moleculare/radiatum border of area CA1 in whole-cell recordings from rat hippocampal slices. *J. Neurophysiol.* 71: 2217-2235.

Wisden, W., Laurie, D. J., Monyer, H., and Seeburg, P. H. (1992) The distribution of 13 $GABA_A$ receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J. Neurosci.* 12: 1040-1062.

Wojcik, W. J. and Neff, N. H. (1984) gamma-aminobutyric acid B receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells. *Mol. Pharmacol.* 25: 24-28.

Wong, R. K. S. and Prince, D. A. (1979) Dendritic mechanisms underlying penicillin-induced epileptiform activity. *Science* 204: 1228-1231.

Wood, J. D., Tsui, D., and Phillis, J. W. (1979) Structure-activity studies on the inhibition of gamma-aminobutyric acid uptake in brain slices by compounds related to nipecotic acid. *Can. J. Physiol. Pharmacol.* 57: 581-585.

Wood, J. D., Kurylo, E., and Lane, R. (1988) γ-Aminobutyric acid release from synaptosomes prepared from rats treated with isonicotinic acid hydrazide and gabaculine. *J. Neurochem.* 50: 1839-1843.

Woodson, W., Nitecka, L., and Ben-Ari, Y. (1989) Organization of the GABAergic system in the rat hippocampal formation: a quantitative immunocytochemical study. *J. Comp. Neurol.* 280: 254-271.

Xie, X. and Smart, T. G. (1991) A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. *Nature* 349: 521-524.

Xie, X.-H. and Tietz, E. I. (1991) Chronic benzodiazepine treatment of rats induces reduction of paired-pulse inhibition in CA1 region of in vitro hippocampus. *Brain Res.* 561: 69-76.

Xiong, Z., Lu, W., and MacDonald, J. F. (1997) Extracellular calcium sensed by a novel cation channel in hippocampal neurons. *Proc. Natl. Acad. Sci. U. S. A.* 94: 7012-7017.

Yamamoto, C. and McIlwain, H. (1966) Electrical activities in thin sections from the mammalian brain maintained in chemically-defined media in vitro. *J. Neurochem.* 13: 1333-1343.

Yamauchi, A., Uchida, S., Kwon, H. M., Preston, A. S., Robey, R. B., Garcia-Perez, A., Burg, M. B., and Handler, J. S. (1992) Cloning of a Na(+)- and Cl(-)-dependent betaine transporter that is regulated by hypertonicity. *J. Biol. Chem.* 267: 649-652.

Ylinen, A., Kälviäinen, R., and Riekkinen, P. J.,Sr. (1995) Long-term efficacy and cognitive effects of vigabatrin. *Acta Neurologica Scandinavica* Supplementum. 162: 47-50.

Yunger, L. M., Fowler, P. J., Zarevics, P., and Setler, P. E. (1984) Novel inhibitors of γ-aminobutyric acid (GABA) uptake: anticonvulsant actions in rats and mice. *J. Pharmacol. Exp. Ther.* 228: 109-115.

Zanotti, A., Mariot, R., Contarino, A., Lipartiti, M., and Giusti, P. (1996) Lack of anticonvulsant tolerance and benzodiazepine receptor down regulation with imidazenil in rats. *Br. J. Pharmacol.* 117: 647-652.

# Appendix

## 10. Appendix

Table 1. Targets of established anticonvulsants:

| | Na channels | T-Ca channels | Excitatory Transmission | GABA Inhibition |
|---|---|---|---|---|
| Barbiturates | - | - | ?/+ | + |
| Benzodiazepines | - | - | - | ++ |
| Carbamazepine | ++ | - | - | - |
| Phenytoin | ++ | - | - | - |
| Ethosuximide | - | ++ | - | - |
| Valproate | ++ | ?/+ | - | ?/+ |

Table was constructed based on reviews by Rogawski and Porter, 1990 and by Löscher and Schmidt, 1994

Table 2. Targets of novel anticonvulsants:

| | Na channels | T-Ca channels | Excitatory Transmission | GABA Inhibition |
|---|---|---|---|---|
| Felbamate | - | - | + | + |
| Lamotrigine | ++ | - | - | - |
| Oxcarbazepine | ++ | - | - | - |
| Topiramate | + | - | - | + |
| Tiagabine | - | - | - | ++ |
| Vigabatrin | - | - | - | ++ |

Table was constructed based on reviews by Rogawski and Porter, 1990 and by Löscher and Schmidt, 1994