GABAergic MECHANISMS IN ADRENAL ENZYME REGULATION

by

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

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ABSTRACT

This work concerns the GABAergic mechanisms involved in the regulation of adrenomedullary ornithine decarboxylase and tyrosine hydroxylase. These enzymes play important roles in the biosynthesis of polyamines and catecholamines, respectively. They are regulated by central dopaminergic and cholinergic pathways, with net excitatory influence, as well as by a tonic serotonergic inhibitory one. It has now been determined that certain GABA analogues are also able to elicit increases in both of these enzyme activities by means of a central mechanism. These GABAergic drugs appear to act independently of the dopaminergic, serotonergic and cholinergic pathways. The similarity of the inductive effects of the dopamine (DA) agonist, apomorphine, and GABA analogues such as γ-hydroxybutyrate (GOBA) and HA-966 suggests that these transmitter systems share a common mechanism in the regulatory process. Because GABA, HA-966 and apomorphine prevent the firing of dopaminergic fibres (all three through inhibitory action at the nigral cell bodies, the last one at the nigrostriatal terminations also) it is proposed that this mechanism involves a pre-synaptic action of dopamine and GABA agonists, preventing the release and subsequent post-synaptic inhibitory action of DA in the striatum. This hypothesis has been verified by the administration of the DA autoreceptor-selective agonist (+)3-PPP: this drug caused a significant increase in the activities of the two enzymes. It has also been shown that D-1 and D-2 specific antagonists are equally efficacious in bringing about an attenuation of the apomorphine-elicited increase in ornithine decarboxylase, but the D-1 site seems to be more efficient in the case of tyrosine hydroxylase. The results in this thesis thus serve to define a role for a GABAergic system in the central regulation of adrenal enzymes and provide insight into the mechanism of the inductive process as initiated by dopamine agonists.

RESUME

Ce travail porte sur les mécanismes GABAergiques qui interviennent dans la régulation de la tyrosine hydroxylase et de l'ornithine décarboxylase de la médullo-surrénale. Ces enzymes jouent un rôle important dans la biosynthèse des polyamines et des catécholamines, respectivement. Elles sont régies par les voies cholinergiques et dopaminergiques centrales et soumises à une influence excitatrice nette ainsi qu'une influence inhibitrice sérotoninergique tonique. On a déterminé que certains analogues du GABA sont également capables de provoquer une augmentation de ces deux activités enzymatiques par le biais d'un mécanisme central. Ces drogues GABAergiques semblent agir indépendamment des voies cholinergiques, sérotoninergiques et dopaminergiques. La similarité des effets de déclenchement par l'agoniste de la dopamine (DA) comme l'apomorphine et des analogues du GABA tels que l'hydroxybutyrate (GOBA) et le HA-966 laissent entendre qu'un mécanisme commun interviendrait dans le processus de régulation par ces systèmes de transmission. Puisque le GABA, le HA-966 et l'apomorphine empêchent la décharge des fibres dopaminergiques (tous trois en exerçant une action inhibitrice au niveau du corps cellulaire du locus niger, l'apomorphine agissant également aux terminaisons nigrostriées), on croit que ce mécanisme serait associé à une action pré-synaptique des antagonistes du GABA et de la dopamine, ce qui empêcherait la libération de la DA dans le striatum et, par la suite, son action inhibitrice post-synaptique. On a vérifié cette hypothèse en administrant l'agoniste (+)3-PPP, autorécepteur-sélectif de la DA; cette substance a entraîné un accroissement significatif des activités des deux enzymes. On a également démontré que les antagonistes spécifiques de D-1 et D-2 réussissent tout aussi efficacement à atténuer l'accroissement du taux d'ornithine décarboxylase provoqué par

l'apomorphine; toutefois, le site de D-1 semble plus efficace dans le cas de la tyrosine hydroxylase. Les résultats de cette thèse servent ainsi à définir le rôle du système GABAergique dans la régulation centrale des enzymes surrénales et à éclaircir le mécanisme du processus inductif tel qu'il est amorcé par les agonistes de la dopamine.

This work is dedicated, with affection,

to the memory of a close and dear friend,

Jeffrey Schindelheim, 1963-1984.

ACKNOWLEDGEMENTS

I shall be forever indebted to my research supervisor, Dr. T.L. Sourkes. I am very privileged to have benefitted from his scientific expertise and knowledge, as well as his constant moral support and encouragement throughout my stay in his laboratory. For his kindness and genuine concern, I am most grateful.

I am also very grateful to Krystyna Missala. Her technical expertise, assistance and friendly advice was a tremendous asset in the realization of this work.

I owe special thanks to Marc Ekker, whose help, advice and friendship was very important in enabling me to get my project off the ground.

I would like to thank Dr. S. Regunathan for his collaboration in the experiments requiring surgical procedures such as intracerebroventricular injection and hemisplanchnicotomization as well as for his good-natured advice in writing this thesis. Dr. Kiran Yashphal's surgical assistance is also gratefully acknowledged.

I would also like to thank Karen Teff for helping to provide a most enjoyable working atmosphere. Her special friendship was a tremendous plus in helping me complete my work.

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ABBREVIATIONS

ACh	acetylcholine
AD	adrenaline
amODC	adrenomedullary ornithine decarboxylase
AMPT	alpha-methyl-p-tyrosine
AMTP	alpha-methyltryptophan
ANOVA	analysis of variance
APO	apomorphine
ATHA	adrenal tyrosine hydroxylase activity
CA	catecholamine(s)
cAMP	adenosine-3,5-cyclic monophosphate
Ci	curie
CNS	central nervous system
COMT	catechol-O-methyltransferase
cpm	counts per minute
DA	dopamine
DBH	dopamine beta-hydroxylase
DHT	5,7-dihydroxytryptamine
DOPA	dihydroxyphenylalanine
DOPAC	dihydroxyphenylacetic acid
DRN	dorsal raphe nucleus
Expt	experiment
g	gram
GABA	gamma-aminobutyric acid
GABA-T	GABA-transaminase
GAD	glutamic acid decarboxylase
GBL	gamma-butyrolactone

ABBREVIATIONS

GOBA	gamma-hydroxybutyrate
GVG	gamma-vinylGABA
5-HIAA	5-hydroxyindoleacetic acid
5HT	5-hydroxytryptamine (serotonin)
5-HTP	5-hydroxytryptophan
HVA	homovanillic acid
i.c.v.	intracerebroventricular
i.p.	intraperitoneal
i.v.	intravenous
kg	kilogram
Km	Michaelis-Menten constant
M	molar
MAO	monoamine oxidase
MDMT	N,N-dimethyl-5-methoxytryptamine
µCi	microcurie
µg	microgram
µl	microliter
µmol	micromole
µM	micromolar
mCi	millicurie
mg	milligram
ml	milliliter
mmol	millimole
mM	millimolar
min	minute
MRN	medial raphe nucleus

ABBREVIATIONS

N	normal
ng	nanogram
nmol	nanomole
ODC	ornithine decarboxylase
6-OH-DA	6-hydroxydopamine
PCPA	p-chloro-phenylalanine
PLP	pyridoxal-5'-phosphate
pmol	picomole
s.c.	subcutaneous
SE	standard error
TH	tyrosine hydroxylase

A. INTRODUCTION

1. GABA

(a) Introduction

γ-aminobutyric acid (GABA) was synthesized in 1883 and was known for many years as a product of plant and microbial metabolism. Roberts and Frankel (1950) were the first to find this substance in the mammalian central nervous system, over thirty five years ago. During the ensuing years it has become apparent that this chemically simple material may serve an important neurotransmitter function (Roberts et al., 1976). In fact, it is considered to be the most important inhibitory neurotransmittter in the nervous system. Over one third of all nerve endings within the brain may release GABA as a transmitter (Fonnum and Storm-Mathisen, 1978). Deficiencies in GABA function have been associated with such neurological disorders as Huntington's chorea (Perry et al., 1973), Parkinson's disease (McGeer et al., 1971) and epilepsy (Lloyd et al., 1984). Excellent evidence exists linking the development of debilitating anxiety to the GABA system (Olsen, 1982; Williams, 1983). In fact, today's most prevalent medication for anxiety is Valium (diazepam), a drug which interacts with the GABA-receptor complex. GABA has also been implicated in the regulation of blood pressure (Defeudis, 1983). The data accumulated to support a neurotransmitter role for GABA have been crucial for the development of the general concept of amino acid neurotransmission. The important findings with GABA have become the impetus for studies which have suggested that agents such as glutamic acid, glycine and taurine may also act as neurotransmitters in the central nervous system (CNS) (Enna, 1979).

(b) Synthesis and Metabolism

GABA does not readily penetrate the blood-brain barrier (Roberts, 1962); hence, the transmitter must be synthesized de novo within the

neuron. The major portion of cerebral GABA is derived by the α-decarboxylation of glutamic acid catalyzed by L-glutamic acid decarboxylase (GAD:EC 4.1.1.15; Roberts and Frankel, 1951). This conversion requires pyridoxal 5'-phosphate (PLP) as cofactor (Roberts et al., 1964). A significant amount of GABA is stored in nerve terminals, and numerous studies (Iversen et al., 1971; Ryan and Roskoski, 1975) have shown that it is released by a calcium-dependent process. GABA is taken up into synaptic terminals by an active, high-affinity, Na^+-dependent transport system. Thus, like other neurotransmitters, the termination of the synaptic action of GABA may be brought about by an efficient recapture mechanism (Szerb, 1982). It eventually undergoes metabolic degradation by the action of the mitochondrial enzyme 4-aminobutyrate α-ketoglutarate aminotransferase (GABA-T; EC 2.6.1.19). This PLP-dependent enzyme produces glutamic acid, thereby providing a continuous supply of the GABA precursor. The rate-limiting step in GABA metabolism is at its point of synthesis (Roberts and Kuriyama, 1968). The regulation of steady-state concentrations of GABA is thus, for the most part, the regulation of GAD activity. A possible mechanism for the control of the production of GABA at nerve endings might be the inhibition of synthesis of its biosynthetic enzyme. Evidence for a feedback repression of GAD has been advanced (Sze, 1970) on the basis of the demonstration that when GABA levels are elevated in developing mouse brain, GAD activity is concomitantly reduced.

(c) Distribution

GABA is widely distributed in the nervous system of vertebrate species. Although its presence is almost exclusively confined to the brain and spinal cord, there is evidence for the presence of GABA in peripheral tissues also (Wu et al., 1986). Furthermore, GABAergic neurons have been

found in the enteric nervous system (Jessen et al., 1986). The distribution of GABA in monkey, rabbit and rat brains, described by Fahn and Côté (1968) and others, reveal that there are regional differences in GABA concentrations within the CNS. The highest concentrations are found in the substantia nigra, globus pallidus, hypothalamus and other cell body-rich brain areas, whereas the lowest concentrations are found in white matter. Nevertheless, GABA is more extensively and more evenly distributed in brain tissue than acetylcholine or the monoamines (Fonnum and Storm-Mathisen, 1978). Moreover, unlike the monoamines, the concentrations of GABA found in the CNS is of the order of μmoles/gm rather than nmoles/gm (Cooper et al., 1982).

(d) GABA Receptors

Although the early biochemical studies suggested that GABA behaves like a neurotransmitter, the evidence for such a function was not compelling until electrophysiologists were able to demonstrate a specific response for this substance. The most important finding in this regard was the discovery that the convulsant drugs picrotoxin and bicuculline selectively block the electrophysiological action of GABA (Curtis et al., 1971). These data indicated the presence of physiologically active receptors on mammalian neurons, firmly establishing GABA as a neurotransmitter.

GABA receptors are generally classified as being bicuculline-sensitive or bicuculline-insensitive. The former category defines the GABA-A site while the latter refers to the GABA-B site. GABA-A receptors are present on cell bodies, dendrites and axon terminals whereas GABA-B receptors are found only on the nerve terminals containing neurotransmitters other than GABA (Enna, 1983).

Iontophoretic studies have shown that GABA inhibits the firing of neurons in mammalian CNS in practically every area tested (Feldman and Quenzer, 1984). GABA seems to be involved in two types of inhibitory process: postsynaptic inhibition and presynaptic inhibition. Electrophysiological studies indicate that GABA-A receptors are coupled to a chloride ion channel. Activation of the recognition site triggers a net influx or efflux of chloride ion, depending upon the prevailing concentration gradient (Enna and Gallagher, 1983). GABA can induce postsynaptic inhibition by hyperpolarizing the postsynaptic membrane. This is achieved by activating GABA-A receptors located on the soma or dendrites of the postsynaptic neuron. Activation at these sites allows for the passage of chloride ions down its concentration gradient into the neuron, causing hyperpolarization and a decrease in cellular activity. If the chloride concentration of the surrounding medium is substantially lowered GABA's hyperpolarizing effect can be blocked. The other form of GABA-mediated inhibition, presynaptic inhibition, is commonly found in sensory systems and serves to modulate sensory input (Feldman and Quenzer, 1984). Activation of GABA-A receptors at presynaptic sites, i.e. at nerve terminals, leads to a net efflux of chloride ion causing a partial depolarization. When a nerve impulse reaches the slightly depolarized terminals, there is a relatively small influx of calcium ion and a smaller amount of transmitter is released (Feldman and Quenzer, 1984). This results in a smaller excitatory effect at the postsynaptic site.

Much less is known about the mechanisms associated with GABA-B receptor activation. In contrast to GABA-A receptors, binding to GABA-B sites is calcium-dependent and is not blocked by bicuculline (Hill and Bowery, 1981). Because this receptor is coupled to the neurotransmitter release process (Bowery et al., 1980), it would seem likely that GABA-B

receptors may influence intracellular levels of calcium.

In general, despite the differences between the GABA-A and GABA-B receptor subtypes, cellular activity is diminished following GABA-receptor activation. Therefore, GABA is designated an inhibitory neurotransmitter. The studies carried out in this thesis are based on this premise; however, although GABA is considered inhibitory at the neuronal level, the possibility that GABA can have net excitatory effects as a result of complex interneuronal connections cannot be excluded.

2. GABAergic Agents Used In This Work:

The emerging importance of GABA in the neuropathology of Huntington's chorea and epilepsy has been the impetus for a great deal of research devoted to the development of GABAmimetic agents. Several have been synthesized or isolated to date, but for the purpose of studying the GABAergic mechanisms presented in this thesis, only five GABA analogues were used (see Figure I). What follows in this section is a brief review of some of the physiological and biochemical properties of these compounds.

(a) Muscimol, Progabide and Bicuculline

i) Muscimol:

Muscimol (3-hydroxy-5-aminomethylisoxazole,) isolated from the mushroom Amanita muscaria, has a striking structural similarity to GABA (Curtis and Watkins, 1965). Iontophoretic application of muscimol to selected neuronal cell populations reveals that muscimol has a pronounced GABA-like inhibitory activity which can be antagonized by bicuculline but not strychnine (Johnston et al., 1968). Receptor-binding and electrophysiological tests suggest that muscimol is a GABA-A agonist (Hill

and Bowery, 1981). In fact, in vitro studies have indicated that muscimol's affinity for GABA receptors is greater than that of GABA itself (Enna et al., 1977). The higher affinity of muscimol has been attributed to its structure; the distance between the two charged groups on the isoxazolol derivative has been shown to be ideal for agonistic action on GABA receptors (Krogsgaard-Larsen et al., 1975). Furthermore the rigid structural configuration prevents an interaction between muscimol and GABA reuptake (Krogsgaard-Larsen et al., 1975). The lack of a recapture mechanism prolongs the synaptic action of muscimol; this is in accord with the views of Naik et al. (1976), who suggest that muscimol has a potent and long-lasting GABA-like action.

As opposed to other GABA agonists, muscimol manifests central nervous system activity in man after systemic administration, causing lethargy in low doses and hallucinations at high doses (Waser, 1967). Similarly, Naik et al., (1976) have shown that intravenously injected muscimol acts on the rat brain as a potent GABA agonist. In general, conformationally restricted analogues of GABA, like GABA itself, do not cross the blood-brain barrier to influence central GABA receptors after systemic administration. The exceptions appear to be those analogues, such as muscimol, which contain 3-isoxazole moieties as masked carboxyl groups (Johnston, 1978). After intravenous injection, muscimol enters the brain and is distributed unevenly to various regions. According to Baraldi et al. (1979), the regions with the highest concentrations are substantia nigra, the colliculi and the hypothalamus. However, only 0.02% of authentic muscimol is found in rat brain 30 minutes after i.v. injection of a pharmacologically active dose (8 μmole/kg or <1 mg/kg; Baraldi et al., 1979). The drug disappears rapidly from the blood and this is paralleled by a rise in plasma levels of various metabolites (Baraldi et al., 1979).

These observations appear to suggest two factors that are responsible for the relatively low levels of muscimol in brain. (1) The first is the slow diffusion of muscimol through the blood-brain barrier. Studies by Maggi and Enna (1979) indicate that muscimol accumulation in brain after i.v. administration is not significantly greater than that of GABA itself. Thus, while muscimol does enter the brain, it does so at a very slow rate. Nevertheless, some of the pharmacological actions of muscimol following systemic administration are probably attributable to the uptake of authentic muscimol by the brain. For example, the injection of 0.8-1.6 nmol of muscimol directly into the brain has an effect on the haloperidol-induced activation of the nigrostriatal system which is comparable to that obtained by i.v. injection of 4-8 μmole/kg of this drug (Gale et al., 1978). Thus, the small aliquot of an intravenously injected dose of muscimol that enters the brain (0.02%; Baraldi et al., 1979), is sufficient to explain some of the pharmacological actions observed.

(2) The second factor contributing to low brain levels of muscimol is its surprisingly rapid metabolism in the periphery. The catabolic pathways for this drug could be oxidative deamination or transamination of the side chain (Baraldi et al., 1979; Maggi and Enna, 1979). Animals given aminooxyacetic acid, a GABA-T inhibitor, have tissue and brain concentrations of muscimol that are several times greater than those of untreated animals (Baraldi et al., 1979; Maggi and Enna, 1979). This suggests that transamination is the major pathway for muscimol breakdown. However, the possibility exists that muscimol metabolites may also penetrate the brain. Hence, the effects observed after systemic administration of muscimol may not be due to an action of muscimol alone but rather, to a combination of muscimol and its derivatives (Maggi and

Enna, 1979).

Like all GABAergic agents, muscimol appears to depress nigrostriatal dopaminergic transmission. However, this agent is unique in that this inhibitory effect is exerted only at the level of the substantia nigra (Wood, 1982). Systemic as well as intranigral administration of muscimol antagonizes the haloperidol-induced activation of striatal tyrosine hydroxylase (TH) (Gale et al., 1978), suggesting a mechanism for the GABAergic regulation of nigrostriatal dopamine neurons. However, other authors claim that muscimol's effect on dopamine metabolism differs from that of other GABA analogues, such as GOBA or HA-966 (see section 2 (b) below), in that it is not associated with DA accumulation (Biggio et al., 1977). Accordingly, Wood (1982) has suggested that a GABA receptor exclusive of dopaminergic nerve endings exists within the striatum which is sensitive to muscimol. Some have suggested that muscimol might even facilitate dopamine release in certain circumstances (Matsui and Kamioka, 1978). Indeed, the effect of muscimol on dopamine transmission is complex. Even the route of administration must be considered: whereas i.v. muscimol produces significant decreases in DA concentration, i.p. muscimol causes increases (Gundlach and Beart, 1981).

ii) Bicuculline:

The convulsant alkaloid bicuculline, isolated from Corydalis and Dicentra plants, is now generally accepted as a selective GABA antagonist (Johnston, 1978). This antagonism of inhibitory synaptic transmission mediated by GABA was shown by experiments in which drugs were applied iontophoretically to neurons in the vertebrate central nervous system (Curtis et al., 1971). The alkaloid appears to act directly at the GABA-A receptor recognition site, hence it is a competitive antagonist (Enna,

1979). Bicuculline-sensitive synaptic inhibitions are found in all areas of the brain. This may reflect the widespread involvement of GABA as an inhibitory transmitter; however, other bicuculline-sensitive inhibitors, such as β-alanine and taurine, are found in the brain, so that not all bicuculline-sensitive synaptic inhibitions necessarily involve GABA. The neurochemical data appear to support the view that GABA is involved in the vast majority of bicuculline-sensitive inhibitions (Andrews and Johnston, 1979).

Early doubts regarding the usefulness of bicuculline as an investigative drug have been related to its instability under certain conditions. At physiological pH and 37° C it is hydrolyzed to the relatively inactive GABA antagonist bicucine (Olsen et al., 1975). The conversion is effected by cleavage of the lactone ring (see structure, Figure I) and it occurs within a few minutes.

Quaternary salts of bicuculline such as bicuculline methiodide (N-methylbicuculline) and bicuculline methochloride are stable in the pH range 2-8 and are much more water-soluble than simple salts of bicuculline (Andrews and Johnston, 1979). For these reasons, they are considered quite useful for many studies involving the GABAergic system. Simple salts of bicuculline are essential, however, for studies of central effects following systemic administration because the quaternary salts of the alkaloid do not readily cross the blood-brain barrier. The experiments carried out in this thesis make use solely of the free base of bicuculline.

iii) Progabide:

Progabide is a synthetic compound defined as the Schiff base of gamma-aminobutyramide and a substituted benzophenone. It was synthesized by Dr. J.P. Kaplan of Laboratoires d'Etudes et de Recherches Synthélabo, Paris

(Kaplan et al., 1980). The benzylidene core, linked to the gamma-aminobutyramide side chain, provides the mechanism for transport across the gut and the blood-brain barrier (Bergmann, 1985).

Progabide undergoes extensive metabolic degradation. The major metabolite is the acid derivative (SL 75.102), formed by either oxidative deamination or transamination. By cleavage of the imine bond, progabide and its acid derivative may give rise to GABAmide or GABA, respectively (Worms et al., 1982). After i.p. and oral administration, progabide appears to be distributed extensively and rapidly to various body tissues and organs (Worms et al., 1982). The metabolites and GABA appear in the circulation and in the brain a few minutes after administration. Brain concentrations of unchanged progabide are 2-3 times higher than the corresponding plasma levels over the entire observation period, suggesting that indeed, progabide crosses the blood-brain barrier. In contrast, the higher plasma levels and lower brain concentrations of SL75.102 as compared to progabide are consistent with the higher polarity and poorer penetrability of the acid derivative (Worms et al., 1982). Levels of GABA and GABAmide continue to rise in the brain quite slowly, peaking 4-6 hours after administration (Worms et al., 1982). These two metabolites most probably originate there, as a result of conversion centrally, because both cross the blood-brain barrier with difficulty.

It should be noted that the GABAmimetic properties of progabide are due to the direct action of SL75.102 and progabide on GABA receptors as well as the fact that progabide is a source of exogenous GABA (Bergmann, 1985). The order of potency of binding has been described as: GABA > SL75.102 > GABAmide > progabide.

Pharmacological studies of the action of progabide have been carried out not only to learn about the action of this agent itself but also to

understand the normal physiology of GABA-mediated neuronal systems. In the rat, progabide increases the rate of turnover of norepinephrine by increasing the liberation of the neurotransmitter. It also effects a decrease in the synthesis and release of both dopamine and serotonin (5HT) (Scatton et al., 1982). The physiological effects of progabide on the cholinergic system indicate a reduction in transmission of this neurotransmitter also, probably due to a local striatal effect (Scatton and Bartholini, 1982).

Unlike muscimol and its metabolites, progabide is relatively free of toxicity. It is the first GABA agonist that is useful in exploring the role of GABA in human disease. Preliminary clinical studies have already shown progabide to be effective in the treatment of epilepsy, spasticity and movement disorders (Bergmann, 1985).

(b) Gamma-Hydroxybutyrate (GOBA) and HA-966

i) GOBA:

About 40 years ago, GOBA and its lactone precursor, γ-butyrolactone (GBL) were found to produce a sleep-like state in mice and cats (Rubin and Giarman, 1947). Over the years much effort has been devoted to understanding the neuropharmacology of these substances. GOBA has been postulated to be a putative neurotransmitter in the CNS (Roth et al., 1980). The following lines of evidence have been developed to support this idea.

GOBA is a metabolite of GABA, although it may be derived from other sources (Gold and Roth, 1977). It occurs naturally in mammalian brain with a discrete regional distribution (Doherty et al., 1978). High-affinity binding sites and a sodium-dependent, high-affinity neuronal uptake system for GOBA have been identified in rat brain (Benavides, et al., 1982a;

Benavides et al., 1982b). GOBA is synthesized in two steps: First, GABA-T converts GABA to succinic semialdehyde. The latter compound is then transformed into GOBA by a specific succinic semialdehyde reductase a specific (Cash et al., 1979). Finally, GOBA itself has been isolated in nerve endings (Snead, 1987).

When injected in an anesthetic dose, GOBA's half-life is about one hour (Roth and Giarman, 1966). The principal mode of metabolism is oxidation to succinic acid, which then enters the tricarboxylic acid pathway. Small amounts of Krebs cycle-related amino acids such as aspartic acid, glutamic acid and even GABA can be derived from GOBA (Doherty et al., 1975).

GOBA causes an increase in brain levels of acetylcholine (Sethy et al., 1976). However, all classes of CNS depressants appear to cause increases in Ach, so this effect is not specific.

Gessa and coworkers found that GOBA causes an increase in brain dopamine (Gessa et al., 1966). The onset and duration of the anesthetic effect induced by this agent, as measured by the loss in the righting reflex, were subsequently shown to coincide with the increased levels of dopamine in striatum (Roth and Suhr, 1970). The increases in striatal dopamine were later attributed to GOBA's ability to inhibit neuronal firing in the nigrostriatal pathway. This conclusion was based on the parallelism between GOBA administration and lesions of the nigrostriatal tract. Both treatments lead to a sharp increase in striatal dopamine (Walters et al., 1973). In both cases, the rise in brain DA was markedly inhibited by injection of amphetamine, presumably owing to the latter's capacity to release newly synthesized DA (Andén et al., 1973). Similarly, both treatments effected an increase in the rate of DA synthesis and an

abolition of the DA depletion induced by α-methyl-p-tyrosine (Walters et al., 1973). That GOBA can antagonize the K^+-induced release of newly formed DA also supports the theory that this agent causes a rapid and selective increase in brain DA by inhibiting release of the transmitter (Bustos and Roth, 1972). GOBA does not inhibit COMT or MAO (Gessa et al., 1968); thus, inhibition of catabolizing enzymes is not responsible for the increase in dopamine.

Further insight into the mode of action of GOBA may be obtained from studies involving GABA, its structural analogue. When applied microiontophoretically, GABA inhibits nigral cell firing (Zivkovic et al., 1974). Treatment with a GABA-T inhibitor, which increases the GABA concentration in the synaptic cleft, inhibits DA turnover (Andén, 1974). GABA, injected into the substantia nigra increases brain DA, as does GOBA (Andén and Stock, 1973). Contrarily, injection of GABA into striatum has no effect. Given these data and the fact that GOBA is a structural analogue of GABA, the suggestion that the action of GOBA on DA neurons is through an inhibitory GABAergic mechanism in the substantia nigra is not unreasonable. Moreover, GOBA may be acting at GABA receptors on the cell bodies of these nigrostriatal dopaminergic fibres.

ii) HA-966

HA-966 (1-hydroxy-3-amino-pyrrolidone-2) was prepared in 1959; it is chemically related to the cyclic anhydride form of GABA; hence, it was used in this work as a GABA agonist. In rat brain, HA-966 selectively elevates the dopamine content of the striatum, with no changes in the levels of norepinephrine and serotonin (Bonta et al., 1971). As was the case with GOBA, HA-966 markedly counteracted the α-methyl-p-tyrosine (AMPT)-elicited depletion of dopamine (Bonta et al., 1971). It has also been shown that

HA-966 increases the DA synthesis rate (Van Zwieten-Boot and Noach, 1975). After HA-966 administration, the content of homovanillic acid (HVA), the major extraneuronal metabolite of DA, decreases in the striatum, while the intraneuronal metabolite dihydroxyphenylacetic acid (DOPAC) increases. This effect is not due to an inhibitory effect on COMT (Hillen and Noach, 1971), but more probably to inhibited release of DA, as was suggested for the action of GOBA (Roth and Suhr, 1970). All the data, taken together, suggest that HA-966, like GOBA, has a GABA-like inhibitory effect on nerve impulses in the dopaminergic nigrostriatal pathway, resulting in inhibition of DA release and a concomitant increase in synthesis. The site of action for both of these GABA analogues is thought to be the dopaminergic cell bodies in the substantia nigra (Murrin and Roth, 1976; Van Valkenburg and Noach, 1978).

Initial pharmacological studies had shown that HA-966 might exert a marked influence on the extrapyramidal system, with a possible beneficial effect in Parkinson's disease as well as an ameliorating effect on tremors of extrapyramidal origin (Bonta et al., 1971). Because of these potential therapeutic benefits, the metabolism of the drug in vivo and its penetrability of the blood-brain barrier was studied. It was a consistent observation during experiments with HA-966, that there was a delay in the onset of action following i.v. administration (Bonta et al., 1971). This observation suggested that a metabolic conversion of the drug is necessary for many of its effects. Experiments showing reduced activity of the compound in hepatectomized mice supported this idea and indicated the liver to be the site of metabolic conversion (Bonta et al., 1971). It is, therefore, tempting to explain the delay of onset of action of HA-966 on the basis of the chemical analogy between HA-966 and the cyclic anhydric form of GOBA or GABA. After conversion of the former drug into a GABA-like

derivative, effects resembling those elicited by GOBA are observed. Indeed, certain pharmacological actions of GOBA are similar to those of HA-966. As was mentioned earlier, they both selectively increase striatal dopamine levels, probably by inhibiting DA release. Furthermore, the sleep-like state produced by GOBA is strikingly similar to the behavioral effects of HA-966 (Bonta et al., 1971).

Other explanations for the action of HA-966 have been suggested. In vivo voltammetric investigations into the action of HA-966 on central dopaminergic neurons permit simultaneous recording of the effect of HA-966 on DA release and the appearance of the drug itself in rat striatum (Mos et al., 1981). The measurements are not specific enough to exclude the possibility that instead of HA-966, a metabolite of the drug is being measured. However, if such was in fact the case, the molecule is probably not extensively rearranged, because its oxidation potential is the same as for HA-966 in vitro (Mos et al., 1981). Also, the fact that the half-life of the drug in blood agrees fairly well with the decay of the drug measured in brain further suggests that some HA-966, although hydrophilic, is transported into the brain.

Whether HA-966 is acting in its original form or as some closely related metabolite, it clearly exerts a central, GABA-like influence on dopaminergic and, possibly, other neurotransmitter systems. This warrants its consideration as a GABA agonist in this work.

3. Pathways of Stress

The study of the physiology of stress dates back about 70-80 years. In 1911, Walter Cannon coined the term "homeostasis". He spoke of the complex physiological reactions that maintain the stability or equilibrium of the internal environment of the organism. For the healthy animal, this

internal equilibrium or homeostasis must be preserved in the face of constant challenges from external sources. For the purposes of this thesis, a stress can be defined as anything which can furnish an anti-homeostatic stimulus to the organism. This definition (Sourkes, 1983) is more of a qualitative concept, it ascribes no quantitative value to the strength of the stimulus. Cannon's studies of the sympathoadrenal system demonstrated that, during stress, the medullary hormone adrenaline (AD) is released from the adrenal glands (Cannon, 1929). Later, it was recognized that the repeated exposure to certain stimuli results in adaptive changes by the organism that preserve the internal conditions. Selye introdued the concept of the "general adaptation syndrome" (Selye, 1936). This syndrome is a response to harmful interactions from the environment, with particular emphasis on the role of the adrenal glucocorticoids. Thus, the hypothalamo-hypophyseal-adrenocortical axis took on a great deal of importance in the studies of the endocrine response to stress (Sourkes, 1985). Selye's stress theory described a non-specific response, manifested by physiological and tissue changes (Selye, 1976). Later studies, however, have indicated that there exist many different patterns of endocrine response to stress agents; in fact, Mason has stated that "there is no single non-specific hormonal response to all stimuli" (Mason, 1971).

In Cannon's view, the release of adrenaline was a short-term response to stress, resulting from the activation of the sympathoadrenal system. It is now known that under stressful circumstances, it is adrenaline which is released from the adrenal medulla and noradrenaline from postganglionic sympathetic fibers to initiate the stress response. It has become evident that with continuing or repeated application of a stressor there are specific increases in some parts of the catecholamine-synthesizing

machinery in the adrenal gland, particularly in the levels of tyrosine hydroxylase (TH, EC 1.14.16.2; Hoeldtke et al., 1974) and dopamine β-hydroxylase (DBH, EC 1.14.17.1; Patrick and Kirshner, 1971). That physiological changes occur in the amounts of enzyme protein in tissue as a result of growth of the organism is a well-established fact, but these increases in response to stress is a novel phenomenon (Sourkes, 1983). This rapid increase in amount of enzyme is referred to as induction.

An important consideration in studying the stress responses discussed above is the source which controls these responses. The discovery of transyaptic or transneuronal inductions of adrenal TH about eighteen years ago (Thoenen et al., 1969) provided a novel approach to the study of stress and the mechanisms of response to it. This approach has been used extensively in this laboratory in an effort to determine the center(s) which control the stress response. The rationale for this approach, as explained by Sourkes (1985), is as follows: If intact innervation of the adrenal gland is indeed a prerequisite for the stress-elicited induction of adrenal enzymes, then one could conceivably trace backward from the responding adrenal through the CNS to the site of the initiating impulses. Thus, one may work out the particular sequence of neurons that a stressor brings into play to provide induction of a particular enzyme. It is possible that different types of stress will result in considerable overlapping in many parts of these sequences. Alternatively, the neural pathways for different stressors might be highly divergent. This could have some therapeutic benefit as it would be possible to suggest the use of a pharmacological blocking agent to counteract the effects of a particular stress prevailing in a given situation. This, then, is the ultimate aim of such work.

The increase in activity of TH in the adrenal glands of immobilized

rats has been attributed to the increase in nervous impulses in the branch of the splanchnic nerve serving the adrenal medulla (Kvetňanský et al., 1970). The analysis of the problem of the relation of induction to stressor is, thus, a matter of tracing the paths that impulses, initiated by stressful stimuli applied to the experimental animal, follow in the CNS in order to affect the cell bodies of the intermediolateral horns of the spinal cord (Sourkes, 1983). These neurons are cholinergic and they constitute the preganglionic sympathetic cells whose fibres project to the adrenal gland. The work described in this thesis began with TH and has also considered ornithine decarboxylase.

4. Neural Regulation of ODC and TH

(a) Ornithine Decarboxylase

Ornithine decarboxylase (ODC, EC 4.1.1.17), the rate-limiting enzyme in polyamine biosynthesis (Tabor et al., 1958), catalyzes the conversion of ornithine to putrescine (1,4-diaminobutane). The polyamines are important in cellular growth and development (Slotkin, 1979). ODC (along with serotonin N-acetyltransferase) has the shortest turnover time of enzymes in mammalian tissues, estimated at about 12 minutes (Jänne and Räina, 1969). Its activity changes sensitively with many different stimuli (Morris and Fillingame, 1974). This enzyme is present in many tissues of the body, including the adrenal medulla and adrenal cortex. The cortical enzyme is essentially under pituitary control (Almazan et al., 1983a). On the other hand, the medullary enzyme is subject to transynaptic induction (Russell and Byus, 1976). Thus, ODC activity is increased in both adrenal cortex and medulla after subjecting the animal to immobilization, cold stress or injection with reserpine (Ramirez-Gonzalez et al., 1981); however, the cold-induced medullary increase in enzyme activity can be prevented by

denervation of the adrenal gland with no effect on cortical ODC activity (Byus and Russell, 1975). With regard to the neural regulation of the medullary enzyme, studies by Ekker et al. (1985) have demonstrated the existence of both ipsilateral and contralateral components of descending spinal pathways for the induction of the enzyme, with the ipsilateral fibre exerting the predominant effect. Experiments with actinomycin D and cycloheximide suggest that increased enzyme activity is due to de novo synthesis of enzyme protein through transcriptional control (Byus and Russell, 1976). This induction is mediated through the formation of cyclic AMP (Byus and Russell, 1976). Because the focus of the work in this thesis is on understanding the neural regulation of adrenal enzymes, only adrenomedullary ODC (amODC) is considered.

(b) Tyrosine Hydroxylase

Tyrosine hydroxylase is regarded as the rate-limiting enzyme in the conversion of tyrosine to the catecholamines (Levitt et al., 1965). It catalyzes the transformation of L-tyrosine to L-dihydroxyphenylalanine (L-DOPA) with dimethyltetrahydropterine ($DMPH_4$) as cofactor. In the chromaffin cells of the adrenal medulla, L-DOPA is converted to dopamine (DA), noradrenaline (NA) and, finally, adrenaline (AD). Regulation of this adrenal enzyme is thought to involve two main types of mechanism. The first requires a short-term, rapid alteration in enzyme activity which can be mediated through feedback inhibition (Spector et al., 1967), allosteric modulators (Cloutier and Weiner, 1973) or changes in substrate concentrations (Davis and Carlsson, 1973). The second mechanism involves delayed, long-term changes in enzyme protein and can be neuronally mediated. Immobilization stress leads to an increase in preganglionic nerve activity and an increase in adrenal TH in 24-48 hours; this induction can be prevented by splanchnic nerve transection (Kvetňansky et al., 1970).

It had been suggested (Dairman and Udenfriend, 1971) that the concentrations of adrenal catecholamines (CA) play a role in the induction of adrenal TH. However, through a series of experiments carried out in this laboratory (Quik and Sourkes, 1976), it has been shown that it is not local adrenal CA concentrations, but rather the state of firing of preganglionic nerve fibres which regulates adrenal TH induction. This conclusion is based on the following observations:

1) Apomorphine, given in a dose known to be effective for adrenal TH inhibition (Goldstein et al., 1970), actually results in an increase in TH activity and a temporary decrease in adrenal CA levels.

2) Kinetic studies have shown that there is no alteration of the K_m of adrenal TH (as a result of apomorphine treatment) with respect to cofactor ($DMPH_4$) or substrate. However, a significant increase in V_{max} was observed in the treated group as compared to controls. Hence, the increase in TH activity is due not to activation of the already present enzyme molecules but rather to de novo synthesis of enzyme protein.

3) The temporary decrease in adrenal CA, as well as the increase in adrenal TH are both prevented by adrenal denervation.

4) The administration of α-methyl-p-tyrosine (AMPT) to rats decreases the concentration of adrenal CA and yet does not affect adrenal TH activity.

5) L-DOPA administration is able to prevent the short-term decrease in adrenal CA occasioned by apomorphine, yet the TH activity remains highly elevated.

The evidence favoring the neuronal regulation of both ODC and TH is compelling. As mentioned in section 3., the phenomenon of a transynaptic induction of adrenal enzymes (Thoenen et al., 1969) provided a means of studying pathways of stress. Through the use of various pharmacological

agents, it is possible to stimulate nervous pathways centrally and determine if such a stimulation causes an increase in adrenal enzyme activity. By using substances that activate specific nerve fibres in the CNS, one can map out the sequence of neurons that are responsible for eliciting enzyme induction. This work has been attempted with three different neurotransmitters; a brief summary of the information obtained to date is presented in sections 4 (c), (d) and (e) below.

(c) Dopaminergic Influences

The use of apomorphine in establishing the neuronal control of adrenal TH (Quik and Sourkes, 1976) suggested that the increased neuronal firing which initiates the increase in that enzyme is due to stimulation of central dopamine receptors. That a dopaminergic system may be important is evident from the work of Beuning and Gibb (1974). Methamphetamine, a drug which releases brain dopamine, increases adrenal TH activity; however, when haloperidol, a dopamine-receptor blocker, is given in combination with methamphetamine, the increase in TH is diminished by about 50 per cent. Administration of the dopamine-receptor agonists piribedil, bromocryptine as well as apomorphine cause an increase in ATHA which is also attenuated by prior injection of haloperidol (Quik and Sourkes, 1977). Furthermore, when L-DOPA is administered together with carbidopa, a peripheral decarboxylase inhibitor, in order to increase the brain concentrations of dopamine, there is a highly significant increase in enzyme activity (Quik and Sourkes, 1977).

Adrenomedullary ODC (amODC) is also induced after repeated administration of dopamine-receptor agonists such as apomorphine and piribedil (Almazan et al., 1982a; Ekker et al., 1984). This increase is dose-related for both drugs and time-dependent for apomorphine (Almazan et

al., 1982a). Pre-treatment of the rats with haloperidol blocks the induction of the enzyme elicited by apomorphine (Almazan et al., 1982a). Unilateral splanchnicotomy, section of the spinal cord and transection of the diencephalon result in a decrease in the responsiveness of amODC to apomorphine. These observations strongly suggest that this enzyme is regulated mainly by a central system, originating primarily in the diencephalon-telencephalon region and including a facilitatory dopaminergic component (Almazan et al., 1983b).

The inductive effect of apomorphine on TH is also centrally mediated, inasmuch as section of the splanchnic nerve (Quik and Sourkes, 1976) or transection of the thoracic spinal cord (Gagner et al., 1983) results in abolition of the apomorphine-induced increase in enzyme activity.

In an attempt to pinpoint further the supraspinal dopaminergic centre controlling these adrenal enzymes, the induction of the activities of ODC and TH by apomorphine was examined in animals also receiving antagonists that act preferentially on different dopaminergic systems of the brain (Creese, 1983). The drugs chosen were metoclopramide and thioridazine, two blocking agents that act primarily on the A9 (striatal) and A10 (mesolimbic and mesocortical) dopaminergic systems of the brain, respectively. Metoclopramide impaired to a large extent the apomorphine-induced increase in ODC and almost completely blocked the induction of TH by apomorphine. In contrast, thioridazine did not prevent the induction of either enzyme by apomorphine. These results (Ekker and Sourkes, 1985a) suggest that it is the stimulation of DA receptors in the striatum that is responsible for the apomorphine-elicited induction of adrenal ODC and TH. The neural mechanism by which activation of the nigrostriatal dopaminergic system (A9) results in changes in adrenal enzyme activity remains to be determined and was one

of the aims of this thesis.

(d) Serotonergic Influences

The results of many experiments have implicated the neurotransmitter serotonin (5HT), not only in the regulation of TH and ODC, but in the regulation of DBH also. A role for a serotonergic system in the control of these adrenal enzymes is based on results from studies using drugs or surgical procedures which can alter the levels of endogenous serotonin. A brief review of some of these studies follows.

The administration of the tryptophan hydroxylase inhibitor p-chlorophenylalanine (PCPA), which leads to a decrease in levels of endogenous serotonin, results in increasing adrenal TH activity (Breese et al., 1974). The intraventricular injection of the neurotoxin 5,7-dihydroxytryptamine (DHT) causes the destruction of serotonin nerve terminals and, hence, the loss of much of the serotonin stores; this also results in a large increase in adrenal TH (Quik and Sourkes, 1977). Splanchnicotomy abolishes the effect of DHT on TH activity, thus illustrating the neural mediation of this effect on TH. Furthermore, the DHT-elicited increase in TH can be partially prevented by administration of L-5-hydroxytryptophan (in combination with the peripheral decarboxylase inhibitor carbidopa) (Quik and Sourkes, 1977). Serotonergic blockade by the centrally acting 5HT antagonist methiothepin also produces an increase in adrenal TH activity (Quik and Sourkes, 1977). To determine whether control of adrenal TH activity by a serotonergic system could be localized to a specific brain region, the effect on TH activity of electrolytic lesions of the raphe nuclei, areas rich in 5HT cell bodies, was studied. Such experiments (Quik et al., 1977) show that an increase in the activity is obtained when the medial but not dorsal raphe nucleus is lesioned.

Furthermore, lesions of the medial raphe nucleus (MRN) result in only a modest reduction of brain 5HT in comparison with lesions of the dorsal raphe nucleus (DRN). This implies that the MRN exerts a tonic inhibitory influence on the transneuronal induction of TH, a conclusion which is consistent with the previous work with PCPA and DHT. In the case of TH, there seems to be a tonic serotonergic input to the adrenal which is inhibitory in nature.

Unlike TH, amODC activity is not affected by cerebral serotonin depletors or by lesions of the MRN (Alamzan et al., 1982b). However, all the treatments described above can effectively potentiate an apomorphine-induced increase in ODC activity. It is also of interest to note that the 5HT antagonist methiothepin given alone can spontaneously increase the resting levels of ODC in the absence of apomorphine (Ekker et al., 1984). Hence, the experiments with PCPA, DHT and the lesions of raphe nuclei suggest that a serotonergic pathway, originating in the MRN, exerts an inhibitory influence over at least the apomorphine-elicited induction of amODC activity.

A serotonergic regulatory mechanism has also been sought for the noradrenaline-producing enzyme DBH. Administration of reserpine to rats causes a time-dependent increase in adrenal DBH activity (Lima and Sourkes, 1986a). Because reserpine is known to interfere with the vesicular storage of monoamines and thus diminish their endogenous levels in the brain, it seemed of interest to determine whether the action of reserpine in DBH activity is mediated specifically via a serotonergic or by some other pathway. Lima and Sourkes (1986a) found that administration of DHT or PCPA, or introduction of a lesion of the MRN, all of which deplete cerebral 5HT stores, does not alter the control values of DBH activity, but they potentiate reserpine. Conversely, serotonin agonists given to reserpinized

rats diminish the increase in DBH activity, although by themselves they do not modify adrenal DBH activity. The studies described above for DBH are consistent with the theory developed from the earlier work on TH (Quik and Sourkes, 1977) and amODC (Almazan et al., 1982b). All three of these adrenal enzymes seem to be influenced by a central serotonergic regulatory system which is inhibitory in nature.

(e) Cholinergic Influences

The cholinergic system's influence on adrenal enzyme activity has previously been investigated. Oxotremorine is a potent, direct stimulant of muscarinic receptors, devoid of nicotinic activity and it also elevates central acetylcholine (ACh) levels (Koelle, 1975; Nordberg, 1978). This centrally active muscarinic agonist increases the activity of tyrosine hydroxylase in the locus coeruleus of rat brain (Lewander et al., 1975); this change in enzyme activity is due to enzyme activation. This agent was subsequently shown to effect an increase in adrenal TH activity also (Lewander et al., 1977). The increase was dose-dependent, peaking at 1.5 mg/kg. The response occurred in adrenal medulla within 4-8 hours, reaching a peak response at 16 hours. The increased TH activity persisted up to 2 weeks. The inductive effect was abolished by denervation of the splanchnic nerve, indicating that the increase was transynaptically mediated (Lewander et al., 1977). Immunotitration with anti-TH serum demonstrated that the increase in enzyme activity in the adrenal medulla is due to an actual increase in the amount of enzyme protein. Administration of centrally acting muscarinic antagonists such as atropine was able to block the oxotremorine-elicited increase in ATHA whereas methylatropine, which penetrates the blood-brain barrier poorly (Witter et al., 1973), was unable to antagonize the inductive effect. Thus, blockade of peripheral

muscarinic sites exclusively does not interfere with the oxotremorine-elicited induction. That methylatropine does not affect this process is in agreement with the views of Guidotti and Costa (1977), who maintain that the transynaptic induction of ATHA operates only through the nicotinic receptors of the adrenal gland. Experiments carried out by Gagner et al. (1983) confirmed the inductive effect of oxotremorine on this adrenal enzyme. Furthermore, pilocarpine, a muscarinic agonist with central actions (Zablocka and Esplin, 1963), also induced adrenal TH (Lewander et al., 1977). In summary, the above observations suggest that the cholinergic system does regulate adrenal TH activity. The site of action of oxotremorine in initiating this cholinergic effect on the adrenal appears to be central; moreover, work in this laboratory has localized this site to a supraspinal level (Gauthier et al., 1979; Gagner et al., 1981).

Evidence for a central cholinergic mechanism regulating the induction of medullary ODC was first put forward by Russell and Byus (1976). In this laboratory (Ramirez-Gonzalez et al., 1980), oxotremorine brought about a large increase in amODC, starting after 30 minutes and remaining evident up to at least 4 hours after injection. This stimulation of medullary ODC is blocked by section of the spinal cord (at the level of the 5th thoracic vertebra) as well as by severing the splanchnic nerve (Ramirez-Gonzalez et al., 1980). Hence, these results are consistent with the evidence for a transynaptic induction of amODC through cholinergic stimulation proposed earlier (Russell and Byus, 1976).

(f) Regulation of ODC vs. TH: Similarities and Differences

A great deal of work in this laboratory has been directed toward the control mechanisms that regulate the activity of adrenal enzymes, particularly ODC and TH. These two enzymes have quite different functions.

Nevertheless, the study of their induction may shed light on the differential effects of various stressors.

One major difference between ODC and TH lies in the time-scales of their respective inductions. The induction of adrenal TH requires a transcriptional stage of 18-24 hrs. and a translational stage, requiring an additional 24-36 hours (Otten et al., 1973). This suggests that a significant increase in ATH is elicited on the third day of treatment with an inducing agent. This is indeed the case with apomorphine, as long as the treatment is maintained over the three-day period. It should be pointed out, however, that small yet significant increases are discernible even at the end of the first day (Quik and Sourkes, 1976). By contrast, just two injections of apomorphine are sufficient to generate large increases in amODC activity within hours (Almazan et al., 1982a). Furthermore, insofar as the apomorphine-elicited induction is concerned, in the case of ODC, the activity of the treated group is several times greater than control levels whereas for TH the apomorphine only causes a doubling of control levels.

In the preceding sections, the effects of various forms of stress, including pharmacological stimulation, on amODC and TH have been briefly described. Because of the many similarities between the responses of these two enzymes, their respective central regulatory mechanisms may be related. Adrenomedullary ODC and TH are both induced by physical stressors such as cold exposure (Thoenen, 1970; Byus and Russell, 1975) and immobilization (Kvetnansky et al., 1970; Ramirez-Gonzalez et al., 1981). Both ODC and TH are induced following the administration of apomorphine (Quik and Sourkes, 1976; Almazan et al., 1982a). Quipazine, which is believed to have a dopamine-like action (Ekker et al., 1984), has also been shown to have an

inducing effect on both TH (Gagner et al., 1983) and amODC (Ekker et al., 1984). Thus, both enzymes respond similarly to dopaminergic stimulation. Intact innervation of the adrenal medulla by the splanchnic nerve is essential for the induction of ODC and TH following dopaminergic stimulation (Quik and Sourkes, 1976; Almazan et al., 1983b). The net inhibitory role of serotonergic fibres originating from the MRN on the induction of the two enzymes by apomorphine has been described in this laboratory (Quik and Sourkes, 1977; Quik et al., 1977; Almazan et al., 1982b). The transneuronal cholinergic stimulation by oxotremorine represents yet another similarity in the mechanisms of control of amODC and TH (Ramirez-Gonzalez et al., 1980; Lewander et al., 1977).

Studies have been made to localize the central site of action of apomorphine in the induction of amODC and TH. This work has involved surgical lesions of parts of the brain or spinal cord (Gagner et al., 1985; Almazan et al., 1983b). Using a pharmacological approach, Ekker and Sourkes (1985a) have presented evidence indicating that the neural impulses, resulting from apomorphine administration and responsible for inducing both TH and ODC, emanate from the striatum.

Acetylcholine liberated by the splanchnic nerve terminals on the chromaffin cells of the adrenal medulla is generally accepted as the first messenger in induction of ODC and TH upon neural stimulation. Cyclic AMP has been suggested as the second messenger for the induction of these adrenal enzymes (Guidotti and Costa, 1977; Byus and Russell, 1976).

5. GABAergic Effects in the CNS

(a) GABA-Serotonin Interactions

Studies have been carried out to determine whether the 5HT-containing neurons of the midbrain raphe nuclei are subject to inhibitory control by

GABA. It has been shown that injection of the GABA antagonists bicuculline and picrotoxin (in sub-convulsive doses) into the MRN increase the 5HT turnover in rat hippocampus. The hippocampus was studied because its serotonergic innervation is derived primarly from the medial raphe nuclei (Bobillier et al., 1976; Pierce et al., 1976). Conversely, administration into the MRN of GABA agonists decreases the 5HT turnover (Forchetti and Meek, 1981). The effects of the GABA antagonists were virtually blocked when administered together with muscimol. Furthermore, diazepam, which as a benzodiazepine potentiates GABA, was able to potentiate the reversal by muscimol of the picrotoxin-induced 5-HIAA increase (Forchetti and Meek, 1981). These experiments do not distinguish which types of GABA neurons specifically control 5HT firing, but the ability of GABA agonists and antagonists to decrease or increase, respectively, the turnover of 5HT suggests that an inhibitory GABAergic influence exists and it is perhaps tonic in nature.

Investigators using in vivo differential pulse voltammetry have found that the anatomical site of the GABAergic influence on serotonergic transmission in the rat (Scatton et al., 1984) is at the level of the dorsal raphe cells, the source of striatal serotonergic afferents (Azmitia and Segal, 1978). Other studies have also indicated that the midbrain raphe nuclei are the anatomical sites for the GABAergic influence on cerebral serotonergic neurons (Nishikawa and Scatton, 1985). For example, injection of GABA, muscimol, SL75102 or GVG into the DRN significantly reduced 5HT synthesis in striatum, olfactory tubercle and substantia nigra, areas which receive serotonergic afferents mainly from the raphe dorsalis (Azmitia and Segal, 1978). Similarly, injection of these drugs into the MRN diminished 5HT synthesis in hippocampus and septum, regions which receive their serotonergic innervation from this nucleus.

Experiments similar to those described above have been carried out by Didier and his coworkers (Didier et al., 1985) to determine the pharmacological effects of GABA-related drugs on the serotonin and 5-HIAA contents of various brain regions. The GABA agonist muscimol increased the 5HT content and reduced the 5-HIAA levels in structures containing serotonergic terminals. This suggests an inhibitory effect of GABA on firing of 5HT neurons and a reduction of 5HT utilization. Conversely, bicuculline stimulated 5HT turnover since its administration produced significant increases in 5HT and 5-HIAA levels (Didier et al., 1985). These data are in agreement with a transynaptic inhibitory control of GABA on 5HT neurons.

In addition to studies in vivo, Mennini et al. (1986) have conducted in vitro binding studies in an attempt to localize GABA receptors on serotonergic neurons. After the selective destruction of 5HT-containing neurons with DHT, the binding of the GABA-A receptor agonist muscimol and the GABA-B receptor agonist baclofen was investigated in various rat brain regions. [^{3}H]Muscimol binding was reduced only in the mesencephalon (midbrain) whereas [^{3}H]baclofen binding was unchanged in all regions considered. These results suggest that GABA receptors may be localized on serotonergic terminals only in the mesencephalon, and furthermore, these receptors could only be of the GABA-A type.

Thus, a great deal of work has implicated the involvement of GABA in the regulation of central serotonergic transmission. This influence of GABA seems to be inhibitory in nature and localized to the midbrain regions, particularly the anterior raphe nuclei (MRN and DRN). However, the fact that GABA influences 5HT in the MRN does not imply that these two neurotransmitter systems jointly interact in the further regulation of

adrenal enzymes, as is known for the MRN itself. Nevertheless, this conceivable interaction is intriguing, and experiments have been designed in the course of this thesis to investigate this possibility.

(b) GABA-Cholinergic Interactions

Work in this laboratory has localized the central cholinergic regulation of adrenomedullary enzymes to a supraspinal level (Gagner et al., 1981; Ramirez-Gonzalez et al., 1980). Our attention then turns, in the first place, to the striatum. This structure contains the highest levels of acetylcholine (ACh), choline acetyltransferase (CHAT), ACh esterase, high-affinity choline uptake activities and muscarinic receptor sites in the central nervous system (Scatton, 1987). The cholinergic activity in the striatum is limited to intrinsic cholinergic neurons and these account for between 1% and 2% of the total striatal neuron population (Lehman and Langer, 1983). These nerve cells appear to play an important functional role in extrapyramidal motor function and in doing so, they interact with various other central neurotransmitters. For example, the dopamine-ACh balance appears to be a major mechanism involved in controlling the extrapyramidal system (Scatton, 1987).

GABAergic neurons are also able to regulate striatal cholinergic activity (Scatton and Bartholini, 1980a). A great deal of evidence suggests that GABA exerts an inhibitory control over striatal cholinergic interneurons. This conclusion is based on the following observations (Scatton and Bartholini, 1982):

The systemic administration into rats of GABA receptor agonists (e.g. progabide or muscimol):

(1) causes an increase in striatal ACh concentrations, probably due to a decreased activity of striatal cholinergic cells and, therefore, diminished release of ACh;

(2) reduces the synthesis of ACh from pyruvate in striatal slices; and

(3) diminishes the rate of utilization of striatal ACh after infusion of hemicholinium-3 (a choline uptake inhibitor) into the striatum. The increase of striatal ACh levels is not linked to alterations in CHAT or ACh esterase activity, as these enzymes are not affected by GABAergic substances. Also, the decrease in ACh turnover is not connected to a reduction in the amount of choline available for ACh synthesis, as GABAergic agents do not influence the striatal levels and uptake of choline. All in all, these data (Scatton and Bartholini, 1982) indicate a reduction of striatal ACh turnover by GABA agonists. Thus, a GABA input may be involved in the regulation of the activity of striatal cholinergic cells, and the inhibitory influence is most probably mediated by intrastriatal mechanisms. This view is supported by the fact that intrastriatal infusion of muscimol or GABA increases ACh concentrations in striatum (Scatton and Bartholini, 1980a). Furthermore, intrastriatal infusion of picrotoxin reduces striatal ACh levels and antagonizes the increase of striatal ACh concentrations caused by a systemic injection of muscimol. Finally, GABA reduces the ACh release evoked by potassium in perfused striatal slices (Stoof et al., 1979).

The inhibitory action of GABA on striatal cholinergic neurons does not seem to involve dopaminergic mechanisms (Scatton, 1987). GABA mimetics cause a similar elevation of striatal ACh after chemical or surgical lesions of the nigrostriatal dopaminergic pathway or after pharmacological alteration of the activity of dopaminergic neurons by apomorphine or neuroleptics (Scatton and Bartholini, 1980a, 1982). Thus, the GABAergic effect on ACh levels in striatum is independent of the integrity of the nigrostriatal pathway.

Because the striatum contains a dense population of GABAergic interneurons (McGeer and McGeer, 1975) and because GABA's effect on ACh is independent of the nigrostriatal dopaminergic pathway, it is possible that these GABA neurons interact directly with cholinergic nerve cells. However, the GABA influence on cholinergic neurons may also be exerted indirectly by modulating the activity of the corticostriatal tract. Indeed, lesions of the corticostriatal projections, which are glutamatergic in nature (McGeer et al., 1977), almost completely abolish the increase in striatal ACh levels elicited by GABA analogues administered systemically (Scatton and Bartholini, 1980b). Accordingly, the GABA-mediated inhibition of striatal ACh neurons may result from the stimulation of GABA receptors located on excitatory glutamatergic afferents to cholinergic interneurons: an increase in GABAergic transmission would reduce excitation by glutamate of striatal cholinergic neurons and thus lead to a decrease in ACh turnover (Scatton, 1987).

In addition to the intrastriatal inhibitory GABA control, the cholinergic neurons also appear to be under an indirect facilitatory GABAergic influence mediated by nigrostriatal DA neurons. Systemic administration of picrotoxin increases striatal ACh levels, and this is dependent on the integrity of the nigrostriatal dopaminergic pathway (Javoy et al., 1977; Ladinsky et al., 1976). Since DA neurons receive striatonigral inhibitory GABA projections (Fonnum et al., 1974) and, in turn, tonically inhibit striatal cholinergic neurons (Bartholini and Stadler, 1977), activation of GABAergic fibres is expected to reduce the inhibitory dopaminergic input on cholinergic cells, thus yielding an increase in ACh turnover. However, the reduced ACh turnover observed with GABA analogues indicates that this indirect GABAergic facilitatory influence on striatal cholinergic neurons is only of minor importance; the

intrastriatal inhibitory GABA influence prevails.

Hence, there seems to be a discrepancy regarding the GABAergic influence on cholinergic transmission. On the one hand, GABA facilitates striatal ACh turnover by means of a DA-dependent mechanism, one that presumably originates in the substantia nigra; on the other hand, GABA can also effect a reduction in striatal cholinergic transmission in a DA-independent manner. Yet this differential GABAergic effect can be explained by the fact that the threshold dose of progabide or muscimol needed to inhibit cholinergic neurons is much lower than that reducing the activity of the nigrostriatal dopaminergic system (Scatton et al., 1982). Thus, the predominance of the GABAergic inhibitory influence is attributed to the high sensitivity of cholinergic cells to GABA agonists relative to DA cells.

The information presented in this section suggests that there are extensive interactions between GABA and ACh in the striatum. The fact that the cholinergic regulation of adrenal enzymes is supraspinal (Gagner et al., 1981; Ramirez-Gonzalez et al., 1980), taken together with the fact that the striatum contains the highest levels of ACh and its associated enzymes in the CNS seems to imply that the cholinergic nerve impulses controlling adrenal enzyme activity emanate from this cerebral structure. Consequently, it seems reasonable to postulate that the GABAergic influence on striatal cholinergic transmission plays a role in the oxotremorine-elicited increases in adrenal enzyme activity. In the course of this research, experiments have been carried out to determine if, in fact, GABA and ACh interact insofar as adrenal enzyme regulation is concerned.

(c) GABA-DA Interactions

Research on the interaction between dopamine and GABA was initially

concerned with the putative, regulatory activity of the inhibitory transmitter GABA on dopamine systems. This work was believed to have potential clinical value, possibly resulting in an additional treatment of schizophrenia (Christensen et al., 1980). At present, it seems that several GABAergic mechanisms are directly and indirectly involved in the regulation of the activity of the dopamine systems (Scheel-Krüger, 1986).

The nigrostriatal dopaminergic pathway is an ascending projection, passing from substantia nigra in the midbrain to the corpus striatum in the forebrain. It is subject to numerous synaptic influences at several sites in the CNS. Of these neuronal contacts, the GABAergic ones are found on 1) presynaptic striatal receptors (Campochiaro et al., 1977) and 2) nigral cell body receptors (Ribak et al., 1976). Most of these GABAergic processes emanate from the striatum and globus pallidus and project to the dendrites of dopaminergic neurons in both the pars compacta and pars reticulata of the substantia nigra (Fonnum et al., 1974; Hattori et al., 1973; Hattori et al., 1975). The relative roles of these receptor populations in GABA-DA interactions is as yet unclear, but the mere existence of these distinct receptor groups suggests an inherent complexity in these neurotransmitter interactions.

Most biochemical studies suggest that GABA exerts an inhibitory control on the nigrostriatal dopamine systems (Wood, 1982; Bartholini, 1980). Indeed, Dray and coworkers (1976) demonstrated that GABA inhibits the firing of cells in the pars compacta of the rat; and Chéramy et al. (1977a) showed that the nigral application in the cat of the GABA antagonist picrotoxin stimulated the release of labelled DA in the caudate nucleus. GABA receptor agonists such as muscimol or progabide decrease the activity of dopaminergic neurons in the striatum. These substances, when

applied systemically (Scatton et al., 1982; Bartholini et al., 1979):

1) reduce the rate of disappearance of DA following administration of alpha-methyl-p-tyrosine.

2) block the accumulation in vivo of DOPA after inhibition of L-aromatic amino acid decarboxylase.

3) prevent the in vitro formation of $^{14}CO_2$ from L-[1-^{14}C] tyrosine in tissue slices, this effect being opposed by picrotoxin.

4) decrease the levels of 3-methoxytyramine, an extraneuronal metabolite of DA.

These effects are particularly evident when the DA neurons are activated by neuroleptics. The data clearly indicate that GABA-receptor stimulation reduces both striatal DA synthesis and release. Furthermore, because GABA added to the perfusion fluid of the caudate nucleus inhibits DA release in vivo (Bartholini and Stadler, 1977) and because the intranigral administration of picrotoxin enhances DA release in the striatum (Chéramy et al., 1977a), it is likely that the GABAergic inhibition of nigrostriatal dopaminergic transmission is effected by activating GABA receptors localized on both somatodendritic (i.e. nigral cell bodies) and terminal (i.e. striatal) areas of DA neurons. This direct, inhibitory striatonigral GABAergic influence on the nigrostriatal DA pathway is often referred to as the "feed-back loop".

While the existence of the striatal "feed-back loop" is well established, there are nevertheless regional differences in the degree of the GABA-induced inhibition of dopaminergic transmission. For example, DA turnover is reduced in the striatum and limbic areas such as the septum and nucleus accumbens but not in other DA-rich brain areas (e.g. cerebral cortex, brainstem, olfactory tubercle and hypothalamus) (Scatton et al.,

1982). Moreover, striatal dopaminergic neurons are more susceptible to GABAergic inhibition than limbic DA neurons. This differential sensitivity, the reasons for which are not yet understood, was observed with both progabide (Scatton et al., 1982) and muscimol (Scatton et al., 1980).

In accordance with its inhibitory effect on DA neurons, GABA-receptor agonists also downregulate DA transmission by controlling the number of receptors. It has been shown that repeated treatment with neuroleptics causes an increase in DA receptor density, as demonstrated by the increase in [^{3}H] spiperone-binding sites in the rat striatum (Burt et al., 1977). However, the conjoint administration of progabide and haloperidol prevents the neuroleptic-induced supersensitivity of the rat to apomorphine (Lloyd et al., 1981). Thus, GABA has an additional function in the control of DA transmission: it regulates DA-receptor density on postsynaptic cells.

Although the bulk of work in this field suggests an inhibitory role for GABA in the nigrostriatal DA system, some authors have argued that, under certain experimental conditions, GABA may promote DA release. GABA, muscimol and GOBA stimulated the release of ^{3}H-DA in the caudate nucleus when introduced for 15 minutes into a superfusion medium; however, the initial stimulation of ^{3}H-DA release was followed by an inhibition of transmitter release when GABA was introduced for 60 minutes (Chéramy et al., 1978). The unexpected stimulatory action of GABA and other GABA agonists might be attributed to their effect on non-dopaminergic neurons. Inhibition of nigral inhibitory interneurons which directly contact the dopaminergic cells could lead to an activation of the dopaminergic pathway. These interneurons could be glycinergic (Chéramy et al., 1978), as the nigral application of glycine reduced ^{3}H-DA release in the caudate nucleus (Chéramy et al., 1977b). Which GABAergic effect predominates, excitatory

or inhibitory? Chéramy and coworkers (1978) have postulated that the direct inhibitory influence is preferentially involved when DA neurons are in an activated state since inhibition of DA release by GABA followed an initial stimulation of the dopaminergic pathway. This theory would be consistent with the antagonistic effect of muscimol on the neuroleptic-induced stimulation of striatal DA turnover (Gale and Guidotti, 1976).

The complexity of GABA-DA interactions is further illustrated in experiments by Reimann et al. (1982), who showed that GABA can both prevent and facilitate DA release in the caudate nucleus of the rabbit; the determining factor was the presence or absence of nipecotate, a blocker of GABA uptake in both neurons and glial cells (Bowery et al., 1976; Schousboe et al., 1979), in the medium superfusing the striatal slices. It seems that the facilitatory effect of GABA can be suppressed by inhibiting cellular uptake of GABA into dopaminergic nerve terminals. It may be that the increase in DA release requires entry of GABA into dopaminergic axons and perhaps an action on the neurotransmitter storage granules (Reimann et al., 1982). Alternatively, because the inhibitory effect occurs in the presence of nipecotate, it could be mediated by a receptor on the cell membrane of the dopaminergic neuron (Reimann et al., 1982). However, in these studies, unlike previous reports in the literature (e.g. Chéramy et al., 1977a), picrotoxin and bicuculline had no effect on DA release. Thus, the inhibitory receptor mechanism theorized by Reimann et al. (1982), resembles that described by Bowery and coworkers (1980), i.e. an action of GABA at a novel (GABA-B?) receptor site.

Much less attention has been paid to the possible effects of DAergic drugs on the turnover of brain GABA. However, if the striatonigral GABAergic feed-back tract is monosynaptic, then a stimulation of post-

synaptic DA receptors in the striatum should increase the GABAergic activity in the substantia nigra. The systemic administration of apomorphine or DA receptor antagonists such as haloperidol and clozapine did not change the accumulation or the disappearance of GABA in the striatum and substantia nigra of the rat (Lindgren, 1987). In contrast to these findings, other studies indicate that DA exerts an inhibitory action on GABA release from rat striatum (Van der Heyden et al., 1980a). This inhibition was also seen in the rat substantia nigra (Van der Heyden et al., 1980b). To complicate matters even further, in vitro studies with slices of substantia nigra revealed that the addition of DA stimulated the release of ^{3}H-GABA (Reubi et al., 1977). This dopaminergic facilitatory effect was confirmed with in vivo studies, where the release of endogenous GABA was measured using a push-pull cannula (Van der Heyden et al., 1979).

Clearly, GABA and dopamine have extensive interconnections in the A9 region and their individual actions are inextricably related. Thus, insofar as the neural regulation of adrenal enzymes is concerned, it may well be that what was hitherto considered a DA-mediated phenomenon is in fact a combined GABA/DA-elicited effect. In this regard, a series of experiments are described in this thesis in an attempt to delineate the roles of these two neurotransmitters systems in the induction of TH and ODC.

(d) GABAergic Effects on DBH

Central muscarinic activity plays a role in the regulation of TH, DBH and ODC (Lewander et al., 1977; Ramirez-Gonzalez et al., 1980). Because GABA blocks the activity of cholinergic neurons in the striatum and elsewhere (Scatton and Bartholini, 1979, 1980b) and GABA agonists such as progabide and muscimol diminish the rate of turnover of brain acetylcholine

(Scatton and Bartholini, 1980a, 1982), it was considered worthwhile to study the effects of GABAergic drugs on the oxotremorine-elicited induction of adrenal enzymes. This has already been done with respect to DBH (Lima and Sourkes, 1986b).

Progabide, a synthetic substance which acts as a GABA-A and GABA-B agonist, significantly decreases the effect of oxotremorine on DBH; this effect seems to be mediated by GABA-A receptors, because bicuculline blocks the action of progabide on the induction of adrenal DBH by oxotremorine (Lima and Sourkes, 1986b). Other GABA agonists, such as muscimol, gamma-vinyl GABA (GVG) and baclofen were also tested. Muscimol, a GABA-A agonist, decreases the resting activity of adrenal DBH but does not significantly impair the action of oxotremorine. Infusion of muscimol into the brain at a small constant dose produces a great decrease in adrenal DBH activity and this suggests that muscimol has a central action (Lima and Sourkes, 1986b). These results support the possibility of a central inhibitory pathway that involves GABA as a neurotransmitter and affects adrenal function. A general inhibitory effect of GABA on tonic stimulatory pathways that maintain the resting levels of adrenal DBH is yet another possibility. Because baclofen, a GABA-B agonist (Hill and Bowery, 1981) had no effect on adrenal DBH activity, the GABA actions are probably mediated by GABA-A receptors.

The above observations suggest that GABA plays a role in the regulation of DBH, thus setting a precedent for the use of GABA in the study of adrenal enzyme regulation, particularly for ODC and TH in this thesis.

(e) Local Vs. Central Effects of GABA

The neurotransmitter substances that effect changes in the activities

of ODC or TH have hitherto all been shown to act via central mechanisms (see sections 4. (c), (d), (e)). Because GABA is a ubiquitous neurotransmitter in the CNS, it is possible that any effect that it might have on adrenal enzyme activity would be centrally mediated. However, a great deal of evidence suggests that there are extensive GABAergic influences in the periphery; in particular, GABA plays a role in the calcium-dependent secretion of catecholamines from the adrenal medulla (Sangiah et al., 1974).

The chromaffin cells of the adrenal medulla specialize in the production, storage and secretion of CA. These processes are modulated by nicotinic receptors located in chromaffin cell membranes and are innervated by splanchnic cholinergic axons. This, however, is too simplistic a view of the neuronal modulation of adrenal medullary function. Histochemical and biochemical studies demonstrate that GABA, GAD, GABA-transaminase and a GABA-benzodiazepine receptor complex similar to that found in brain are present in bovine adrenal chromaffin cells (Kataoka et al., 1984). These GABA receptors, like those of brain, are activated by GABA or muscimol and are blocked by bicuculline. Moreover, chromaffin cells in culture contain GABA uptake, storage and release mechanisms similar to those in brain. In addition to the GAD-positive immunoreactivity of chromaffin cells, immunocytochemical analysis (Kataoka et al., 1986) suggests that GABAergic fibres reach the adrenal medulla in association with the terminals of the splanchnic nerve.

Functionally, GABA receptors on chromaffin cells modulate the ACh-induced release of CA. By means of a technique of perfusion of the adrenal gland [thus eliminating central effects of drugs or their metabolites on CA release (Hilton et al., 1958)], it was shown that GABA-mimetic drugs cause the release of CA into the circulation, whereas a GABA antagonist reduces

the CA content of adrenal effluent blood (Kataoka et al., 1986). The magnitude of this release is comparable to that obtained by maximally efficient electrical stimulation of the splanchnic nerve. The increase of the CA content in adrenal effluent blood elicited by muscimol or THIP, two GABA-A agonists, was not blocked by splanchnicotomy, but it was prevented by bicuculline methiodide, a specific GABA-A receptor antagonist that does not cross the blood-brain barrier (Kataoka et al., 1986). The data suggest that the CA release elicited by GABA or other GABA-mimetics is not the consequence of an activation of transynaptic mechanisms but rather the result of stimulation of GABA-A receptors located on membranes of adrenal chromaffin cells. It has also been shown (Kataoka et al., 1986) that the extent of CA release elicited by splanchnic nerve stimulation was decreased by administration of GABA agonists and was increased by bicuculline. Thus, endogenous GABA may reduce the responsiveness of nicotinic receptors on chromaffin cells.

In summary, GABA modulates the spontaneous release of CA and the release elicited by electrical stimulation of the splanchnic nerve. In studying the GABAergic mechanisms of adrenal enzyme regulation, the observations discussed above warrant serious consideration. The peripheral administration of GABA analogues should not immediately suggest that GABA acts exclusively in the central nervous system to affect changes in adrenal enzyme activity; the possibility of a local effect at the chromaffin cell membrane is not altogether unreasonable.

(f) GABA and Polyamine Metabolism

There appear to be at least five different pathways that can contribute to GABA formation in the periphery; one of these is the production of GABA during the course of polyamine metabolism (Fogel, 1986).

It has been found that GABA may be derived from putrescine (Fogel, 1986); this polyamine is the product of the decarboxylation of ornithine by ODC. However, in the CNS, the contribution of putrescine-derived GABA to the total GABA pool is considered to be negligible. The conversion of putrescine to GABA in mammalian brain (Seiler and Al-Therib, 1974) proceeds through acetylation of putrescine to monoacetylputrescine. This compound is then oxidatively deaminated by monoamine oxidase (MAO). Subsequent steps include oxidation of N-acetyl-γ-aminobutyraldehyde to N-acetyl GABA, and its deacetylation. This pathway has also been found in rat intestine and kidney (Seiler and Al-Therib, 1974). Alternatively, GABA can be formed from putrescine by direct oxidative deamination; the first step catalyzed by diamine oxidase (DAO) and the second by aldehyde dehydrogenase. This pathway for GABA formation appears to be restricted to the peripheral tissues (Fogel, 1986).

Because GABA is present in fibers and chromaffin cells of the adrenal medulla in various species (Alho et al., 1985), it is conceivable that the pathways utilizing putrescine for GABA formation discussed above are functional in the adrenal medulla. Consequently, the regulation of amODC, which catalyzes the formation of putrescine, might be influenced by levels of GABA in that gland. Thus, the regulation of ODC by GABA must take into account the possibility of local end-product inhibition at the protein level rather than a neural mechanism mediated by the neurotransmitter GABA. As intriguing as this possibility may be, it is rather unlikely; GAD is also found in adrenal medulla (Alho et al., 1985; Kataoka et al., 1984) and this enzyme is probably responsible for synthesizing most of that tissue's GABA supply.

6. DOPAMINE RECEPTORS

The dopamine receptor was initially considered to be a single protein in dynamic equilibrium between two configurational states (Creese et al., 1975). At present, it is generally agreed that there are, in fact, two categories of DA receptor, designated D-1 and D-2 (Kebabian and Calne, 1979). The D-1 receptor has been defined as being coupled to the stimulation of adenylate cyclase, as demonstrated in rat striatum (Kebabian et al., 1972). In many tissues, the initiation of the physiological response to DA is associated with the accumulation of cyclic AMP. The 7-halogenated benzazepine, SCH23390, is a DA antagonist (Iorio et al., 1983) and has proved useful as a D-1-selective ligand. It has thus provided information regarding the properties of this receptor. For example, the regional distribution of the high-affinity D-1 sites in rat brain was determined by using [^{3}H]-SCH23390:receptor densities were greatest in corpus striatum, nucleus accumbens and olfactory tubercle. Interestingly, however, it was also revealed that only some of the (D-1) receptors binding [^{3}H]-SCH23390 are linked to adenylate cyclase and, hence, to cAMP synthesis (Mailman et al., 1986). In contrast to this, stimulation of the D-2 receptor effects an inhibition of adenylate cyclase activity (Onali et al., 1985; Stoof and Kebabian, 1981). The D-2 site can be labelled with butyrophenone ligands such as [^{3}H]-haloperidol (Seeman et al., 1975). The two DA receptor subtypes are also distinguished by the fact that adenylate cyclase-linked receptors are stimulated by micromolar concentrations of dopamine, but the D-2 sites respond to nanomolar ranges (Kebabian and Calne, 1979).

The most extensively studied dopaminergic brain region is the nigrostriatal system; five DA receptor loci have been identified (Kebabian and Calne, 1979). Figure III attempts to schematize the localization of D-

1 and D-2 receptors in the A9 (nigrostriatal) region. In the substantia nigra, there are presynaptic D-1 sites residing on striatonigral GABAergic nerve-endings, and D-2 receptors located on dopaminergic cell bodies ("autoreceptors", Nagy et al., 1978) which regulate the electrical activity of these cells. In the striatum there are: (1) presynaptic D-2 receptors on dopaminergic nerve terminals which regulate the biosynthesis of the amine (Kehr et al., 1972); (2) D-2 sites on neurons projecting to the striatum from the cerebral cortex (Schwarcz et al., 1978) and (3) post-synaptic D-1 sites on neurons intrinsic to the caudate nucleus. It has also been suggested that D-1 and D-2 receptors co-exist on the same caudate nucleus neurons (which receive input from the substantia nigra) and that these receptor subtypes exert inhibitory and excitatory influences, respectively (Ohno et al., 1987).

The functional consequences of receptor binding reflect the cellular location of the receptors. Thus, DA metabolism which is increased in the presence of DA antagonists, is affected to a greater extent by D-2-specific blockers than by D-1-specific blockers. Similarly all DA agonists decrease DA metabolism, but the decrease is more appreciable with the administration of the D-2-selective drugs (Boyar and Altar, 1987). Furthermore, DA release is increased by D-2 but not D-1-specific antagonists and D-2 agonists decrease DA release whereas D-1 agonists have minimal effects (Boyar and Altar, 1987). The predominance of the D-2 receptor in controlling DA metabolism and release is probably attributable to the presence of D-2 but not D-1 sites on the nigrostriatal projection presynaptically.

The behavioral consequences of D-1 and D-2 specific receptor stimulation has also been investigated. Experiments with mice have

implicated D-2 receptors in the mechanisms of locomotion and rearing whereas D-1 receptors seem to be involved in the expression of grooming (Starr and Starr, 1986).

Many studies have suggested that D-1 and D-2 receptors in the striatum can interact and thus regulate their responsiveness. For example, D-2 receptor stimulation can suppress the responsiveness of the D-1 receptor within the striatum (Stoof and Kebabian, 1981). The receptors may also interact "co-operatively", whereby activation at the D-1 site allows for the expression of the consequences of D-2 receptor stimulation. For example, Walters et al., (1987) reported that D-1 and D-2 receptors exert synergistic effects on the firing rates of basal ganglia neurons and on the expression of stereotyped behavior in rats. Furthermore, using AMPT-treated rats these authors showed that the ability of D-2 agonists to induce changes in the electrophysiology of basal ganglia neurons and in spontaneous motor activity requires the availability of endogenous dopamine to stimulate D-1 receptors. Thus, the currently held view that D-2 and D-1 receptors are each individually responsible for governing certain biochemical or behavioral phenomena may have to be modified.

Previous work in this laboratory has established a role for DA in the induction of amODC and ATHA (Almazan et al., 1982a; Quik and Sourkes, 1976). The exact mechanism of this DA-elicited induction is not known. Do DAergic agents increase adrenal enzyme activity by stimulating D-1 receptors or D-2 receptors, or is each of the receptor subtypes equally important? Do the receptors act synergistically to elicit this inductive phenomenon? Looking at this problem from a different perspective, one might consider whether the mechanisms involved bring into play the postsynaptic or presynaptic DA receptors. In this work, various pharmacological manipulations have been carried out by utilizing D-1 and D-

2-specific agents as well as substances which selectively activate presynaptic dopamine receptors. The aim has been to shed light on the DA-mediated induction of TH and ODC.

7. RESEARCH OBJECTIVES

Studies of the neural regulation of adrenal enzymes have provided useful information in elucidating the central nervous pathways involved in the stress response (Sourkes, 1983). The aim of the present work is to contribute to this information by determing whether GABAergic nerve fibres play a role in the induction of ODC and TH. In pursuing this main objective answers to the following questions were sought:

1) Other CNS neurotransmitters each have similar effects on TH and ODC; do these two adrenal enzymes respond differently or similarly to treatment with GABA analogues?

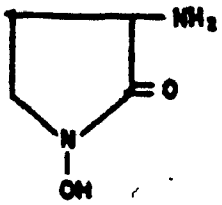
2) If GABA does influence the regulation of ODC and TH, is this through a central or peripheral mechanism?

3) Because GABA, a ubiquitous neurotransmitter in the central nervous system, is known to interact with the neurotransmitters which do, in fact, cause an increase in TH or ODC, does it exert its influence on TH and ODC independently or via an interaction with these other CNS neurotransmitters?

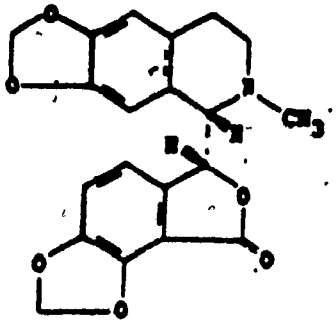
Another aspect of this project was to study the neural mechanism by which activation of the nigrostriatal dopaminergic system (A9) results in changes in adrenal enzyme activity. In this regard, the efficacy of D-1 vs. D-2 dopamine receptor stimulation in the induction of ODC and TH was determined. In addition, because certain GABA analogues were found to cause an increase in the two adrenal enzymes at the same time as they prevented dopamine release, the possibility that dopaminergic agonists might effect changes in adrenal enzyme activity through a presynaptic

mechanism was explored.

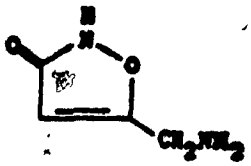
i) GABAergic Analogues:



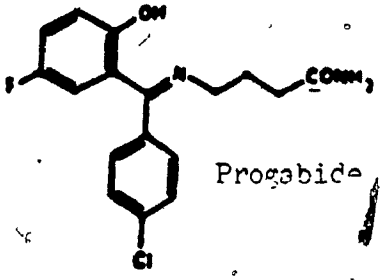
HA-066
(1-hydroxy-3-amino
-pyrrolidone-2)



Bicuculline

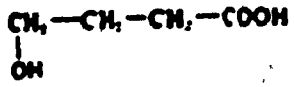


Muscimol



Progabide (SL 76002)

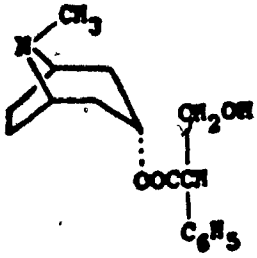
4-{[(4-chlorophenyl) (5-fluoro-2-hydroxy phenyl)methylene]amino}butanamide



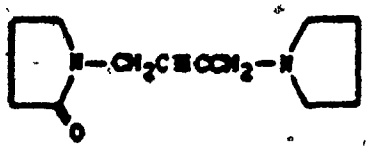
Gamma-hydroxy butyric acid

GOBA

ii) Cholinergic Analogues:

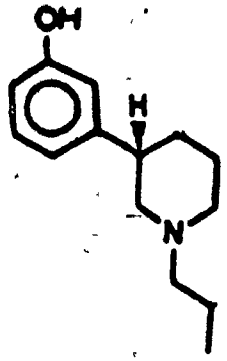


Atropine



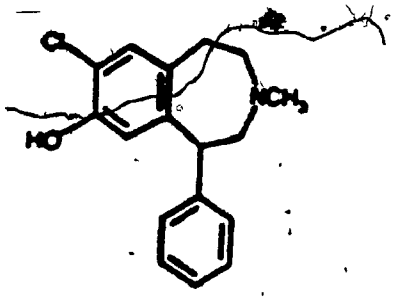
Oxotremorine

Figure I: Structures of GABAergic and Cholinergic Analogues Used.



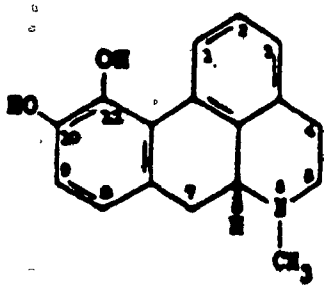
R(+)-3-PPP

(R)-3-(3-hydroxyphenyl)
-N-n-propylpiperidine

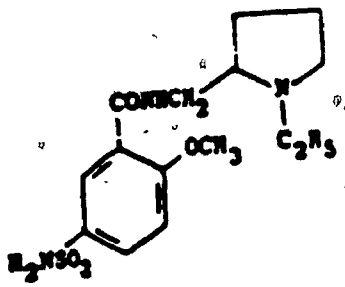


SCH23390

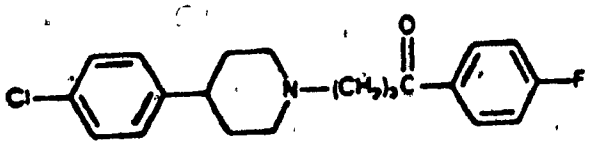
7-chloro-2,3,4,5-tetrahydro
-3-methyl 5-phenyl-1H-3-
benzazepine-7-ol.



Apomorphine

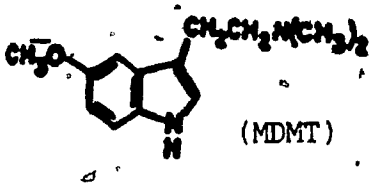


Sulpiride



Haloperidol

5HT Agonist:



(MDMT)

N,N-dimethyl-5-methoxytryptamine

Figure II: Structures of Dopaminergic and Serotonergic Analogues Used

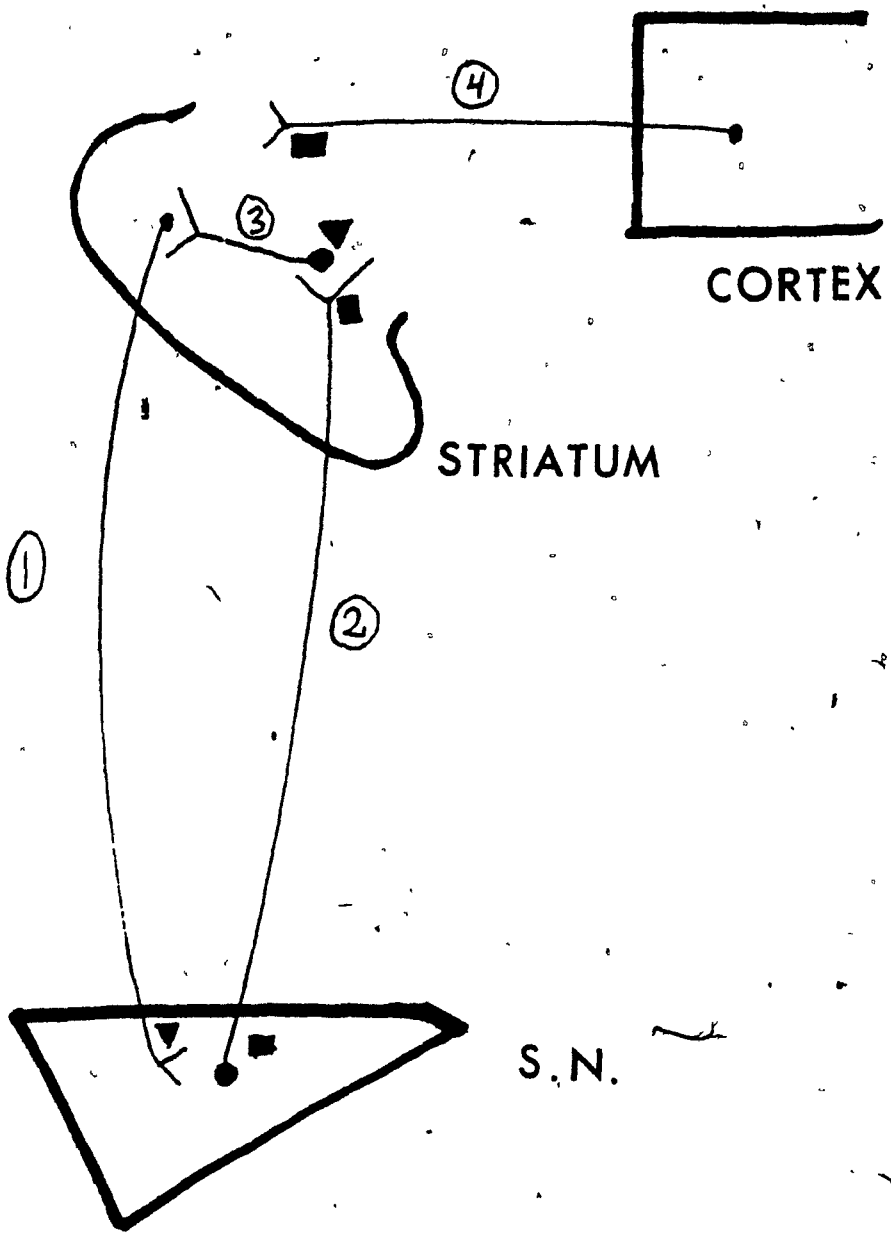


Figure III: A Schematic Representation of D-1 and D-2 Receptors in the A9 Region.

Legend: ▼ - D-1 Receptor Site
■ - D-2 Receptor Site
S.N.- Substantia nigra
1.- GABAergic striatonigral pathway
2 - DAergic nigrostriatal pathway
3 - Cholinergic striatal intrinsic neuron
4 - Corticostriatal neuron

B. - MATERIALS AND METHODS

1. ANIMALS

Male Sprague-Dawley rats weighing between 180 and 250 grams were used, except for experiments involving splanchnicotomy. They were obtained from the breeding farms of Charles River Canada Incorporated in St. Constant, Quebec. The animals were usually received from the supplier at approximately 1 p.m. They were then tail-marked and distributed to cages on the day of arrival. The rats were frequently handled before use in experiments in order to accustom them to the experimenter. Experiments were begun the following morning. The rats were kept 4 or 5 to a cage in an animal room with a light-dark cycle of 12 hours and with a thermostatically controlled temperature of 22°C. In experiments involving the administration of apomorphine, the animals were placed in individual wire cages. There was free access to tap water and Purina Checkers. Control animals received vehicle by the same route as the experimental group and also received the same volume and number of injections. At the end of an experiment, the rats were sacrificed by decapitation.

2. SURGERY

(a) INTRACEREBROVENTRICULAR INJECTION OF DRUGS

Rats weighing between 200 and 210 grams were anaesthetized with chloral hydrate, 300 mg/kg i.p. (USP, Fisher Scientific Company, Montreal, Canada) and positioned in the stereotaxic instrument. With the skull flat, the injections were given at the point 1.0 mm posterior to bregma, 1.5 mm lateral to midline and 3.5 mm vertical (Paxinos and Watson, 1982). The ventricular site was confirmed by injection of methylene blue. The drugs were given in a volume of 10 µl at the rate of 5 µl/minute delivered from a Hamilton microsyringe. Animals were sacrificed 24 hours after drug administration. In i.c.v. experiments, only adrenal TH activity was determined.

(b) SPLANCHNICTOMY

Left-sided hemisplanchnicotomy was performed in rats under chloral hydrate anaesthesia, 300 mg/kg i.p. The animals weighed between 200 and 250 grams at the time of surgery. With a dissecting microscope, the tissue surrounding the adrenal gland was completely dissected, except for protection of the vascular supply of the gland. The mean weights of the adrenals on the denervated and intact sides were not significantly different. To verify the efficacy of the operation, in a few rats the site of the transection was electrically stimulated and heart rate and blood pressure were measured. Electrical stimulation should elicit an elevation in these two physiological parameters only in animals with an intact splanchnic nerve; with the technique used, no increase in heart rate or blood pressure was detected. Injections were begun on the fifth post-operative day in order to allow for sufficient recovery from surgical stress. In all experiments involving splanchnicotomy, only adrenal TH activity was determined.

3. DRUGS

Over the course of this work, a variety of neurotransmitter systems were studied and, consequently, many drugs were used. They are listed in Table B.1. The following drugs were purchased from the Sigma Chemical Company, St. Louis, MO: sodium γ-hydroxybutyrate (GOBA), muscimol, oxotremorine, methylatropine bromide, α-methyl-DL-tryptophan (AMTP), N,N-dimethyl-5-methoxytryptamine (MDMT) and p-chlorophenylalanine methylester HCl (PCPA). Atropine sulfate was purchased from the J.T. Baker Chemical Company, Phillipsburg, NJ; bicuculline from K & K Laboratories, Plainview, NY; apomorphine HCl from F.E. Cornell and Co., Montreal, Quebec; and R(+)3-PPP (3-(3-hydroxyphenyl)-N-n-propylpiperidine; Hjorth et al., 1981) from

Research Biochemicals Incorporated, Wayland, MA. The following drugs were gifts and are gratefully acknowledged: progabide (SL76002) from L.E.R.S.-Synthélabo, Paris, France; HA-966 from Dr. Noach, U. of Leiden, The Netherlands; haloperidol (McNeil Laboratories, Don Mills, Ontario); SCH23390 (Schering Co., Bloomfield, NJ); sulpiride from Delagrange International, Paris, France, and from Ravizza S.P.A., Milan, Italy.

Most of the drugs were dissolved in saline; however there were some exceptions. Progabide was suspended in 0.5% methyl cellulose and injected in this form. GOBA was dissolved in water and titrated to approximately pH 7.2 with a few drops of 1N HCL. Haloperidol, sulpiride, bicuculline, HA-966 and MDMT were each dissolved in a few drops of glacial acetic acid, diluted with deionized water and titrated to pH 6.3. SCH23390 was dissolved in a 25% solution of propylene glycol. All drugs were injected in a volume of either 2.5 or 5.0 ml/kg body weight except for oxotremorine, which was injected in a volume of 1.25 ml/kg. Control animals received the vehicle in the same volume as the drug and with the same number of injections.

The doses of drugs used are presented in Table B.1. They refer to milligrams of the commerically available form of the drug. For example, GOBA was injected in a dose of 0.5 g/kg as the sodium salt and apomorphine was injected in a dose of 3 mg/kg as apomorphine hydrochloride. There were two exceptions to this rule. The doses given for methylatropine bromide and atropine sulfate (5 mg/kg and 10 mg/kg, respectively) refer to the base itself, i.e. larger amounts of the salts were dissolved in order to get effective doses of methyl atropine and atropine, respectively. The drug doses were based on body weight, except for i.c.v. experiments, where the dose was a fixed amount per animal.

4. BIOCHEMICAL PROCEDURES:

a) Determination of Ornithine Decarboxylase Activity in Adrenal Medulla.

(i) CHEMICALS

L-[1-^{14}C]Ornithine (specific activity, 52.4 - 54.3 mCi/mmol) and 2,5-diphenyloxazole (PPO) were purchased from New England Nuclear, Boston, MA. Pyridoxal 5'-phosphate (crystalline) and DL-dithiothreitol were purchased from Sigma Chemical Company, St. Louis, MO. EDTA, sodium phosphate (dibasic), potassium phosphate (monobasic), ethyleneglycol monomethyl ether, toluene and monoethanolamine were all purchased from the Fisher Scientific Company, Montreal.

(ii) TISSUE PREPARATION

In experiments involving the determination of ODC activity, the animals were sacrificed 4 hours after the initial injection of drugs, unless otherwise noted. After decapitation, the adrenal glands were removed immediately and placed on ice. Both medulla and cortex contain ODC, the former under neural, the latter under endocrine regulation (Almazan et al., 1982a). Because the focus of this work is on the neural regulation of adrenal enzymes, it was necessary to separate the medullary from the cortical tissue. To do this the glands were first freed of capsular tissue and weighed. The dissections were done at 4°C by visual inspection under a magnifying lamp and they were completed within 4 hours of sacrifice. The pairs of medullae were kept on ice and the cortices were discarded. After all pairs of glands were dissected, the medullae were homogenized, in 200 µl of the ODC assay buffer (see below), with a motor-driven Teflon pestle (A. H. Thomas Co., Philadelphia, PA) rotating at 140 rpm for 30 seconds. The homogenate was centrifuged for 30 minutes at 12,000g in an Eppendorf table-top centrifuge (Brinkmann 5412). The supernatant was kept at -70°C

overnight and assayed the following day.

(iii) ASSAY of ODC ACTIVITY in vitro

The in vitro assay of ODC activity used in this work is a method that combines elements of the assays described by Russell and Snyder (1968) and Jänne and Williams-Ashman (1971), with some minor modifications (Ramirez-Gonzalez et al., 1980). Essentially, the method involves the measurement of the $^{14}CO_2$ produced by the enzymic decarboxylation of L-[^{14}C]ornithine. The ODC assay buffer consisted of 0.05M sodium-potassium phosphate buffer, pH 6.8; 1.0 mM dithiothreitol; 0.1 mM EDTA; and 0.05 mM pyridoxal phosphate. The incubation mixture contained, in a final volume of 0.5 ml, the following ingredients (final concentrations given): 100 µl of the 12,000g supernatant fraction of the tissue homogenate (homogenized in 200 µl of the above ODC assay buffer); 1 µCi of L-[^{14}C] ornithine, 0.04 mM; pyridoxal phosphate 0.05 mM; dithiothreitol 1.0 mM; EDTA 0.1 mM and sodium-potassium phosphate, 0.05 M, pH 6.8. The reaction was carried out in 25 ml Erlenmyer flasks equipped with a plastic well hanging from a rubber stopper (Kontes Glass Co., Vineland, NJ). The plastic well contained a filter paper (2cm^2, Whatman no. 3) impregnated with 100 µl of a mixture of ethyleneglycol monomethyl ether and monoethanolamine (2:1). After a preincubation period of 10 minutes at 37°C, the reaction was started by injecting the substrate (1 µCi of L-[^{14}C] ornithine in a volume of 0.3 ml) through the rubber stopper, and was allowed to continue for 45 minutes at 37°C in a shaking water bath (Haake SWB 20). The reaction was stopped with the injection of 0.5 ml of 6 M sulfuric acid through the rubber stopper. The flasks were incubated for an additional period of 45 minutes in order to trap all the CO_2. The filter paper was then collected and placed in a vial containing 10 ml of a mixture of toluene and ethyleneglycol monomethyl

ether (2:1) that contained 0.4% 2,5-diphenyloxazole. The radioactivity contained in each sample was determined with the use of a scintillation counter (Beckman LS-250). The activity of ornithine decarboxylase is expressed as pmoles CO_2 produced per mg protein per 45 minutes at 37°C. The protein content of the sample was estimated by Lowry's method (Lowry et al., 1951).

(b) Determination of Adrenal Tyrosine Hydroxylase Activity

(i) Chemicals

Aquasol-2 and L-[ring-3,5-^{3}H] tyrosine (54.2 Ci/mmol) were purchased from New England Nuclear Corporation, Boston, MA. The labelled tyrosine was purified before use by a modification of the procedure of Coyle (1972) and stored in 2% ethanol at 4°C. An appropriate volume of that solution was lyophilized to dryness prior to each assay and the residue dissolved in 1 mM unlabelled L-tyrosine, pH 3.1. Dowex 50W-X8 (H+ form, 200-400 mesh) from Bio-Rad laboratories (Canada) Ltd., Mississauga, Ontario and alumina (Woelm, neutral activity, Grade I) from ICN (Canada), Montreal, Quebec, were used to purify the radioactive substrate. L-Tyrosine, catalase (2x crystallized from beef liver) and 6,7-dimethyl-5,6,7,8-tetrahydropterin HCl ($DMPH_4$) were obtained from Sigma Chemical Company, St. Louis, MO. Potassium phosphate (monobasic) and trichloroacetic acid were purchased from J.T. Baker Chemical Company, Phillipsburg, NJ. Sodium acetate was from Fisher Scientific Company and L-ascorbic acid was purchased from BDH Chemicals. Brocresine (p-bromo-m-hydroxybenzyloxyamine phosphate, NSD 1055) was a gift of Lederle Laboratories, Pearl River, NY.

(ii) Tissue Preparation

In experiments involving the determination of TH activity, the injections were carried out over a period of 3 days, except in the case of

HA-966, where the duration of the experiment was only one day. In all cases, the animals were sacrificed by decapitation on the morning following the last injection. The adrenal glands were immediately removed and put on ice. After being freed of capsular tissue and weighed, pairs of glands (each gland separately in the case of splanchnicotomized rats) were homogenized in 0.9 ml of ice-cold saline with a Teflon pestle rotating at 140 rpm for 30 seconds. The homogenizations were completed within 4 hours of sacrifice.

(iii) Adrenal TH Assay

The activity of adrenal tyrosine hydroxylase was determined in 100 µl portions of tissue homogenate according to the method of Nagatsu et al. (1964) as modified by Gauthier et al. (1979). The method involves the measurement of the tritiated water produced as a result of the hydroxylation of L-[ring 3,5-^{3}H] tyrosine. The radioactive substrate (54.2 Ci/mmol) was purified before use, first by passage through an alumina column and then through a Dowex 50W-X8 (H+ from, 200-400 mesh) column, 0.5 x 3.0 cm and stored in 2% ethanol at 4°C.

The incubation mixture, in a total volume of 0.5 ml, contained 100 µl of the tissue homogenate and 0.2 ml of a solution of 198 µmol sodium acetate, 9.9 µmol potassium phosphate (monobasic), 1950 U catalase, and 69 nmoles of brocresine (free base), pH 6.1. After addition of 0.05 ml solution containing 50 nmoles L-[ring-3,5-^{3}H] tyrosine, pH 6.1 (approximately 350,000 cpm), the samples, in open 13 x 100 mm borosilicate tubes, were equilibrated for 5 minutes at 30°C in a Dubnoff metabolic shaking incubator. The enzymatic reaction itself was started with the addition of 6,7-dimethyl-5,6,7,8-tetrahydropterin hydrochloride, 0.42 µmol free base, and ascorbate, 1.25 µmol in 0.05 ml. After 15 minutes, the reaction was stopped by addition of 0.1 ml 25% trichloracetic acid. The

samples were cooled on ice for at least 45 minutes and were then centrifuged for 15 minutes at 20,000 g. The supernatant was passed through a Dowex 50W-X8 column 0.5 x 3.0 cm previously equilibrated with 1.8 ml trichloroacetic acid, pH 1.5. The protein pellet was washed once with 0.8 ml trichloroacetic acid, pH 1.5; the washings were passed through the column also. Both effluents were collected, combined with 10 ml of Aquasol-2 and the radioactivity of the tritiated water produced by the enzymatic reaction measured in a liquid scintillation counter.

Recovery of tritiated water was about 95% and counting efficiency approximately 40%. The enzyme activity was linear for 20 minutes, about 4% of the labelled substrate having disappeared at that time (Ekker, 1985). Adrenal tyrosine hydroxylase activity is expressed as nmol of L-dihydroxyphenylalanine (DOPA) formed per hour per pair of adrenal glands at 30°C.

5. STATISTICAL METHODS

(a) Student's t-test

Values of enzyme activities in all Tables and Figures are expressed as mean ± standard error. The significance of the difference between two different treatment groups was determined by Student's t-test. In most cases, particularly in pilot studies, a two-tailed test of t was used. However, when the directionality of a particular treatment's effect was already established, a one-tailed test was often applied for comparing two different treatment groups.

(b) Analysis of Variance

In addition to Student's t-test, the data were subjected to an analysis of variance (ANOVA). Initially, a one-way ANOVA was carried out to determine the significance of the experiment as a whole. In certain 2 x

2 experiments, a two-way ANOVA was also done to assess the degree of interaction between two drugs administered to the same animals. In many instances, the data from several similar experiments involving the administration of a particular drug were pooled and subjected to ANOVA. In these cases, the significance of a drug's effect was considered after accounting for variations between experiments.

Table B.1

Drugs used in the experiments described in this thesis: Doses, Route and Schedule

Neurotransmitter System	Name of Compound	Mol. Wt.	Route	Dose(s)	Injection Schedule ODC	TH
GABAergic						
(a) Agonists	Muscimol	114.1	s.c.	3 mg/kg	1/d*	b.i.d.
	Progabide (SL76002)	334.5	s.c.	100 mg/kg or 100 mg/kg+50 mg/kg	1/d	b.i.d.
	Sodium hydroxybutyrate (GOBA)	126.1	i.p.	0.5 g/kg	1/d	1/d
	HA-966	111	i.p. or i.c.v.	150 mg/kg or 5 µg	-	1/d
(b) Antagonist	Bicuculline	367.34	s.c.	1 mg/kg	1/d	b.i.d.
Dopaminergic						
(a) Agonists	Apomorphine hydrochloride	303.81	s.c.	1.5 mg/kg or 3 mg/kg	t.i.d.	q.i.d.
	R(+)3-PPP	255.79	s.c. or i.c.v.	10 mg/kg or 2 µg	1/d	1/d
(b) Antagonists	SCH23390	403.57	i.p.	3 mg/kg	1/d	b.i.d.
	DL-Sulpiride	341.43	s.c.	40 mg/kg or 50 mg/kg	1/d	b.i.d.
	Haloperidol	375.88	i.p.	5 mg/kg	-	1/d

Table B.1

Drugs used in the experiments described in this thesis: Doses, Route and Schedule

Neurotransmitter System	Name of Compound	Mol. Wt.	Route	Dose(s)	Injection Schedule ODC	TH
Cholinergic						
(a) Agonist	Oxotremorine	206.28	s.c.	0.5 mg/kg or 0.35 mg/kg	1/d	b.i.d.
(b) Antagonists	Methylatropine bromide	384.5	s.c.	5 mg/kg	1/d	b.i.d.
	Atropine sulfate	694.82	i.p.	10 mg/kg	-	1/d
Serotonergic						
(a) Agonists	MDMT	218.3	s.c.	1 mg/kg	-	b.i.d.
	DL-AMTP	218.2	s.c.	100 mg/kg	-	1/d
(b) Antagonist	PCPA	250.1	i.p.	300 mg/kg	-	1/d

*1/d = once a day

C. RESULTS

1. Effects of GABAergic Drugs on Adrenal Enzymes

(a) Tyrosine Hydroxylase

The influence of the GABA system on TH was studied as follows: Various GABAergic agents were injected into rats to determine whether these drugs can effect an increase in TH activity. A series of experiments was carried out with each of five GABAergic agents and the results obtained from individual experiments for a particular drug were pooled.

i) The Effects of Muscimol, Bicuculline, Progabide, HA-966 and GOBA ATHA.

Muscimol, a GABA-A receptor agonist, was administered in a dose of 3 mg/kg twice daily for 3 days. As shown in Table C.1, it caused a significant increase in ATHA ($P < 0.01$), almost doubling the control levels. This inductive effect was clearly apparent despite the variability among the experiments ($F = 24.94, P < .01$), as shown in the ANOVA (Table C.1).

The above result was obtained with muscimol, 3 mg/kg. To determine if a lower dose of muscimol could also elicit a significant increase in ATHA, a dose-response experiment was carried out. The results revealed that a dose of 1 mg/kg of muscimol given twice daily for 3 days was unable to cause a significant increase in ATHA. Thus, muscimol gave 20.2 ± 1.19 ($n=4$) nmoles L-DOPA per hr. per pair adrenals, compared to control: 21.4 ± 3.88 ($n=4$) ($P > 0.05$) whereas a dose of 3 mg/kg b.i.d. for three days significantly elevated the enzyme activity: 33.6 ± 1.93 nmoles ($n=4$) L-DOPA per hr. per pair of adrenals with muscimol as agonist, 21.4 ± 3.88 ($n=4$) in the controls ($P<0.05$).

In addition to varying the dose of muscimol, the injection schedules were also altered. In a single experiment, muscimol injections for two days, 3 mg/kg b.i.d., produced a 38% increase in enzyme activity. The

actual values were 45.8±4.33 nmoles L-DOPA per hr. per pair adrenals compared to 33.3±1.92 for saline controls (n=5) ($P<0.05$). In 2 experiments, injection of muscimol for one day also produced a significant increase in ATHA, with a mean increase of 48% over saline controls (Table C.2).

It seems reasonable to conclude that muscimol, given in a dose of 3 mg/kg can cause a significant increase in ATHA. While these experiments illustrate that one-day and two-day periods are sufficient to elicit significant enzyme induction, the largest relative increase in enzyme activity occurs after three days of muscimol administration (see Figure IV).

Bicuculline was also administered to rats in a series of experiments. This alkaloid blocks the action of certain GABA agonists, which then (by definition) are said to act at GABA-A receptors. Results of the individual experiments are shown in Table C.3. In five out of six trials bicuculline-treated rats showed a mean increase in ATHA, but in only two experiments were the increases statistically significant ($P<0.01$). The overall mean increase, based on six experiments, was 23.8±9.61%. After separating the experiments into two groups, based on the number of injections per day, it was found that bicuculline injections once daily produced an average mean increase equal to 35±5% over controls (experiments #1,2, Table C.3) and twice daily injections produced an average mean increase of only 18±11%. Furthermore, of the two experiments producing significant increases in ATHA, one was run on a one per day injection schedule and one on a twice daily injection schedule. It seems then, that increasing the number of injections of bicuculline from one to two per day does not effect a greater induction of the enzyme nor does it ensure a statistically significant result.

The results with bicuculline are in contrast to those with muscimol. The latter drug consistently effected a significant increase in ATHA, even under different conditions. Although the data show that bicuculline may elicit a significant increase in ATHA, its action is inconsistent. Whether the GABA antagonist plays a role in the regulation of TH is uncertain. The reasons for the ambiguity of the data are not obvious, but they may simply reflect the unstable nature of bicuculline in solution. (Andrews & Johnston, 1979).

The efficacy of progabide, a drug which is metabolized to GABA in the CNS, in bringing about induction of adrenal TH was tested as well. Two experiments were carried out, with results shown in Table C.4. It is evident that progabide is unable to elicit an increase in enzyme activity. The mean enzyme activities of the two groups were almost identical.

GOBA and HA-966, GABA analogues which temporarily prevent dopamine release from nigrostriatal fibres, were also tested for their effects on ATHA. GOBA was administered to rats once daily for 3 days, 0.5 g/kg i.p., in 4 experiments (Table C.5, set II). It produced a significant increase in enzyme activity (Table C.5 $P < 0.01$). HA-966 was given by two routes: peripherally, 150 mg/kg i.p. (set I, 4 expts., Table C.6), and centrally, 5 µg i.c.v. (set III, 2 expts). This compound effected significant increases in ATHA by both routes of injection ($P<0.01$ and $P< 0.05$, respectively). The mean enzyme activities resulting from these 3 treatments are shown in Table C.5; the data for experiments in each set were pooled and subjected to ANOVA, with removal of variance owing to differences between the experiments; the statistics are summarized in Table C.6.

Despite the variability among the experiments involving peripheral injection of the drugs ($P< 0.01$), the data show that both HA-966 and GOBA are able to elicit significant increases in ATHA ($P<0.01$ for both drugs).

HA-966 is the more potent in effecting an induction, a single injection of 150 mg/kg for only one day being sufficient. In an experiment not shown here, GOBA was unable to cause a significant increase in ATHA after only 1 day but, as Tables C.5 and C.6 show, it did so after 3 days of injection. The results of the i.c.v. experiments with HA-966 are very striking. The application of a minute amount of it (5 µg in contrast to 150 mg/kg given peripherally) injected directly into the brain cavities produced a significant increase in enzyme activity (30% over controls). This suggests that the inductive effect of this GABA analogue is centrally mediated.

Another approach was used to study the possibility that HA-966 is acting centrally; this involved the peripheral administration of the drug to rats which had been hemisplanchnicotomized previously. In these animals, the nervous input to the left adrenal gland was severed while that of the right gland remained intact. If the GABA agonist is acting centrally, then its administration would cause an increase in ATHA in the right but not the left gland. The results from such an experiment are shown in Table C.7.

Whereas HA-966 had no inductive effect on ATHA in the denervated gland (in fact, it appeared to decrease activity), it caused a significant increase in enzyme activity in the intact gland, as compared to saline controls. The decrease in ATHA in the denervated gland might be a manifestation of a local GABAergic phenomenon, as discussed by Kataoka et al. (1986). However, this lowering effect was not explored further in this thesis. The fact that splanchnicotomy prevents the increase in ATHA elicited by HA-966 corroborates the findings of the i.c.v. experiments with this compound. It seems that the induction of adrenal tyrosine hydroxylase by HA-966, not unlike that of dopamine agonists (Quik and Sourkes, 1976) is

mediated by central neuronal mechanisms.

(ii) GABA Agonist-Antagonist Interactions in the Regulation of ATHA

Because certain GABA agonists increase ATHA, as shown above, the possibility that co-administration of a GABA antagonist might abolish this inductive effect was examined. In three separate experiments, rats previously treated with bicuculline were given one of three GABA agonists. The results are set out in Table C.8.. The increases in enzyme activity effected by GOBA and muscimol were not blocked by the GABA antagonist. Progabide, which was unable to elicit an increase in ATHA when given alone, produced a statistically significant increase when administered to bicuculline-treated animals.

These experiments suggest that GABA-receptor blockade (by bicuculline) might paradoxically facilitate a GABA-mediated inductive effect occurring elsewhere. This idea is fostered by the knowledge that the striatum (and presumably other regions of the brain) contains numerous GABAergic neurons, some of them in series (Scheel-Krüger, 1986). Whatever the explanation of that result may be, the data clearly demonstrate the fact that bicuculline does not attenuate the increase in ATHA elicited by GABA agonists.

(b) Ornithine Decarboxylase

In these studies GABAergic agents were administered to rats to test effects on adrenal medullary ornithine decarboxylase (amODC) activity. In four experiments (see Table C.9) muscimol given in a single dose of 3 mg/kg s.c. was able to elicit a significant increase only once. This is in sharp contrast to the results observed with ATHA, where muscimol consistently provided significant increases in ATHA (Tables C.1 and C.2). The baseline levels of amODC activity were quite variable, three of the control values lying in the low range of ODC activity as observed over many years in this laboratory, but one in the moderately high range (149±45.3

pmoles CO_2 per 45 minutes per mg protein). This is not surprising as ODC is very readily induced (Morris and Fillingame, 1974) Progabide had no effect on amODC in two experiments. The data from these experiments are summarized in Table C.9 also.

GOBA and HA-966 were also administered to rats to determine if they can induce amODC. The data from 2 similar experiments for each drug were pooled and subjected to ANOVA. Whereas HA-966 elicited a significant increase in amODC ($P < 0.01$, Table C.10), GOBA did not do so (Table C.10). The increase caused by HA-966 amounted to 155% above baseline; this is quite small compared to the effect of other drugs, such as apomorphine (Almazan et al., 1983b) or oxotremorine acting centrally (Ramirez-Gonzalez et al., 1980).

To summarize, of four GABAergic drugs tested, only HA-966 caused significant induction of amODC. Muscimol and GOBA had no significant effect under the conditions tested.

2. Interactions of GABAergic Agents with the Cholinergic System

(a) Ornithine Decarboxylase

Despite the limited result obtained in section C.1 (b), it is plausible that GABA analogues might influence the well established inductive effect of the cholinergic agent oxotremorine on that adrenal enzyme (Ramirez-Gonzalez et al., 1980). Thus, the administration of GABAergic agents to rats also receiving oxotremorine might elicit a greater induction in amODC than with the latter drug alone. Initially, a dose-response study was undertaken to establish a dose of oxotremorine that can induce amODC submaximally. Methyl atropine was administered thirty minutes prior to the muscarinic agonist in order to antagonize its peripheral, but not central, effects. Sixty per cent of the induction that was elicited by

1.0 mg/kg of oxotremorine occurred at a dose of 0.35 mg/kg (see Figure V). By administering this submaximal dose of oxotremorine with a GABA analogue, any additional increment in enzyme activity owing to the latter drug should be readily seen.

Two experiments involving the combination of muscimol and oxotremorine were carried out and the results are presented in Table C.11. As was reported earlier, muscimol by itself does not cause a significant increase in amODC. When it was given in combination with oxotremorine it caused a greater mean induction than observed with the latter drug alone but this augmentation was not statistically significant ($P > 0.05$, Table C.11). Thus, it can be concluded that muscimol does not contribute to an interaction between GABAergic and cholinergic neurotransmitter systems in the induction of amODC.

Two similar experiments were performed, this time with progabide as the GABA agonist. The results are summarized in Table C.12. Progabide, as was shown earlier, had no inductive effect on amODC ($P > 0.05$). In the present experiments the presence of progabide potentiated the induction of amODC elicited by oxotremorine in one of the experiments, but not the other.

The data from the experiments with progabide and with muscimol suggest that these GABAergic agents do not reliably affect the increase in amODC owing to oxotremorine administration. It is concluded that under the experimental conditions used, interaction of the GABA and central muscarinic systems has not been demonstrated to occur.

(b) Tyrosine Hydroxylase

In the case of adrenomedullary TH, certain GABA analogues elicit a significant increase in enzyme activity (see section C.1 (a)). The

possibility that the increase in ATHA mediated by a cholinergic analogue might be influenced by GABAergic agents was investigated. Bicuculline, a GABA antagonist, was administered conjointly with oxotremorine, a muscarinic agonist which significantly increases the activity of the adrenal enzyme (Lewander et al., 1977). In two separate experiments (Table C.13), each with a different dose of oxotremorine, the enzyme activity in the group receiving both drugs was not significantly different from that of the group receiving oxotremorine alone ($P > 0.05$).

Thus, the centrally-mediated increase in ATHA caused by oxotremorine is not dependent on functionally intact GABAergic fibres, as judged from the use of bicuculline.

GABA agonists were also tested conjointly with oxotremorine (Table C.14). Thus, progabide, which has no inductive effect on ATHA, was given to rats receiving oxotremorine and oxotremorine caused a significant increase in ATHA either in the absence or presence of progabide. The enzyme activity of animals receiving both drugs was not significantly different from that of animals receiving oxotremorine alone ($P > 0.05$).

Muscimol, a GABA agonist with a significant inductive effect on ATHA (Table C.1) was also given to oxotremorine-treated rats. In this case the effects were additive, i.e. the respective increases produced by muscimol and oxotremorine separately were conserved in the group receiving both drugs simultaneously. This additive effect suggests that each drug causes an increase in ATHA by independent mechanisms.

Another approach in investigating a possible interaction between GABAergic and cholinergic systems is to treat animals with the cholinergic blocker atropine and determine if muscimol still elicits an increase in ATHA. If there is an attenuating effect, this would suggest that cholinergic pathways are involved in the GABAergic induction of the enzyme.

Atropine penetrates the blood brain barrier and blocks muscarinic sites centrally as well as peripherally. Thus, whether the GABA agonist is acting centrally or not, atropine would have the potential of interacting with it. The results (Table C.15) reveal the fact that even in the presence of atropine, muscimol is capable of causing a significant increase in enzyme activity.

When taken together, the data from experiments involving a combination of GABAergic and cholinergic compounds suggest that these two neurotransmitter systems do not interact insofar as their regulation of ATHA is concerned.

3. Interaction of GABAergic Agents With Serotonergic Systems in the Regulation of ATHA

The possibility that GABA might interact with serotonin (5HT) in bringing about an increase in ATHA was explored. Initially, the effect of a serotonin agonist, N,N-dimethyl-5-methoxytryptamine (MDMT), on enzyme induction was determined. Four experiments involving the use of MDMT were pooled and its effect on ATHA vs. saline controls was determined by ANOVA. The data are presented in Table C.16. The ANOVA indicates that MDMT has no effect on ATHA when given alone ($P > 0.05$). The injection schedule of the drug was different in each experiment, but this did not influence the effect of MDMT on ATHA (F for experiments = 0.39, $P > 0.05$).

The serotonin agonist was then administered in combination with muscimol to determine if the enzyme induction elicited by the latter drug could be attenuated. The data from 3 separate experiments were pooled and subjected to ANOVA (Table C.17). The experiments were carried out over a 1,-2, or 3-day period. The presence of MDMT did not hinder the induction caused by the GABA agonist ($F = 1.00$, $P > 0.05$); there was little

variation between the experiments ($F = 1.36$, $P > 0.05$).

PCPA, which causes depletion of central 5HT stores, increases ATHA (Breese et al., 1974). This effect was verified against saline controls in 2 experiments; the results were pooled and subjected to ANOVA (Table C.18). Thus PCPA, caused a significant elevation of enzyme activity, approximately 65% over control values ($P << 0.01$). The results were consistent in 2 experiments ($F = 0.329$, $P >> 0.05$). This serotonin-depleting agent was then administered to rats receiving either bicuculline or muscimol. It was felt that if GABA and 5HT interact in the regulation of ATHA, then an increase in enzyme activity elicited by a GABAergic agent might be potentiated by PCPA. The results are shown in Table C.19. In one experiment, PCPA and muscimol were injected into rats either separately or together. Each drug elicited a significant increase in ATHA when given alone and their inductive effects were additive. In the second experiment the results were similar when bicuculline was used in combination with PCPA (Table C.19). Because, in both experiments, the induction elicited by each drug alone persisted in the presence of the other drug, and because the increase over saline controls caused by the two drugs together was approximately equal to the sum of the increases produced by each drug individually, it seems that these two neurotransmitters make use of independent neural pathways in bringing about enzyme induction.

In a final experiment involving a combination of GABAergic and serotonergic agents, α-methyl-DL-tryptophan (AMTP) was administered to rats also receiving HA-966. AMTP was tested because it is converted to methyl serotonin in vivo (Roberge et al., 1972) and might then act on ATHA as does serotonin itself, exerting a braking influence on enzyme induction. Thus, AMTP, was administered, 100 mg/kg s.c., in the morning (zero time) and was allowed 8 hours to reach its maximum concentration in the brain.

HA-966, 150mg/kg i.p., was given at this time and the animals were sacrificed at 32 hours. The results are summarized in Table C.20. AMTP, being in effect a serotonin agonist, did not elicit an increase in ATHA ($P > 0.05$ vs. saline controls). However its administration to rats also receiving HA-966 did not hinder the induction caused by the latter compound (HA-966 vs. HA-966 + AMTP, $P > 0.05$).

The experiments reported in this section suggest that the GABAergic and serotonergic pathways leading to induction of adrenal TH are independent of one another; there seems to be no interaction between their respective neurotransmitters.

4. Dopaminergic Regulation of Adrenal Enzymes

(a) DA Autoreceptor Stimulation with (+)3-PPP

(1) Tyrosine Hydroxylase

The induction of ATHA resulting from the administration of GOBA and HA-966 suggested a need for further study of the dopaminergic regulation of adrenal enzymes. This is because both of these GABA analogues prevent the firing of dopaminergic neurons in the striatum (Walters et al., 1973; Hillen and Noach, 1971), thus implicating dopamine as a potentially significant contributor to the GABAergic induction process.

A dopamine autoreceptor-selective agonist should also inhibit DA release (Zetterström and Ungerstedt, 1984) hence, its administration should elicit an increase in adrenal enzyme activity in a manner similar to that of GOBA and HA-966. To investigate this possibility, (+)3-PPP (3-(3-hydroxyphenyl)-N-n-propylpiperidine), was administered to rats in a dose of 10 mg/kg, s.c. once daily for three days. As shown in Table C.21, (+)3-PPP did not elicit a significant inductive effect. The drug was also administered directly into the cerebroventricular system, in order to

circumvent the blood-brain barrier. The data from this experiment is also summarized in Table C.21. Interestingly, although the i.c.v. administration of the drug produced the same per cent increase in ATHA as did the peripheral injection (27% over saline controls in both cases), only in the case of the former was the effect statistically significant ($P<0.05$).

(ii) Ornithine Decarboxylase

Because HA-966 elicited a significant increase in amODC activity also, the rationale behind the administration of (+)3-PPP was applicable to this enzyme as well. The data from 3 similar experiments involving the injection of (+)3-PPP, 10 mg/kg s.c., were pooled and subjected to ANOVA. (Table C.22). In this case, the drug elicited a significant increase in amODC ($P<0.01$), amounting to 62% over saline controls. Despite the significant variation between the three experiments ($F=39.443$, $P<0.01$), the results were consistent as indicated by the non-significant interaction of drug on experiments ($F=2.00$, $P>0.05$). To illustrate the ready inducibility of this enzyme, a comparison of the effects of (+)3-PPP on the two adrenal enzymes is warranted. Whereas TH required the direct administration of the drug into the cerebroventricular system in order to achieve a statistically significant effect, amODC responded to a peripheral injection. Moreover, the per cent increase over controls was 62% for amODC but only 27% for TH (see Tables C.21 and C.22).

The baseline variation in these experiments with (+)3-PPP, as is the case for most amODC experiments in this thesis (for example, see Table C.9), was statistically significant. However, these variations are well within the limits of variation normally observed in this laboratory. The fact that statistical significance was attained merely illustrates the sensitivity of the statistical methods used. At the same time, it should

also be mentioned that although (+)3-PPP elicited a significant increase in amODC, (62% over controls, Table C.22), this increase is much less than the many-fold increase in enzyme activity produced by apomorphine (Almazan et al., 1983b).

(b) D-1 vs. D-2 Receptor Stimulation

(i) Ornithine Decarboxylase

The inductive effect of DA agonists on amODC (Almazan et al., 1982a). can be abolished by the prior injection of dopamine antagonists. However, the relative importance of DA- receptor subtypes in the regulation of this adrenal enzyme is unknown. To study this question, experiments were carried out with D-1 and D-2-specific antagonists with the aim of establishing whether one dopamine receptor subtype is more effective in attenuating the apomorphine-elicited increase in amODC activity than the other.

In two separate but similar experiments, the D-1-specific blocker SCH23390 was tested (Table C.23). It had no effect by itself on amODC activity in comparison to saline-treated controls ($P > 0.05$). However, in both experiments SCH23390 was able to diminish significantly the increase in enzyme activity elicited by apomorphine.

These data were subjected to ANOVA. For this particular analysis, only the effect of SCH23390 on apomorphine was considered; thus, only lines 3 and 4 from experiments A and B (Table C.23) were used. The ANOVA revealed (Table C.24) that there was no variation between experiments ($F < 1.00$) and that SCH23390 did indeed significantly attenuate the inductive effect of apomorphine on amODC ($F = 15.23$, $P < 0.01$). The mean amODC activity for the group receiving both drugs was less than 50% that of the activity of the APO-treated group (157 ± 15.81 vs. 70 ± 15.81 pmoles CO_2 per 45

minutes per mg protein; APO vs. both drugs).

To investigate the effect of D-2 specific blockade, sulpiride was used. To create optimal conditions for showing an attenuation of induction, a submaximal dose of the inducing agent (1.5 mg/kg APO) and a very high dose of the blocking agent (50 mg/kg sulpiride) were used. In two similar but separate experiments (Table C.25) sulpiride alone had no effect on amODC. However, in both experiments, the D-2 antagonist was able to lower the apomorphine-elicited increase in amODC (see Table C.25). The magnitude of the attenuating effect was somewhat different in each experiment, and only in the case of Expt. A was there a significant difference between the group receiving both drugs and the group receiving APO alone.

For a better assessment of the effect of sulpiride on apomorphine induction, the data were subjected to ANOVA; this increased the number of degrees of freedom available for critical comparisons. As before, our interest was in the possibility of a real attenuation of the effect of apomorphine, Hence, only lines 3 and 4 from experiments A. and B. were used in Table C.26. The ANOVA indicates first of all that the results of the two experiments were not dissimilar. Secondly, it is seen that sulpiride, indeed, has a significant blocking effect on the apomorphine-elicited induction of amODC ($F = 5.66$, $P < 0.05$). Thus, the mean increase elicited by apomorphine was 107 ± 20 pmoles CO_2 per 45 minutes per mg protein but this was diminished to 45 ± 20 (58% reduction) by sulpiride.

The data from the four experiments described in this section and taken as a whole suggest that as far as the attenuation of the APO-elicited increase in amODC is concerned, the D-1 and D-2 receptor-selective antagonists are equally efficacious.

ii) Tyrosine Hydroxylase

As is the case for ODC, the administration to rats of dopamine receptor agonists results in an induction of TH activity (Quik and Sourkes, 1976) and this induction can be blocked by injecting haloperidol (Quik and Sourkes, 1977). The site of action of apomorphine responsible for the induction of TH and ODC has been localized to the striatum (Ekker and Sourkes, 1985). Whether the effect of dopamine agonists on TH is mediated through the D-1 or D-2 receptor subtype has yet to be determined. Just as in the experiments with ODC, the D-1 and D-2-specific antagonists, SCH23390 and sulpiride, respectively, were administered to apomorphine-treated rats to investigate the relative importance of dopamine receptor subtypes in the regulation of ATHA.

The results of two similar experiments designed to study the effect of SCH23390 on apomorphine are summarized in Table C.27. In the one experiment, where it was tested alone, the D-1 antagonist had no effect on TH activity ($P > 0.05$). Both experiments were designed to test the increase in ATHA elicited by apomorphine in the presence and absence of SCH23390. The results show that apomorphine's effect was attenuated, but this lowering effect was statistically significant in only one of the experiments (Expt.B:$P < 0.025$ vs. APO).

Again, to provide a more detailed analysis of these results, the data from the two experiments were subjected to ANOVA. It was only of interest to determine whether SCH23390 can antagonize the apomorphine-elicited increase in ATHA. Hence, from each experiment only the group treated with apomorphine and with apomorphine plus the antagonist were considered (Table C.28). The results of ANOVA show that, in fact, the two experiments are consistent ($F = 1.24$, $P > 0.05$) in demonstrating attenuation of the induction ($F = 7.27$, $P < 0.05$): The level of ATHA resulting from

apomorphine, 55.9±4.0, being reduced to 41.2±3.74 nmoles L-DOPA per hour per pair adrenals by SCH23390 treatment.

The effect of sulpiride was also studied. This drug was injected into apomorphine-treated rats in two experiments. These results are summarized in Table C.29. In the one experiment where it was tested alone, the D-2 blocker had no effect on ATHA ($P > 0.05$). In each of the two experiments, the increase in enzyme activity elicited by apomorphine was diminished in the presence of sulpiride; however, this effect was not statistically significant. ($P > 0.05$ vs. APO for each experiment). The data from the 2 experiments were then subjected to ANOVA (see Table C.30). As was the case with SCH23390, this analysis considered only the effect of sulpiride on the apomorphine-elicited increase in ATHA. Hence, from each experiment, only the groups treated with apomorphine and with apomorphine plus the antagonist were considered. This analysis reveals that sulpiride caused a slight, but not statistically significant, diminution of the apomorphine-elicited induction (58.5±4.12 nmoles L-DOPA per hour per pair adrenals for APO-treated rats vs. 50.4±4.12 for APO + sulpiride rats). Thus, while the D-1 blocking agent SCH23390 was able to attenuate significantly enzyme induction, sulpiride was unable to do so.

(c) GABA-Dopamine Interactions in the Regulation of ATHA

The increased activity of TH by HA-966, a drug that blocks striatal dopamine release, is comparable to the induction of the enzyme by certain other substances, including DA agonists. To determine whether these inductive effects are independent of each other, the results obtained from the joint administration of HA-966 and apomorphine were compared to those obtained by each drug given alone (Table C.31). ATHA for the group receiving both drugs was not significantly different from that of the group

receiving apomorphine alone. However, treatment with apomorphine increased significantly the action of HA-966. That HA-966 could not potentiate the effect of apomorphine is presumably due to the already maximal dosage of the latter drug used for enzyme induction. Nevertheless, the quantitative similarity of the effects produced by the drugs suggests the possibility that they share a common mechanism.

To investigate further possible GABA-DA interactions in the induction of ATHA, dopamine-receptor blocking agents, which antagonize the apomorphine-elicited induction of ATHA, were used. The administration of dopamine antagonists to rats receiving GABA analogues might differentiate between a dopamine-mediated and a dopamine-independent GABAergic induction. To this end, haloperidol, a non-specific dopamine antagonist; SCH23390, a D-1 selective blocking agent; or sulpiride, a D-2-specific antagonist was administered to rats receiving either GOBA, HA-966 or muscimol. The results are summarized in Table C.32. SCH23390 and sulpiride failed to block the induction in enzyme activity produced by GOBA or HA-966 ($P > 0.05$ for all cases). Similarly, haloperidol was unable to prevent the muscimol-elicited increase in ATHA. Thus, while all three of these DA antagonists are capable of attenuating, at least to some extent, the induction of TH by DA agonists, they are ineffective in preventing an increase caused by GABAergic substances.

Table C.1: Effect of Muscimol on ATHA (Given for 3 days); Means and ANOVA

Treatment	N	ATHA*	P
Controls	23	31.6±2.05	
Muscimol	25	59.7±1.97	<0.01

ANOVA

Sources of Variation	df	Mean Squares	F	P
Total	47	-	-	-
Effect of muscimol	1	9420.45	97.40	<0.01
Experiments	4	2413.39	24.94	<0.01
Residual error	42	96.76	(1.0)	-

*ATHA is expressed as nmoles L-DOPA per hr. per pair adrenals. The results represent data pooled from 5 similar experiments. Muscimol, 3mg/kg s.c., was given b.i.d. for 3 days, 5 hrs. between injections. Controls received vehicle alone. In each experiment muscimol gave a mean increase, ranging from 53% to 104% over saline controls; average mean increase = 79% ± 19%. The probabilities are based on Fisher's F-test.

Table C.2: Effect of A One Day Injection of Muscimol on ATHA; Means and ANOVA

Treatment	N	ATHA*	P
Controls	8	25.6±1.66	
Muscimol	9	37.9±1.57	<0.01

Sources of Variation	df	Mean Square	F	P
Total	16	-	-	-
Effect of muscimol	1	654.06	29.15	<0.01
Experiments	1	469.55	21.22	<0.01
Residual error	14	22.13	(1.0)	-

*ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. The results represent data from 2 similar experiments. Muscimol, 3 mg/kg s.c. b.i.d., was given for 1 day, 5 hrs. between injections. Controls received vehicle alone.
The probabilities are based on Fisher's F-test.

Table C.3: Effect of Bicuculline on ATHA

Expt. #	Injection Schedule	ATHA*				P vs. Control
		Control	(n)	Bicuculline	(n)	
1	1/d	34.2±4.74	(4)	44.9±3.50	(4)	>0.05
2	1/d	28.0±1.00	(4)	39.5±2.90	(4)	<0.01
3	2/d	43.6±0.94	(5)	54.5±2.91	(5)	<0.01
4	2/d	31.7±2.71	(5)	40.3±7.47	(5)	>0.05
5	2/d	36.0±2.83	(5)	34.2±2.00	(5)	>0.05
6	2/d	14.6±1.17	(3)	18.0±1.26	(4)	>0.05

* ATHA is expressed as nmoles L-DOPA per hr. per pair adrenals. Bicuculline was given, 1 mg/kg s.c., either once daily (1/d) or twice daily (2/d) for 3 days. Probabilities are based on Student's t-test.

Table C.4: The Effect of Progabide on ATHA

Expt.	ATHA (nmoles L-DOPA per hour per pair adrenals)		P* vs. Control
	Controls (n)	Progabide (n)	
A	38.2±3.75 (4)	35.0±6.00 (4)	>0.05
B	31.7±2.71 (5)	34.5±1.48 (5)	>0.05
Means	34.6±2.53	34.7±2.53	

* The probabilities are based on Student's T-test. (2-tailed). Progabide was administered s.c., 100 mg/kg in the morning and 50mg/kg 5 hrs. later, for 3 days. Controls received vehicle alone. The data from the experiments were pooled and subjected to ANOVA. Effect of progabide: 1df, F = 0.00, P > 0.05; Experiments: 1df, F = .945, P > 0.05; Interactions: 1df, F = .703, P > 0.05; Mean Square for Error (14df) = 57.53.

Table C.5: The Effect of GOBA and HA-966 on ATHA

	Treatment	Route	N	ATHA	P vs. Control
I	Controls	i.p.	15	24.8±1.12	
	HA-966		16	35.1±1.08	<0.01
II	Controls	i.p.	14	33.1±1.40	
	GOBA		16	41.2±1.31	<0.01
III	Controls	i.c.v.	8	39.2±3.25	
	HA-966		6	51.5±3.76	<0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. For each drug, the results represent data pooled from replicate experiments (I and II, 4; III, 2). HA-966 was given once, 150 mg/kg i.p. GOBA, 0.5 mg/kg i.p., was administered once daily for 3 days. HA-966 was also given i.c.v., 5 µg per animal, in a volume of 10µl. Controls received vehicle alone. Probabilities are based on Fisher's F-test.

Table C.6: The Effect of GOBA and HA-966 on ATHA - ANOVA

A. Effect of HA-966, i.p.

Sources of Variation	df	Mean Square	F	P
All	30	-	-	-
8 treatment subgroups[a]	7	347.34	18.54	<<0.01
Effect of HA-966, 150 mg/kg	1	821.44	43.86	<<0.01
4 experiments	3	513.10	27.39	<<0.01
Interactions	3	23.54	1.26	>0.05
Error	23	18.73	(1.0)	-

[a] 4 experiments x 2 treatments (drug/no drug).

B. Effect of GOBA, i.p.

Sources of Variation	df	Mean Square	F	P
All	29	-	-	-
8 treatment subgroups[a]	7	547.16	20.02	<<0.01
Effect of GOBA, 0.5mg/kg	1	488.57	17.88	<<0.01
4 experiments	3	1034.99	37.88	<<0.01
Interactions	3	78.86	2.89	>0.05
Error	22	27.32	(1.0)	-

[a] 4 experiments x 2 treatments (drug/no drug)

C. Effect of HA-966, i.c.v.

Sources of Variation	df	Mean Square	F	P
All	13	-	-	-
4 treatment subgroups[a]	3	246.96	2.92	>0.05
Effect of HA-966	1	519.38	6.14	<0.05
2 experiments	1	139.14	1.64	>0.05
Interactions	1	82.36	0.973	>0.05
Error	10	84.63	(1.0)	-

[a] 2 experiments x 2 treatments (drug/no drug)

Table C.7: The Effect of Hemisplanchnicotomy on the Induction of ATHA by HA-966

Treatment	Left (denervated) gland ATHA* (n)	Right (innervated) gland ATHA* (n)
Hemi-splanchnicotomy + saline	a) 24.03±1.17 (3)	b) 21.42±2.30 (3)
Hemi-splanchnicotomy + HA-966, 150mg/kg i.p.	c) 13.52±3.84 (3)	d) 27.77±1.56 (4)

*ATHA is expressed as nmoles L-DOPA per hour per adrenal gland. The injection of HA-966 was done on the fifth post-operative day. Probabilities are based on Student's t-test (one-tailed):

a) vs. c), $P < 0.05$; a) vs. b), $P > 0.05$;

b) vs. d), $P < 0.05$; c) vs. d), $P < 0.01$;

Table C.8: The Effect of Bicuculline on the Induction of ATHA by GABA Agonists.

	Treatment	N	ATHA	P vs. Control	P vs. GABA Agonist Alone
A.	Control	5	43.6±0.946	-	-
	Bicuculline	5	54.5±2.91	<0.01	
	Muscimol	6	83.9±5.13	<0.001	-
	Bic. + Muscimol	4	101.5±6.25	<0.001	>0.05
B.	Control	5	31.7±2.71	-	-
	Bicuculline	5	40.3±7.47	>0.05	
	Progabide	5	34.5±1.48	>0.05	-
	Bic. + Progabide	5	53.1±7.38	<0.05	<0.05
C.	Control	3	14.6±1.17	-	
	Bicuculline	4	18.0±1.26	>0.05	
	GOBA	4	23.0±1.60	<0.01	-
	Bic. + GOBA	4	30.8±3.90	<0.01	>0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. Muscimol, 3 mg/kg s.c. and bicuculline, 1mg/kg s.c. were administered b.i.d. for 3 days. GOBA, 0.5 g/kg i.p., was given once daily for 4 days. Progabide was given s.c. for 3 days, 100 mg/kg in the morning and 50 mg/kg 5 hrs. later. Bicuculline was administered 30 minutes prior to the agonists. Probabilities are based on Student's t-test.

Table C.9: The Effect of Muscimol and Progabide on amODC

Expt.	Treatment	Route	N	amODC*	P
1.	Control	s.c.	6	6.3± 2.64	
	Muscimol		4	208.0±48.00	<0.001
2.	Control	s.c.	3	18.3± 3.50	
	Muscimol		5	27.9± 3.13	>0.05
3.	Control	s.c.	3	28.9± 3.10	
	Muscimol		3	77.3±21.40	>0.05
4.	Control	s.c.	5	149.4±45.29	
	Muscimol			175.2±55.77	>0.05
5.	Control	s.c.	4	30.5± 7.98	
	Progabide		3	27.7± 3.70	>0.05
6.	Control	s.c.	4	13.8± 6.20	
	Progabide		4	16.3± 2.40	>0.05

*amODC is expressed as pmoles CO_2 per 45 minutes per mg protein. Muscimol was given once, 3 mg/kg. Progabide was given once, 100 mg/kg. Animals were sacrificed 4 hrs. after injection. Probabilities are based on Student's t-test (2-tailed).

Table C.10: The Effect of HA-966 and GOBA on amODC; Means and ANOVA

Means

	Treatment	N	amODC*	P
A.	Controls	7	29.9±8.43	
	HA-966	8	76.2±7.89	<0.01
B.	Controls	7	29.9±4.69	
	GOBA	7	43.7±4.69	>0.05

ANOVA

A. HA-966	Sources of Variation	df	Mean Square	F	P
	All	14	-	-	-
	Effect of HA-966	1	7985.71	16.04	<0.01
	2 Experiments	1	7192.40	14.44	<0.01
	Error	12	497.90	(1.0)	-

B. GOBA	Sources of Variation	df	Mean Square	F	P
	All	13	-	-	-
	Effect of GOBA	1	663.65	4.31	>0.05
	2 Experiments	1	7012.98	45.55	<<0.01
	Error	11	153.94	(1.0)	-

*amODC is expressed as pmoles CO_2 per 45 minutes per mg protein. HA-966, 150 mg/kg and GOBA, 0.5 g/kg were each administered i.p. Animals were sacrificed 4 hours after injection. Probabilities are based on Fisher's F-test.

Table C.11: The Effect of Muscimol on the Oxotremorine-Elicited Induction of amODC

	Treatment	N	amODC	P vs. Control
A.	Control	3	28.9± 3.10	-
	Muscimol	3	77.3± 21.40	>0.05
	Oxotremorine	3	149.8± 22.40	<0.01
	Musc. + Oxot.	5	193.7± 59.70*	
B.	Control	5	149.4± 45.29	-
	Muscimol	4	175.2± 55.77	>0.05
	Oxotremorine	4	572.8±150.95	<0.025
	Musc. + Oxot.	5	705.4±145.50*	

*P > 0.05 vs. oxotremorine alone. amODC is expressed as pmoles CO_2 per 45 minutes per mg protein. Muscimol was given 3 mg/kg s.c., 60 minutes prior to oxotremorine. Methyl atropine, 5 mg/kg s.c., was given 30 minutes prior to oxotremorine to block the latter's peripheral effects. Oxotremorine was administered in a dose of 0.35 mg/kg s.c. Probabilities are based on Student's t-test. Animals were sacrificed 4 hrs. after oxotremorine administration.

Table C.12: The Effect of Progabide on the Oxotremorine-Elicited Induction of amODC

	Treatment	N	amODC	P
A.	Control	4	30.5± 7.98	-
	Progabide	3	27.7± 3.70	>0.05
	Oxotremorine	2	552 ± 64	<0.001
	Prog. + Oxot.	4	936 ±196*	<0.001
B.	Control	4	13.8± 6.20	-
	Progabide	4	16.3± 2.40	>0.05
	Oxotremorine	4	756 ±254	<0.05
	Prog + Oxot.	5	720 ±175*	<0.01

*P > 0.05 vs. oxotremorine alone. amODC is expressed as pmoles CO_2 per 45 minutes per mg protein. Drug administration was as follows: progabide: 100 mg/kg s.c., 60 minutes prior to oxotremorine; oxotremorine: 0.35 mg/kg s.c.; methylatropine: 5mg/kg s.c., 30 minutes prior to oxotremorine. Animals were sacrificed 4 hrs. after oxotremorine injection. Probabilities are based on Student's t-test.

Table C.13: The Effect of Bicuculline on the Oxotremorine-Elicited Induction of ATHA

Expt.	Treatment	N	ATHA	P
A.	Control	4	28.0± 1.00	-
	Bicuculline	4	39.5± 2.90	<0.01
	Oxotremorine	4	67.5±14.80	<0.05
	Bic. + Oxot.	4	63.7± 3.40*	<0.01
B.	Control	4	34.2± 4.74	-
	Bicuculline	4	44.9± 3.50	>0.05
	Oxotremorine	4	87.9± 5.28	<0.001
	Bic. + Oxot.	5	77.7± 2.52*	<0.005

*P > 0.05 vs. oxotremorine alone. ATHA is expressed as nmoles L-DOPA per hr. per pair adrenals. Bicuculline was administered, 1 mg/kg s.c., once daily for 3 days, 2 hours prior to oxotremorine. The doses of oxotremorine were as follows: In experiment A., 0.35 mg/kg s.c.; in experiment B., 1.5 mg/kg s.c. In both cases, methyl atropine, 5 mg/kg s.c., was given 30 minutes prior to oxotremorine. Animals were sacrificed on the morning following the last injection. Probabilities are based on Student's t-test.

Table C.14: The Effect of GABA Agonists on the Increase in ATHA Elicited By Oxotremorine

	Treatment	N	ATHA	P vs. control	P vs. oxotremorine
A.	Control	4	38.2±3.75	-	
	Progabide	4	35.0±6.00	>0.05	
	Oxotremorine	4	81.2±3.40	<0.001	-
	Prog. + Oxot.	4	72.8±7.80	<0.01	>.05
B.	Control	4	24.6±1.69	-	
	Muscimol	4	37.6±2.68	<0.01	
	Oxotremorine	4	58.9±4.04	<0.001	-
	Musc. + Oxot.	5	73.9±5.36	<0.001	<0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. Drugs were administered as follows: progabide: 100mg/kg in the A.M., 50 mg/kg 5 hours later; muscimol: 3 mg/kg b.i.d. 5 hours between injections, oxotremorine: 0.35 mg/kg b.i.d., 5 hrs. between injections, 1 hr. after muscimol and 2 hrs. after progabide; methylatropine 5 mg/kg b.i.d., 30 minutes prior to each oxotremorine injection. All drugs were administered s.c. Probabilities are based on Student's t-test.

Table C.15: The Effect of Atropine on the Muscimol-Elicited Induction of ATHA

Treatment	N	ATHA	P vs. control	P vs. muscimol
Control	5	37.1±4.54	-	
Atropine	5	49.9±4.59	>0.05	
Muscimol	6	69.3±6.85	<0.005	
Atropine + Muscimol	5	72.2±7.68	<0.005	>0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. Drugs were administered for 3 days as follows: atropine : 5 mg/kg i.p. b.i.d., 5 hrs. apart; muscimol : 3 mg/kg s.c. b.i.d., 5 hrs. apart, 30 minutes after atropine. Probabilities are based on Student's t-test.

Table C.16: The Effect of MDMT on ATHA; Means(a) and ANOVA(b)

(a)

Treatment	N	Route	ATHA	P*
Controls	18	s.c.	32.8±1.96	
MDMT, 1 mg/kg b.i.d.	19	s.c.	37.6±1.91	>0.05

(b)

Sources of Variation	df	Mean Square	F	P*
All	36	-	-	-
8 treatment subgroups[a]	7	100.76	1.46	>0.05
Effect of MDMT	1	213.07	3.08	>0.05
4 experiments	3	27.14	0.39	>0.05
Interactions	3	136.903	1.98	>0.05
Error	29	69.17	(1.0)	-

*Probabilities are based on Fisher's F-test. MDMT was administered for either 1,2 or 3 days. The animals were sacrificed on the morning following the last injection of MDMT. ATHA is expressed as nmoles L-DOPA per hour per pair adrenals.

[a]4 experiments x 2 treatments (controls/MDMT).

Table C.17: The Effect of MDMT on the Muscimol-Elicited Induction of ATHA; Means (a) and ANOVA (b).

(a)

Treatment	Route	N	ATHA	P*
Muscimol, 3 mg/kg b.i.d.	s.c.	14	49.0±2.54	
Musc. + MDMT, 1 mg/kg b.i.d.	s.c.	15	52.6±2.54	>0.05

(b)

Sources of Variation	df	Mean Square	F	P*
All	28	-	-	-
6 treatment subgroups[a]	5	153.5	1.70	>0.05
Effect of MDMT on muscimol	1	90.38	1.00	>0.05
3 experiments	2	122.37	1.36	>0.05
Interactions	2	216.18	2.40	>0.05
Error	23	90.07	(1.0)	-

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. Muscimol was administered b.i.d., 5 hrs. apart. MDMT was given 30 minutes prior to each muscimol injection. The drugs were administered for either one, two, or three days. Accordingly, the animals were sacrificed either 24, 48 or 72 hrs. after the first injection.

*Probabilities are based on Fisher's F-test.

[a]3 experiments x 2 treatments (muscimol/muscimol + MDMT)

Table C.18: The Effect of PCPA on ATHA; Means(a) and ANOVA(b)

(a)

Treatment	N	Route	ATHA	P*
Control	10	i.p.	21.0±2.05	
PCPA, 300 mg/kg	10	i.p.	34.7±2.05	<<0.01

(b)

Sources of Variation	df	Mean Square	F	P*
All	19	-	-	-
4 treatment subgroups[a]	3	319.16	7.610	<0.01
Effect of PCPA	1	938.38	22.370	<<0.01
2 experiments	1	13.83	0.329	>0.05
Interactions	1	5.28	0.126	>0.05
Error	16	41.94	(1.0)	-

* Probabilities are based on Fisher's F-test. ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. PCPA was administered once and the animals were sacrificed 24 hours later.

[a] 2 experiments x 2 treatments (control/PCPA).

Table C.19: The Effect of PCPA on Induction of ATHA by Muscimol or Bicuculline

	Treatment	N	ATHA	P vs. con	P vs. PCPA
A.	Control	5	20.7± 1.88	-	
	PCPA, 300 mg/kg i.p.	5	33.4± 1.78	<0.005	-
	Muscimol, 3 mg/kg, b.i.d.	5	34.8± 1.20	<0.001	
	PCPA + Muscimol	6	45.6± 3.52	<0.001	<0.025
B.	Control	5	21.3± 4.32	-	
	PCPA, 300 mg/kg i.p.	5	36.0±10.75	<0.025	-
	Bicuculline, 1mg/kg s.c. b.i.d.	5	27.4± 3.17	<0.05	
	PCPA + Bicuculline	6	43.0± 8.94	<0.001	>0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. Probabilities are based on Student's t-test. Duration of treatment was for 1 day. PCPA was administered once, 30 minutes prior to the GABAergic agent. Muscimol and bicuculline were given b.i.d., 5 hours between injections.

Table C.20: The Effect of AMTP on the Induction of ATHA by HA-966

Treatment	N	ATHA	P vs. control	P vs. HA-966
Control	4	31.6±1.32	-	<0.0125
HA-966, 150 mg/kg i.p.	4	47.1±4.12	<0.0125	-
AMTP, 100 mg/kg s.c.	4	36.4±3.18	>0.05	<0.05
HA-966 + AMTP	4	43.0±2.04	≤0.005	>0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals.
Probabilities are based on Student's t-test.

Table C.21: The Effect of (+)3-PPP on ATHA

Expt.	Treatment	N	Route	ATHA	P
A.	Saline	5	s.c.	20.7±1.28	-
	(+)3-PPP, 10 mg/kg	5	s.c.	26.4±4.98	>0.05
B.	Saline	7	i.c.v.	25.7±1.98	-
	(+)3-PPP, 2µg	9	i.c.v.	32.7±1.74	<0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. In experiment A., (+)3-PPP was given once daily for 3 days. The probabilities are based on Student's t-test.

In experiment B., the drug was administered i.c.v., 2µg/10µl, and the animals were sacrificed after 24 hours. The data for the latter treatment represents a pooling of 2 experiments and were subjected to ANOVA. Variations between replicate experiments could thus be accounted for. The probabilities are based on Fisher's F-test. Effect of (+)3-PPP: 1df, $F = 7.00$, $P < 0.05$; Experiments: 1df, $F = 20.07$, $P < 0.01$; Mean Square for Error (13 df) = 27.4.

Table C.22: The Effect of (+)3-PPP on amODC; Means(a) and ANOVA(b)

(a)

Treatment	N	Route	amODC	P
Control	11	s.c.	31.9±3.27	
(+)3-PPP, 10 mg/kg	11	s.c.	51.9±3.27	<0.01

(b)

Sources of Variation	df	Mean Square	F	P*
All	21	-	-	-
6 treatment subgroups[a]	5	2391.6	20.300	<0.01
Effect of (+)3-PPP	1	2192.21	18.608	<0.01
3 experiments	2	4646.83	39.443	<0.01
Interactions	2	236.07	2.00	>0.05
Error	16	117.81	(1.0)	-

amODC is expressed as pmoles CO_2 per 45 minutes per mg protein. (+)3-PPP was administered once and the animals were sacrificed 4 hrs. thereafter. Probabilities are based on Fisher's F-test.

[a] 3 experiments x 2 treatments (drug/no drug).

Table C.23: The Effect of SCH23390 on the Apomorphine-Elicited Increase in amODC

Expt.	Treatment	N	amODC	P vs. control	P vs. APO
A.	Control	4	25.1 ± 4.02	-	
	SCH23390, 3 mg/kg i.p.	4	24.0 ± 3.97	>0.05	
	APO, 3 mg/kg s.c. t.i.d.	4	154.0 ±34.71	<0.01	-
	APO + SCH23390	4	70.8 ± 9.44	<0.005	<0.05
B.	Control	4	20.3± 0.94	-	
	SCH23390, 3 mg/kg i.p.	4	20.6 ± 2.66	>0.05	
	APO, 3 mg/kg s.c., t.i.d.	4	159.5 ±24.68	<0.005	
	APO + SCH23390	4	68.5 ± 9.92	<0.005	<0.01

amODC is expressed as pmoles CO_2 per 45 minutes per mg protein. In both experiments, SCH23390 was administered 30 minutes prior to the first injection of APO. APO was injected 3 times, at t = 0, t = 1.5 and t = 3 hours. Animals were sacrificed 4 hrs. after first injection of APO. Probabilities are based on Student's t-test (one-tailed).

Table C.24: The Effect of SCH23390 on the APO-Elicited Increase in amODC:ANOVA

Sources of Variation	df	Mean Square	F	P
All	15	-	-	-
4 treatment subgroups[a]	3	10184.75	5.090	<0.05
2 experiments	1	6.79	<1.000	>0.05
Effect of SCH23390 on APO	1	30493.89	15.230	<0.01
Interactions	1	53.58	0.026	>0.05
Error	12	2001.7	(1.0)	-

Treatment	n	amODC*
APO	8	157.0±15.81
APO+SCH23390	8	70.0±15.81

[a] 2 experiments x 2 treatments (APO/APO+SCH23390)

*amODC is expressed as pmoles CO_2 per 45 minutes per mg protein.

Table C.25: The Effect of Sulpiride on amODC Induction By APO

Expt.	Treatment	N	amODC	P vs. control	P vs. APO
A.	Control	4	33.1 ± 3.92	-	
	Sulpiride, 50 mg/kg s.c.	3	28.8 ± 0.88	>0.05	
	APO, 1.5 mg/kg s.c., t.i.d.	4	101.2 ±24.98	<0.025	-
	APO + Sulpiride	3	30.6 ± 0.48	>0.05	<0.05
B.	Control	4	34.1 ± 2.30	-	
	Sulpiride 50 mg/kg s.c.	3	36.1 ± 3.88	>0.05	
	APO, 1.5 mg/kg s.c., t.i.d.	5	110.8 ±32.21	<0.05	
	APO + Sulpiride	3	59.9 ± 9.50	<0.05	>0.05

amODC is expressed as pmoles CO_2 per 45 minutes per mg protein. Sulpiride was injected 30 minutes prior to the first injection of APO. APO was administered 3 times, at t = 0, t = 1.5 and t = 3 hrs. Animals were sacrificed 4 hrs. after first injection of APO. Probabilities are based on Student's t-test (one-tailed).

Table C. 26: The Effect of Sulpiride on the APO-Elicited Increase in amODC: ANOVA

Source of Variation	df	Mean Square	F	P
All	14	-	-	-
Effect of Sulpiride	1	13511.02	5.66	<0.05
2 experiments	1	1602.24	0.67	>0.05
Error	12	2389.02	(1.0)	-

Treatment	n	amODC*
APO	9	107±20
APO + Sulpiride	6	45±20

*amODC is expressed as pmoles CO_2 per 45 minutes per mg protein.

Table C.27: The Effect of SCH23390 on the APO-Elicited Increase in ATHA

Expt.	Treatment	N	ATHA	P vs. control	P vs. APO
A.	Control	4	28.7±1.98	-	
	SCH23390, 3 mg/kg i.p., b.i.d.	4	30.7±4.14	>0.05	
	APO, 3 mg/kg s.c., q.i.d.	4	58.8±9.00	<0.025	-
	APO + SCH23390	5	44.6±3.95	<0.025	>0.05
B.	Control	3	25.6±1.73	-	
	APO, 3mg/kg s.c., q.i.d.	4	52.9±4.30	<0.005	-
	APO + SCH23390	4	36.8±3.36	<0.025	<0.025

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. Both experiments were carried out for 3 days. APO was administered q.i.d., every 2 hours. SCH23390 was given b.i.d., 30 minutes prior to the 1st and 3rd injection of APO. Animals were sacrificed on the morning following the last injection. Probabilities are based on Student's t-test (1-tailed).

Table C.28: The Effect of SCH23390 on the APO-Elicited Increase in ATHA:ANOVA

Sources of Variation	df	Mean Square	F	P
All	16	-	-	-
4 treatment subgroups[a]	3	374.14	2.97	>0.05
Effect of SCH23390 on APO	1	916.77	7.27	<0.05
2 experiments	1	156.66	1.24	>0.05
Interactions	1	49.00	.388	>0.05
Error	13	126.15	(1.0)	-

Treatment	n	ATHA*
APO	8	55.9±4.00
APO + SCH23390	9	41.2±3.74

[a] 2 experiments x 2 treatments (APO/APO + SCH23390)

*ATHA is expressed as nmoles L-DOPA per hr. per pair adrenals.

Table C.29: The Effect of Sulpiride on the APO-Elicited Increase in ATHA

Expt.	Treatment	N	ATHA	P vs. control	P vs. APO
A.	Control	4	31.1±3.63	-	
	Sulpiride, 40 mg/kg s.c.	4	36.2±2.61	>0.05	
	APO, 3 mg/kg s.c.	4	64.2±7.12	<0.005	-
	APO + Sulpiride	4	57.4±6.22	<0.01	>0.05
B.	Control	4	25.6±1.73	-	
	APO, 3mg/kg s.c.	4	52.9±4.30	<0.005	-
	APO + Sulpiride 40 mg/kg s.c.	4	43.4±5.28	<0.025	>0.05

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. APO was administered q.i.d. for 3 days, 2 hrs. between injections. Sulpiride was given b.i.d., 30 minutes prior to the first and third injection of APO. Animals were sacrificed on the morning following the last injection. Probabilities are based on Student's t-test (one-tailed).

Table C.30: The Effect of Sulpiride on the APO-Elicited Increase in ATHA:ANOVA

Sources of Variation	df	Mean Square	F	P
All	15	-	-	-
4 treatment subgroups[a]	3	303.77	2.24	>0.05
Effect of Sulpiride on APO	1	265.69	1.96	>0.05
2 experiments	1	638.32	4.70	>0.05
Interactions	1	7.29	0.05	>0.05
Error	12	135.68	(1.0)	-

Means:	Treatment	n	ATHA*
	APO	8	58.5±4.12
	APO + Sulpiride	8	50.4±4.12

[a] 2 experiments x 2 treatments (APO/APO + Sulpiride)

* ATHA is expressed as nmoles L-DOPA per hour per pair adrenals.

Table C. 31: The Effect of the Joint Administration of APO and HA-966 on ATHA

Treatment	N	ATHA	P vs. Control
Control	4	26.5±2.28	-
HA-966	4	34.8±2.09	<0.025
APO	4	43.5±5.18	<0.01
APO + HA-966	5	42.6±2.85	<0.005

ATHA is expressed as nmoles L-DOPA per hour per pair adrenals. HA-966, 150 mg/kg i.p., was given 30 minutes prior to APO. APO, 3 mg/kg s.c., was given q.i.d. with 2 hours between injections. The injections were given for 1 day and the animals were sacrificed 24 hours after the first injection. The probabilities are based on Student's t-test (1-tailed). Treatment with APO and HA-966 was significantly greater than with HA-966 alone ($P<0.05$). However, it was not significantly different from treatment with APO alone ($P>0.05$).

Table C.32: Interaction of DA Blocking Agents on Rats Treated with GABAergic Drugs

	Treatment	N	ATHA (nmoles L-DOPA per hour per pair adrenals)
I.	dl-Sulpiride, s.c.		
	GOBA	8	46.1±3.16
	GOBA + Sulpiride	8	45.3±3.16
	HA-966+ A	7	34.2±1.86
	HA-966 + Sulpiride	8	32.4±1.74
II.	SCH23390, i.p.		
	HA-966	7	26.8±2.40
	HA-966 + SCH23390	8	28.5±2.24
III.	Haloperidol, i.p.		
	Muscimol	5	53.5±6.92
	Muscimol + Haloperidol	6	68.4±5.49

The results with HA-966 represent data pooled from 2 similar experiments. The results with GOBA and muscimol are each from one experiment. Drugs were administered as follows: GOBA: 0.5 g/kg i.p. once daily for 3 days; HA-966: 150 mg/kg i.p. for 1 day; muscimol: 3 mg/kg s.c. b.i.d., 5 hrs. apart, for 3 days; dl-sulpiride: 40 mg/kg s.c. once daily, 30 minutes prior to either GOBA or HA-966; SCH23390: 3 mg/kg i.p. b.i.d. (3 hrs. apart), 30 minutes prior to HA-966; haloperidol: 5 mg/kg i.p. once daily, 60 minutes prior to muscimol. Animals were sacrificed on the morning following the last injection.

The data were subjected to ANOVA. Variation between replicate experiments. could thus be accounted for ($P>0.005$ for all cases). Probabilities are based on Fisher's F-test.

Effect of dl-sulpiride on GOBA-treated rats: 1df, $F=0.03$, $P>>0.05$, Mean Square for Error (12df)=80.09.
Effect of dl-sulpiride on HA-966-treated rats: 1df, $F=.488$, $P>0.05$, Mean Square for Error (11df)=24.11.
Effect of SCH23390 on HA-966-treated rats: 1df, $F=.256$, $P>0.05$, Mean Square for Error (11df)=40.29.
Effect of haloperidol on muscimol: 1df, $F=2.95$, $P>0.05$, Mean Square for Error (9df)=206.96.

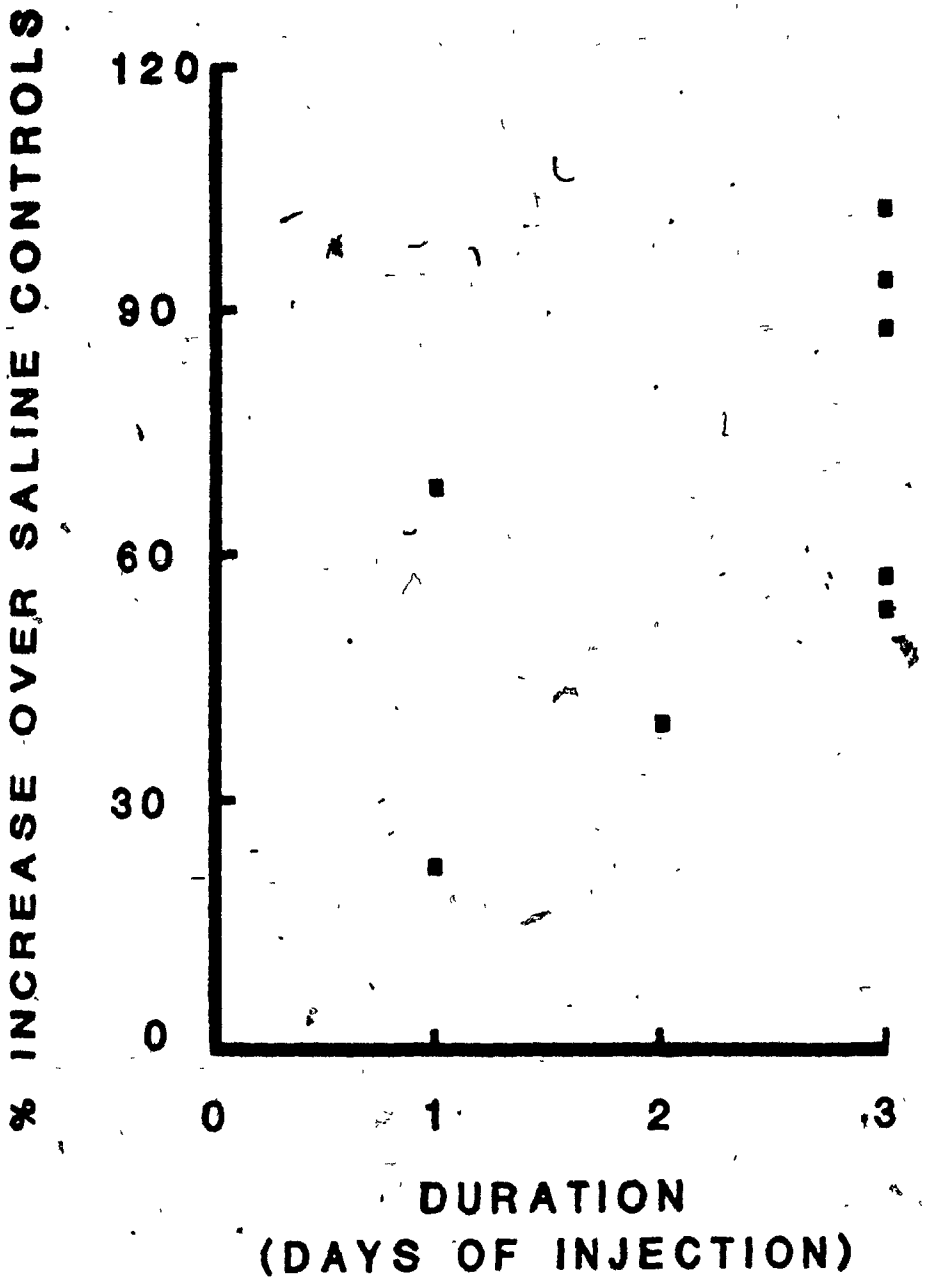


Figure IV: Effect of Repeated Administration of Muscimol on the Per Cent Increase in ATHA over Saline Controls. Muscimol was given in a dose of 3 mg/kg b.i.d., s.c. for either 1,2, or 3 days.

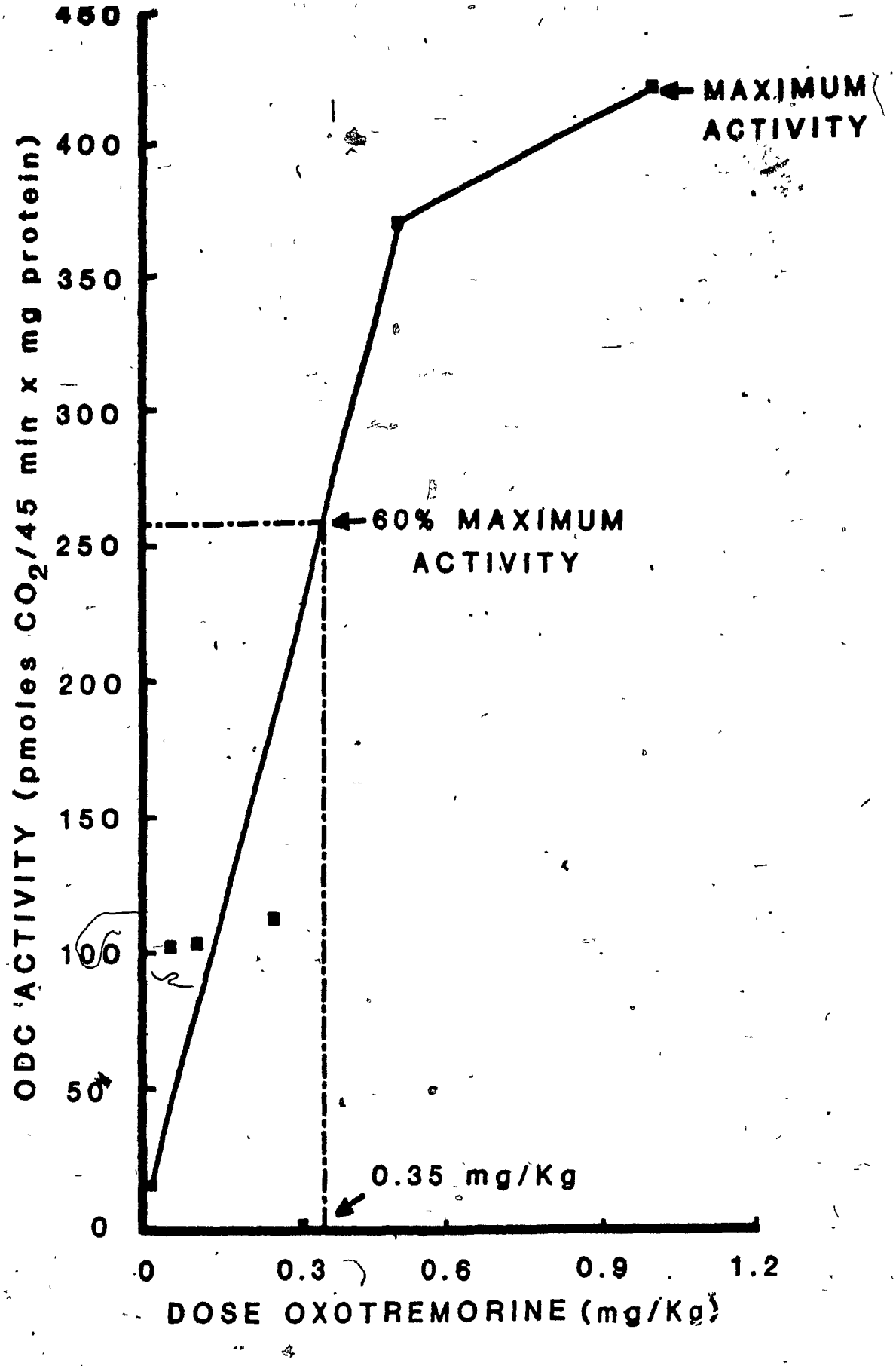


Figure V: Dose Response Curve: ODC Activity vs. Oxotremorine

Rats were treated s.c. with 6.65 mg/kg methyl atropine, 30 minutes prior to injection of variable doses of oxotremorine (s.c.) and were sacrificed 4 1/2 hours after the initial injection.

D. DISCUSSION

1. GABAergic Regulation of Adrenal Enzymes:

The experiments performed in this thesis, involving the use of neurotransmitter analogues, suggest that GABA plays a role in the induction of the adrenal enzymes amODC and TH. However, there is no absolute consistency in the actions of the five GABAergic substances tested in this regard, for they are not equally efficacious in bringing about the increases in enzyme activity. Moreover, a given GABAergic agent may effect a change in ATHA, but may not do so for amODC activity. These inconsistencies may be attributed to the different sites of action or metabolic pathways taken by these drugs.

(a) Tyrosine Hydroxylase

Of the analogues tested muscimol, GOBA and HA-966 consistently effected increases in ATHA. Muscimol almost doubled the control values when given twice daily for 3 days in a dose of 3 mg/kg (Table C.1). A lower dose (1 mg/kg), even when administered for three days, was not effective. However, injections of the higher dose (3 mg/kg) for one or two days were able to produce significant increases in enzyme activity (Table C.2 and Figure IV), although these were not as high as the increases elicited after a three-day injection period.

The conditions required to produce an effect with muscimol correspond well with reports in the literature about its metabolism. Naik et al. (1976) maintain that muscimol penetrates the blood brain barrier and has a potent and long-lasting GABA-like action, but other authors have since demonstrated that only a small aliquot of the injected dose enters the brain (Baraldi et al., 1979). Although the amount of authentic muscimol taken up by the brain is sufficient to explain some of the pharmacological actions obtained by intravenous injection of the drug (Gale et al., 1978; Baraldi et al., 1979), this may not be able to elicit the induction of adrenal enzymes. Thus, in order for an effective amount of muscimol to

enter the brain a dose of at least 3 mg/kg must be administered peripherally. Another facet to consider with the systemic administration of muscimol is the metabolism of the drug in vivo. Muscimol is metabolized rapidly (Baraldi et al., 1979) after intravenous injection (Maggi and Enna, 1979). Thus, the biochemical effects produced by this GABAergic substance may be due not to an action of the drug alone but rather to a combination of muscimol and some derivative, or the derivative alone. This may be the case for the induction of TH. The significant increase in TH activity might result after only a 24 hour period (Figure IV) if, in fact, the effect is due to the metabolite and not the drug itself. Interestingly, in experiments not reported in this thesis, it was found that muscimol applied directly into the cerebroventricular system is not able to cause an increase in TH activity. The reason for this lack of effect may be that muscimol must indeed be metabolized in the periphery, perhaps in the liver, in order for it to be biochemically active. That muscimol has an effect when given s.c. but not i.c.v. is not without precedent. For example, this drug has differential effects on central dopamine metabolism, depending upon the route of administration: Whereas i.v. muscimol produces decreases in nigrostriatal and mesolimbic levels of dopamine, the i.p. administration of the compound increases the concentration of DA in these brain regions (Gundlach and Beart, 1981). Obviously, muscimol has very complex pharmacological and metabolic characteristics, and further work is needed to determine how it exerts its biochemical effects. Nevertheless, it is clear that muscimol in its original form or a metabolite exerts a significant inductive effect on adrenal TH activity.

GOBA and HA-966 were also effective in inducing significant increases in ATHA; the former drug yielded a 25% rise over saline controls while the latter produced a 42% increase. GOBA produced a significant elevation in

adrenal enzyme activity only after three days of injection whereas HA-966 elicited enzyme induction within 24 hours and with less than one-third the dose used for GOBA (Table C.5). The different conditions required for, and the different magnitudes of enzyme induction evoked by, these compounds are probably a function of their differential metabolism. It has been suggested that a metabolic conversion of HA-966, possibly in the liver, is necessary for many of its effects (Bonta et al., 1971), but it is unlikely that this molecule is extensively rearranged (Mos et al., 1981). If HA-966 is, in fact, active in its native form, then it is reasonable to expect this GABA analogue to effect a change in ATHA in as little as 24 hours after injection and with a relatively small dose (150 mg/kg). Contrarily, Roth and Giarman (1966) reported that GOBA's half-life in vivo is about one hour. Thus, in order to supply a biochemically effective amount of GOBA, the drug must be administered repeatedly (i.e. for 3 days). Another approach would be to inject the drug once, but in a very high dose. In fact, in an experiment not reported in this thesis, a single injection of GOBA, in a dose of 2.2 g/kg was able to elicit a significant increase in ATHA. However, this avenue was discontinued because the dose used was found to be lethal to some of the rats receiving it.

Unlike the other GABA agonists tested, progabide was unable to effect an increase in adrenal TH. This finding was unexpected. Because progabide is reported to be effective in treating neurological problems such as epilepsy and movement disorders in clinical studies (Bergmann, 1985) (that is, has an action expected of a GABAergic substance), it was anticipated that the drug would be of use in this particular study as well. Despite its ready penetrability of the blood brain barrier (Worms et al., 1982), progabide is less potent than any of its associated metabolites (i.e. GABA, GABAmide, or SL75.102) in binding to GABA receptors (Bergmann, 1985). This

may explain its ineffectiveness. Unfortunately, even though progabide's metabolites are more potent in binding to GABA sites, their relative polarity renders them incapable of penetrating the brain and they would therefore be poor choices for experiments involving peripheral administration of the drugs. For the very same reasons, these metabolites (i.e. GABAmide ~~or SL75.102~~) would presumably be ~~ideal~~ candidates for the i.c.v. experiments discussed below.

Once it had been established that GABA analogues can cause an increase in ATHA, the next question to consider was whether or not this effect is mediated centrally. As was mentioned in the introduction (Sections 4.(c),(d), and (e)), the neural regulation of both ODC and TH is thought to be controlled by a supraspinal centre in the A9 region, which includes the striatum. Because neurotransmitters such as DA, 5HT and ACh act centrally to effect changes in adrenal enzyme activity, it was reasonable to assume that GABA also acts in this manner. Histochemical and biochemical studies have demonstrated the existence of GABA receptors in bovine adrenal chromaffin cells (Kataoka et al., 1984), so that the possibility of a local GABAergic mechanism controlling adrenal enzyme activity had to be considered. By administering a GABA analogue directly into the cerebroventricular system, it was felt that one could specifically elicit a central effect without stimulating adrenal GABA receptors. The most practical approach to this problem was thought to be a bolus i.c.v. injection of a GABA analogue that would be most effective after only one administration. Based on the data from experiments involving peripheral injections, HA-966 was the most judicious choice. (Other experimental avenues could be the continuous infusion of drug as with an osmotic mini-pump or repeated application through a cannula implanted in the skull. Future work in studying this question could conceivably utilize these

methodologies.) Indeed, the application of 5 µg of this GABA analogue directly into the brain was able to produce a significant increase in enzyme activity within 24 hours (approx. 30%, Table C.5). Although it is possible for a substance given i.c.v. to diffuse from the brain into the periphery, the fact that such a minute amount of drug was administered (5 µg i.c.v. vs. 150 mg/kg i.p.) strongly suggests that the resulting inductive effect is due to an action at the site of injection. Moreover, the fact that HA-966 evoked an increase in ATHA when given i.c.v. suggests that: (a) this compound does not require any modification in the liver to act at GABA sites in the brain or (b) the enzymes that are required to convert HA-966 into an active form are found in the brain. This is in sharp contrast with the i.c.v. experiments with muscimol, where the lack of enzyme induction implied that muscimol must be metabolized in the periphery before it can exert biochemical effects.

The question of central mechanisms of TH regulation can also be studied from a different perspective. Instead of injecting an active compound directly into the brain cavities and then looking for a positive response, one can systemically administer a substance that does not penetrate the blood-brain barrier and look for the lack of induction by something that is confined to the periphery. Accordingly, the i.p. injection of GABA itself, which as a very hydrophilic agent, penetrates the blood-brain barrier only to a negligible extent, would be expected to have no effect on ATHA. In fact, in two experiments not reported in this thesis, the administration of GABA, 300 mg/kg i.p. once daily for 3 days, was unable to induce adrenal TH. This negative finding corroborates the results with HA-966 given i.c.v., thus suggesting that GABAergic agents regulate ATHA centrally. For the sake of completeness, it should be mentioned that GABA was also administered i.c.v.; however, in combination

with the anesthetic used (chloral hydrate, 300 mg/kg i.p.), it proved to be lethal in all cases.

Another approach was used to study the possibility that HA-966 acts centrally. The drug was administered (i.p.) to rats previously hemisplanchnicotomized (Table C.7). While the drug significantly elevated the enzyme activity in the intact gland as compared with controls, HA-966 had no effect on ATHA on the denervated side. Although this approach was attempted in only one experiment, the finding agrees well with the i.c.v. experiments. Dependence of the inductive effect on the intact innervation of the adrenal gland suggests that the GABAergic regulation of ATHA is primarily central and not local. This, however, does not mean to imply that the regulatory process is exclusively under neuronal control. Local effects are possible, especially because of the existence of GABA receptors on chromaffin cells (Kataoka et al., 1986). In fact, in the denervated gland (Table C.7), HA-966 lowered the ATHA. At this point, it is important to distinguish between two types of enzyme regulation: (a) the short term increase in enzyme activity known as "enzyme activation" and (b) the long term changes in enzyme activity due to de novo enzyme protein synthesis referred to as "induction". Insofar as the GABAergic regulation of adrenal enzymes is concerned, one can speculate that the process of induction is directed centrally whereas enzyme activation is probably controlled by local GABAergic mechanisms. The latter phenomenon may be indirectly related to the increased release of adrenal CA caused by GABA-mimetic drugs (Kataoka et al., 1986). As a result of the increased liberation of its end product, in this case due to a local GABAergic effect, TH is said to be disinhibited (Spector et al., 1967) and hence, activated. Actually HA-966 caused a lowering and not an elevation of ATHA in the denervated adrenals (Table C.7). This inconsistency could be explained by the fact that enzyme

activation occurs within minutes whereas the ATHA measured in this experiment corresponded to a 24-hour period. The notion that there are two components to consider in adrenal enzyme regulation is not a novel one. While at first it was thought that local adrenal CA concentrations regulate ATHA (Dairman and Udenfriend, 1971), work in this laboratory with splanchnicotomized rats (Quik and Sourkes, 1976) subsequently showed that the enzyme is predominantly (but not exclusively) controlled by a neuronal mechanism.

The only GABA antagonist used in this project was bicuculline. Although the data show that bicuculline may evoke significant increases in ATHA, this effect is inconsistent. The discrepancies of the various experiments may simply be a reflection of bicuculline's instability in solution (Andrews and Johnston, 1979). Despite this problem, it was felt that bicuculline might be able to antagonize the inductive effects elicited by GABA agonists. Three such experiments were performed and they produced surprising results (Table C.8). In none of the experiments did the antagonist abolish the agonist-elicited increase in adrenal enzyme activity. Moreover, the co-administration of bicuculline with progabide enabled the latter drug to produce a statistically significant elevation of ATHA, an effect which it was unable to produce alone. Evidently, the GABAergic regulation of adrenal enzymes is complex; unlike the DA system (Quik and Sourkes, 1977), where haloperidol can abolish the increase in ATHA produced by apomorphine, the abolition of the agonist-elicited induction by an antagonist does not seem to be a property of the GABA system. Moreover, not only does bucuculline fail to antagonize the inductive effect, it paradoxically facilitates the agonist-elicited increase in enzyme activity. How might this inconsistency by explained?

The simplest solution to this dilemma may be that the unusual results

seen in all the experiments with bicuculline are due to the instability of the antagonist in solution. Thus, in the three experimental groups involving co-administration of agonist and antagonist, the observed mean enzyme activities are really indicative of the effect of the agonist alone. Another explanation is based on the notion that the A9 region contains numerous GABAergic neurons, many of which are linked in series (Scheel-Krüger, 1986). If a pair of GABAergic neurons are combined in a double inhibitory link, the net output efferent to these neurons will be an excitatory one. This phenomenon is well established in the striato-nigrocollicular and striato-nigrothalamic GABAergic pathways (Chevalier and Deniau, 1987). If a third inhibitory GABA fibre emanating from a different brain region, is added afferent to this scheme, and if this fibre's receptor targets are especially sensitive to bicuculline, then the GABA agonist-mediated inductive effect will be facilitated. This explanation assumes that GABA receptors, in different regions of the brain, exhibit differential sensitivities to various GABAergic agents. Indeed, this concept has some experimental basis. For example, Wood (1982) showed that muscimol acts in the substantia nigra, but not in the striatum, to depress striatal DA release. Furthermore, electrophysiological studies have demonstrated that non-DAergic cells of the pars reticulata in the substantia nigra are much more sensitive to GABA than the dopamine cells in the pars compacta (Grace and Bunney, 1979). Also, direct application of GABA or GABA agonists such as muscimol in the pars reticulata results in both inhibition of pars reticulata neurons and activation of DA neurons in pars compacta (Grace and Bunney, 1979; Scheel-Krüger, 1986). Accordingly, it is conceivable that bicuculline or the various GABA agonists act differently in different brain regions, and have dissimilar affinities for different populations of GABA receptors. The complexity of the GABA system

which is, after all, a very significant contributor to the neurotransmitter activity in the CNS (Fonnum and Storm-Mathisen, 1978), is further illustrated by the fact that two GABA agonists exert their effects by different mechanisms: HA-966 selectively elevates the DA content in the striatum by depressing its release from DA neurons (Bonta et al., 1971); muscimol's action is not necessarily associated with dopamine accumulation and depression of striatal DA liberation (Scheel-Krüger, 1986). In some circumstances, muscimol is thought to bind to non-DA neurons which exist in the striatum, but when applied intranigrally it too depresses striatal DA release (Wood, 1982).

(b) Ornithine Decarboxylase

One of the aims of this work was to compare and contrast the GABAergic regulation of ODC and TH. Do ODC and TH respond in the same way to GABA analogues? Previous work with other neurotransmitters has suggested that these two adrenal enzymes are similarly regulated (Introduction, 4(f)). Of the drugs tested, only HA-966 effected a statistically significant increase in amODC. Although muscimol occasioned an increase in amODC in one experiment, it had no consistent effect on this adrenal enzyme. Similarly, GOBA, which caused significant increases in ATHA, was unable to change the levels of amODC. Thus, the latter two drugs have differential effects on the induction of TH and amODC. This should not, however, suggest that the GABAergic regulation of these two enzymes is entirely different. The impotence of some GABA analogues tested is probably attributable to the incompatibility between the time-scale for ODC induction and the time period required for the metabolic conversion of the drugs into active inducing agents. As was mentioned above, muscimol and GOBA seem to require alteration of their original form; their penetration of the blood-brain barrier may also be time-consuming. Contrarily, HA-966 does not appear to

be extensively rearranged and can be effective relatively soon. Because the protocol for the ODC assay used in this laboratory required sacrificing the animals four hours after the initial drug administration, it is conceivable that this interval does not allow for the metabolic conversion of muscimol and GOBA.

The central regulation of ODC by GABA was not investigated. The i.c.v. approach was not feasible as the procedure itself is very stressful and the enzyme levels would not be at resting values as the drug is administered. Thus, it would be difficult to distinguish between elevated enzyme levels resulting from stressing the animals and the increase in amODC due to a drug-elicited induction. Nevertheless, because the data with TH suggest that the GABAergic regulation is centrally mediated, it is not unlikely that ODC is also regulated in this way, especially because of the parallels in the regulation of these two enzymes by other neurotransmitter systems (Introduction, 4(f)).

2. Interactions of GABAergic Agents With Cholinergic, Serotonergic and Dopaminergic Systems

The ultimate goal of the work in this laboratory is to elucidate the nervous pathways mediating the organism's response to stressful situations. Hitherto, the central regulation of adrenal enzymes has implicated the involvement of DA, 5HT and ACh neurotransmitter systems (Sourkes, 1983; Introduction, 4 (c), (d), (e)). Once it was established that certain GABA analogues are able to induce adrenal enzyme activity, it was of interest to determine whether this transmitter substance exerts its influence on adrenal enzyme function independently or by way of an interaction with all or some of the neurotransmitters investigated previously. If the GABA-elicited action is independent of other brain chemicals then one more

pathway has been uncovered which the brain has at its disposal to orchestrate the animal's stress response, illustrating the versatility of the nervous system in its ability to communicate messages to the periphery. On the other hand, if this GABAergic effect is connected to that of the other neurotransmitter substances tested previously, then the role of GABA in adrenal enzyme regulation might be strictly a modulatory one. The notion that GABA interacts with other neurotransmitters in the CNS is not without precedent; this neurotransmitter has been demonstrated to interact with cerebral serotonin, acetylcholine and dopamine in certain systems (Introduction, 5(a), (b) and (c)). Moreover, the concept of two brain chemicals interacting to bring about a change in adrenal enzyme activity has already been addressed in this laboratory. Quik and Sourkes (1977) had studied the joint regulation of ATHA by serotonergic and dopaminergic systems. Their work suggested that an intact DA system is required in order for the serotonergic effect to occur. Their evidence also intimates that the interaction between the two systems is sequential, with the serotonin system preceding the dopamine one. The role of reserpine, which leads to the depletion of adrenal catecholamines, in the serotonergic regulation of DBH was also studied (Lima and Sourkes, 1986a) and the results suggest that a simultaneous depletion of central serotonin and catecholamines is necessary for the induction of this enzyme. It was also shown (Lima et al., 1986) that a lesion of the MRN potentiates the inducing action of the CA-depleting drug. Finally, the investigation of the induction of rat adrenal DBH by oxotremorine was extended to include the effects of GABA-mimetic drugs on this system (Lima and Sourkes, 1986b). In this thesis, GABA analogues were administered conjointly with cholinergic drugs in order to study their combined effects on TH and amODC. In the case of GABA-5HT interactions, only the effects on TH were investigated.

(a) GABA-ACh Interactions in the Regulation of amODC and TH

The degree of interaction between two types of neurotransmitter systems with respect to enzyme regulation can be inferred by comparing the algebraic sum of the enzyme activities resulting from the administration of two neurotransmitter analogues, given separately, with the enzyme activity produced upon simultaneous injection of the drugs. To study the possibility of a GABA-ACh interaction in amODC regulation, muscimol or progabide was combined with oxotremorine in order to determine whether these GABA analogues would amplify the induction elicited by the muscarinic agonist. In two experiments there was no evidence of interaction involving muscimol and oxotremorine (Table C.11). In one of these (Table C.11.A.), muscimol and oxotremorine appeared to act quite independently. Two similar experiments, this time with progabide as GABA agonist (Table C.12), also yielded inconclusive results. Because these GABAergic agents do not reliably affect the oxotremorine-induced increase in amODC, it can be concluded that GABA and central cholinergic systems do not interact in the regulation of this adrenal enzyme. It should be noted that muscimol and progabide were unable to induce amODC activity consistently when given alone (Section D.1).

The possibility of a neurotransmitter interaction was also investigated with adrenal TH. A variety of approaches was considered for this enzyme. First, it was shown that the co-administration of the GABA antagonist bicuculline did not significantly attenuate the oxotremorine-elicited increase in ATHA (Table C.13). Secondly, the increase caused by oxotremorine was not at all affected by the presence of progabide, which alone had no effect whatsoever on the enzyme (Table C.14A). Thirdly, muscimol given to oxotremorine-treated rats had an additive effect, as was the case in one of the amODC experiments. Again, this finding suggests

that the two drugs are causing increases in ATHA by independent mechanisms. In a final experiment in this particular study, the cholinergic muscarinic antagonist atropine did not block the inductive action of muscimol on ATHA. Clearly, this group of experiments, which utilized different combinations of cholinergic and GABAergic compounds, was unable to demonstrate any kind of interaction between these two neurotransmitters insofar as the regulation of amODC and adrenal TH is concerned; and this despite the extensive interactions between GABA and ACh in striatum (Introduction, 5 (b)). This is in sharp contrast to the findings of Lima and Sourkes (1986b), who demonstrated that GABA agonists can impair the inductive effect of oxotremorine on adrenal DBH activity. The fact that a GABAergic inhibitory mechanism is involved with a central cholinergic stimulatory pathway for DBH but not with the cholinergic induction of amODC and TH illustrates the enzyme specificity of the central neuronal influences which regulate the biosynthetic activity of the adrenal gland.

(b) GABA-Serotonin Interactions in the Regulation of ATHA

Serotonin has been implicated in the regulation of ATHA. As was described earlier (Introduction, 4(d)), this neurotransmitter seems to supply a tonic inhibitory input to the adrenal gland. Lesion experiments have demonstrated that this serotonergic control emanates from the MRN (Quik et al., 1977). Because administration into the MRN of GABA agonists decreases 5HT turnover (Forchetti and Meek, 1981), and because depletion of central serotonin stores causes an increase in ATHA (Introduction 4(d)), it was thought that the GABA-elicited increase in this adrenal enzyme (Results, C.1(a)), may be explained by the ability of GABA agonists to remove the inhibitory influence of 5HT. The suggestion that a GABA-serotonergic interaction plays a role in a regulatory process is not without precedent. Harris and McCall (1986) have suggested that an

interaction exists between GABAergic and 5HT neurons in the regulation of central sympathetic nerve activity. These authors have explained this phenomenon by proposing that there is a tonic GABAergic inhibition of serotonergic sympatho-excitatory pathways. In an attempt to demonstrate a similar situation with adrenal enzyme regulation, the effect of the conjoint administration of 5HT and GABA analogues on induction of ATHA was investigated.

At first, the effect of a serotonin agonist, MDMT, was studied. This compound had no effect on ATHA (Table C.16), a finding which is consistent with the results of Quik and Sourkes (1977), who showed that the joint administration of 5-hydroxytryptophan (5-HTP) and carbidopa are inert with respect to TH induction. These negative findings can be explained by theorizing that the tonic inhibitory influence exerted by endogenous 5HT on these adrenal enzymes is already maximal, and cannot be augmented by introducing an agonist.

The next step was to determine whether MDMT could block the muscimol-elicited increase in TH. In the data of Table C.17 it is evident that the serotonin agonist was unable to hinder the induction caused by muscimol. In a similar experiment, AMTP was co-administered with HA-966, in the hope that the former compound, which is converted to α-methylserotonin in vivo (Roberge et al., 1972), would diminish the HA-966-elicited increase in ATHA. Table C.20 reveals that AMTP has no effect of its own, nor did it influence the effect of HA-966 in any way. If the GABAergic mechanism in inducing ATHA involves a decrease in 5HT turnover, then measures to supply an exogenous source of 5HT (administration of MDMT or AMTP) would be expected to antagonize the increase in enzyme activity. The data indicate that this is not so; GABA acts elsewhere than at the MRN in its control over ATHA.

The possibility of a GABA-5HT interaction in adrenal enzyme regulation was also studied from a different perspective. If the GABA-elicited increase in ATHA is actually due to a decrease in 5HT turnover, then a further depletion of central 5HT stores with PCPA should potentiate the GABA-mediated increase in enzyme activity. Two such experiments were done, with muscimol and bicuculline as GABA analogues. (In this particular experiment, bicuculline effected a significant increase in ATHA). The results from the two experiments are similar (Table C.19). Each drug elicited a significant increase on its own, and the inductive effects of the two drugs (PCPA + Muscimol, or PCPA + Bicuculline) were additive (Table C.19.A). This implies the existence of two independent neural pathways involved in the enzyme induction; to suggest an interaction, a significant super-additive or potentiating effect should be detected. In summary then, all the experiments in this section, when taken together, demonstrate that GABA and 5HT exert their respective effects on ATHA independently.

(c) GABA-DA Interactions in the Regulation of ATHA

Pharmacological manipulations similar to those described in the preceding two sections were done in order to ascertain whether the GABAergic effect on ATHA is mediated by the dopamine system. These two neurotransmitter systems are inextricably linked (Introduction 5(c); Scheel-Krüger, 1986), particularly in the A9 region, where they combine to form the nigrostriatal-striatonigral feedback loop. Because the central dopaminergic influence on ATHA seems to be controlled from the striatum (Introduction, 4(c)), it is more likely that GABA interacts with this neurotransmitter in adrenal enzyme regulation than with either 5HT or ACh. Experiments to investigate this possibility are discussed below.

A simple approach to this problem was to examine the effect of injection of GABA analogues to rats receiving various dopamine-receptor

blockers. Interestingly, while the increases brought about by GOBA and HA-966 were not blocked by sulpiride or SCH23390, the increase in ATHA occasioned by muscimol was not diminished; rather, it was facilitated (Table C.32). The dichotomy of the results with HA-966 and GOBA on the one hand and muscimol on the other seems perplexing; nevertheless, they serve to illustrate the complexity of the GABA system and, as was alluded to earlier (D.1), the differential mechanisms by which GABAergic agents act. That the inductive effect of muscimol is amplified in the presence of haloperidol is consistent with the theory that certain GABAergic effects depend on the actual dynamic state of dopamine neurons (Gale and Casu, 1981). Scheel-Krüger (1986) maintains that the inhibitory effect of GABA is more readily apparent under high neuronal activity of DA neurons, such as resulting from the administration of dopamine-receptor antagonists, than during normal conditions. Evidently, neuroleptic-induced activation of dopaminergic neurons is important for the mode of action of muscimol, but not for GOBA and HA-966. Despite this inconsistency, it is clear that all three DA antagonists, sulpiride, SCH23390 and haloperidol fail to diminish in any way the GABAergic inductive effect on ATHA. This is analogous to atropine's inability to attenuate the muscimol-elicited increase in this adrenal enzyme (D.2(a)), and the conclusion is similar: the GABA-mediated induction is not dependent on the functioning of dopaminergic fibres.

If these two neurotransmitter systems have independent effects on TH induction; then joint administration of a DA and GABA agonist would be expected to produce the algebraic sum of the individual inductive influences of each agonist. To investigate whether this is so, the effect of a simultaneous injection of HA-966 and apomorphine (APO) was determined. The data indicate that there is no additive effect; in fact, the increase evoked by the conjoint administration of the drugs was very similar to that

seen with apomorphine alone (Table C.31). It seems reasonable to conclude that these two drugs elicit an increase in enzyme activity by the same mechanism, and that APO, at the dose given (3 mg/kg), is already doing so maximally.

With regard to this putative "common mechanism" shared by GABA and DA agonists, the key to its elucidation may be the neurochemical properties of HA-966. This GABA analogue prevents the firing of DAergic fibres, (Bonta et al., 1971) and increases the DA synthesis rate (Van Zwieten-Boot and Noach, 1975). Its sites of action are the dopaminergic cell bodies in the substantia nigra (Van Valkenburg and Noach, 1978). By comparison, dopamine agonists such as apomorphine have been shown to cause a decrease in DA synthesis (Bitran and Bustos, 1982) and release (Zetterström and Ungerstedt, 1984) in rat brain, these effects being mediated by autoreceptors on dopaminergic nerve terminals (Kehr et al., 1972) or cell bodies (Nagy et al., 1978), respectively. Complementary to this, electrophysiological studies have demonstrated that DA agonists applied microiontophoretically produce a complete inhibition of the firing rate of nigrostriatal DA neurons (Aghajanian and Bunney, 1977). If GABAergic substances such as HA-966 or GOBA cause the induction of TH by preventing DA release; then it is conceivable that apomorphine and other DA agonists act presynaptically in the same manner to cause enzyme induction. If this is so, then GABA and DA would actually be sharing a common mechanism. The hypothesis that DA agonists effect changes in adrenal enzyme activity by a presynaptic and not postsynaptic mechanism will be discussed further in section D.3.

3. Dopaminergic Regulation of Adrenal Enzymes

(a) Presynaptic Mechanisms:

The dopaminergic regulation of adrenal enzymes is a well-established phenomenon, and it is controlled at the level of the striatum (Introduction, 4(c)). Hitherto, it was assumed that the mechanism of the induction process is simply a matter of dopamine or DA agonists binding postsynaptically in the striatum, thereby eliciting a response registered as activation of a series of neural efferents to the adrenal gland with a net excitatory effect. However, the current findings resulting from the administration of GABA analogues provide ample reason to question this hypothesis.

In this work, three of the five GABAergic drugs tested: HA-966, GOBA and muscimol, were successful in inducing ATHA and, of these three, only HA-966 evoked a change in amODC. The reasons for the discrepancies with these GABA drugs might be, in part, due to differences in their specificities as well as to the diversity of mechanisms by which they affect the neurochemistry of the CNS. Nevertheless, the GABA analogues which induce enzyme activity have certain properties in common. GOBA blocks impulse flow in the nigrostriatal pathway, and this blockade may result in an immediate and marked increase in dopamine synthesis (Walters and Roth, 1976). HA-966 also prevents the release of DA and increases its synthesis rate (Introduction, 2(b)). Their site of action is probably the dopaminergic cell bodies in the pars compacta of the substantia nigra (Murrin and Roth, 1976; Van Valkenburg and Noach, 1978). Even muscimol, which is quite diverse in its effects on DA metabolism, depresses striatal DA release by a nigral action (Wood, 1982). Thus, all three GABAergic substances cause an accumulation of DA presynaptically in the striatum, yet they still manage to elicit an increase in the activity of amODC and TH.

This result indicates that the mechanism of these inductions requires the reduction of presumed inhibitory dopaminergic nerve impulses in the striatum. Indeed, Skirboll and coworkers (1979) have postulated that the firing of spontaneously active neurons in the caudate nucleus is suppressed when postsynaptic receptor sites are stimulated by dopamine while conditions that reduce dopaminergic neuronal firing lead to an increase in their activity. Because dopamine agonists can mimic the effects of GABA analogues, in that they too can prevent DA release by binding to the dopaminergic nerve terminals or by interacting with nigral cell body receptors (section D.2 (c)), it is justifiable to hypothesize that DA agonists also elicit enzyme induction by effectively reducing the postsynaptic inhibitory action of dopamine.

To test this "presynaptic hypothesis", animals were treated with 3-PPP, a drug that is believed to be a centrally acting, autoreceptor-selective agonist, decreasing both the rate of DA synthesis and the DA neuronal firing rate (Hjorth et al., 1981). Some recent reports in the literature have suggested that the stereoisomers of 3-PPP have differential effects on dopaminergic transmission. On the basis of a behavioral model (Stahle and Ungerstedt, 1984), it was concluded that (+)3-PPP, but not (-)3-PPP, is a pure agonist on dopamine autoreceptors. As a corollary to this, Arbilla and Langer (1984) demonstrated that (-)3-PPP facilitates release of ^{3}H-dopamine, probably by blocking DA autoreceptors. While it is likely that the (+) enantiomer is a DA autoreceptor agonist, some authors have suggested that a postsynaptic agonist action is not excluded at higher doses.

In this work the peripheral administration of (+)3-PPP elicited a 27% mean increase of ATHA over controls but this was not statistically significant. Although Stahle and Ungerstedt (1984) used a dose of (+)3-PPP

as high as 10 mg/kg s.c. and obtained specific pharmacological activity with it, this dose was insufficient to evoke a response in the biochemical model used here. Instead of raising the dose and risking the possibility of stimulating postsynaptic DA receptors, the drug was administered i.c.v. in subsequent experiments (in a dose of 2 μg), with the aim of determing whether it has a central, autoreceptor-selective effect. In this case, the drug evoked a significant increase in ATHA.

With respect to induction of amODC, peripheral injection of (+)3-PPP produced statistically significant results. Thus, both enzymes are regulated similarly by this putative presynaptic mechanism, but amODC is apparently more sensitive to the drug than TH is. Although the findings with this substance lend support to the hypothesis that DA agonists induce these adrenal enzymes by acting presynaptically, the interpretation of the results cannot be definitive until the discrepancies in the literature regarding the pharmacological properties of (+) and (-)3PPP are clarified.

In view of the above findings, how can the inductive effects of apomorphine, which most certainly is not limited to a presynpatic action, be explained? Moreover, how does one interpret the inability of 6-hydroxydopamine (6-OH-DA), which destroys catecholaminergic nerve terminals, to abolish the apomorphine-elicited increase in TH (Quik and Sourkes, 1977)? The former problem can be explained by evidence which shows that dopamine receptors in the A9 region exhibit differential sensitivity to DA agonists. On the basis of behavioral studies, it has been postulated that presynaptic autoreceptors are more sensitive to DA agonists than are postsynaptic receptors (Strömbom, 1976). Electrophysiological studies by Skirboll et al. (1979) demonstrated that the inhibition of the firing rate of striatal neurons by iontophoretically-applied DA required a larger dose than that required for inhibiting the

firing rate of nigral dopaminergic neurons. In addition, Brase (1980) concluded that presynaptic receptors on dopaminergic nerve terminals in the striatum are more sensitive to apomorphine than are the postsynaptic striatal DA receptors. Admittedly, the low "presynaptic dose" of apomorphine used in this work (3 mg/kg) is higher than the autoreceptor-selective doses used by the authors mentioned above. This may suggest a relative participation of pre- and postsynaptic systems in the regulation of DA release (Skirboll et al., 1979). Thus, small doses of apomorphine do, indeed, preferentially affect the more sensitive presynaptic receptors, and thereby prevent DA release to a large extent; but larger doses may recruit more postsynaptic inhibitory fibres in the striatum, thereby effecting an even greater inhibition of dopaminergic cell firing by striatonigral feedback pathways. The inability of 6-OH-DA to abolish the apomorphine-elicited increase in ATHA, could be due to the fact that the neurotoxin incompletely destroys catecholaminergic nerve terminals.

At this time, it would be difficult to suggest an exact locus for a putative presynaptic mechanism in inducing amODC or TH. Although GABAergic and DA agonists have opposite effects on DA synthesis, they are both capable of blocking the firing of DA neurons and for both classes of compound this effect is thought to be localized to the nigral dopaminergic cell body receptors (Murrin and Roth, 1976; Van Valkenburg and Noach, 1978; Aghajanian and Bunney, 1977). One might speculate then, that the inductive process is initiated at these nigral DA autoreceptors.

(b) D-1 vs. D-2 Receptors in Adrenal Enzyme Regulation:

In the course of establishing the central dopaminergic influences on amODC and TH, it was shown that the increases in these enzyme activities brought about by DA agonists can be attenuated by the prior administration

of the dopamine-receptor blocker haloperidol (Quik and Sourkes, 1977; Almazan et al., 1982a). Subsequently, in an attempt to localize the dopaminergic centre controlling these adrenal enzymes, DA antagonists that act site-specifically were used. As a result of these studies (Ekker and Sourkes, 1985a), it was postulated that the dopaminergic neural impulses controlling the induction of TH and amODC emanate from the striatum. One of the goals of the present thesis was to pinpoint further the site of action of DA agonists in this regulatory process. In this regard, the experiments performed by Quik and Sourkes (1977) and Almazan et al., (1982a) were repeated, this time using antagonists that were specific for the D-1 and D-2 dopamine receptor subtypes.

Initially, it was felt that the dopamine-mediated effect on enzyme induction was simply a postsynaptic phenomenon. Since both receptor subtypes have been reported to exist postsynaptically in the striatum (Introduction, 6; Figure III), one would expect that either D-1 or D-2-specific antagonists would block the apomorphine-elicited increase in TH and amODC. However, in light of the "presynaptic hypothesis" proposed earlier (Discussion, 3 (a),), and because the presence of D-2 but not D-1 sites presynaptically (Kehr et al., 1972; Nagy et al., 1978) enables the D-2 receptor to predominate in controlling DA metabolism and release (Boyar and Altar, 1987) one would anticipate that sulpiride, a D-2 blocker, would hinder the apomorphine-elicited induction to a significantly greater extent than would SCH23390, a D-1-selective antagonist (Hyttel, 1983). Indeed, this neuroleptic, sulpiride, a substituted benzamide, is designated a D-2-specific blocker as it fails to block DA-sensitive adenylate cyclase activity (Trabucchi et al., 1975; Elliot et al., 1977). Mereu et al. (1983) suggested that sulpiride can readily antagonize the inhibitory effect of apomorphine on the DAergic cells of the substantia nigra and that

this interaction takes place at the autoreceptor level. Pinnock and coworkers (1979) have also shown that the agonist-induced decrease in neuronal activity is attenuated by antagonists such as sulpiride, which act at dopamine D-2 receptor sites in the rat substantia nigra. Other studies have shown that (-) sulpiride is especially active upon presynaptic DA autoreceptors (El Mestikawy et al., 1986). In a behavioral model, sulpiride blocked DA autoreceptors at doses that had relatively little postsynaptic receptor effect (Kendler et al., 1982), again illustrating that this neuroleptic has a DA autoreceptor-specific action.

At first glance, however, the results obtained do not correlate with the earlier data which suggested that DA agonists act presynaptically. Tables C.23 through C.26 suggest that the increase in amODC, as occasioned by apomorphine, is diminished significantly both by SCH23390 and by sulpiride ($P < 0.01$; $P < 0.05$ respectively). In the case of TH, the induction by apomorphine was attenuated by both antagonists, but only the D-1-specific blocker effected a significant diminution (Table C.28: $P < 0.05$ for APO vs. APO+SCH23390; Table C.20: $P > 0.05$ for APO vs. APO+Sulpiride). The fact that D-1 and D-2-receptor blockers have very similar effects on the apomorphine-elicited induction of these adrenal enzymes might be looked upon as another example of how the two DA receptor subtypes in the striatum interact "co-operatively" to elicit a certain biochemical action, as suggested by Walters et al. (1987). Thus, the D-1 blockade occasioned by SCH23390 hinders the effect of DA or DA agonists binding to a D-2 site and sulpiride, similarly, diminishes the efficacy of D-1 receptor stimulation. This explanation would be more likely if adrenal enzyme induction were a process initiated postsynaptically. However, alternative arguments may be put forward if the inductive process is, indeed, a presynaptic phenomenon.

As was suggested earlier, sulpiride is an autoreceptor-selective antagonist; thus, it was able, as expected, to attenuate the presumed presynaptic action of apomorphine in increasing amODC activity, but not TH activity. The discrepancy between the two enzymes in this regard cannot be satisfactorily explained; however, it should be noted that the presynaptic autoreceptors modulating dopamine release are chemically stereoselective (Arbilla and Langer, 1981) and only (-) sulpiride increases the firing of DA neurons in the substantia nigra (Mereu et al., 1983). In these experiments, a racemic mixture of sulpiride was administered. Therefore, an insufficient amount of the active enantiomer might have been present to be effective for blocking TH induction. The findings with SCH23390, which indicate that this D-1-selective antagonist can substantially lower the apomorphine-elicited increase in both ATHA and amODC activity, are more difficult to explain in terms of a putative presynaptic action of DA agonists on these adrenal enzymes. The difficulty stems from the notion that, as was mentioned earlier (Introduction, 6.), dopamine receptors located presynaptically are primarily of the D-2 subtype. However, recent evidence has suggested that SCH23390 is not strictly a D-1 receptor antagonist. Plantje and coworkers (1984) suggested that although SCH23390 is the most selective D-1 antagonist known at present, it is also able to antagonize competitively the effects of D-2 agonists. It has also been postulated that SCH23390 can reverse the amphetamine-induced suppression of DA neuronal firing by an interaction with the D-2 receptor (Goldstein et al., 1987). Napier et al. (1986) have suggested that SCH23390 is not exclusively a D-1 antagonist. A D-2 blocking action of this compound may be seen at very high concentrations. Thus, the attenuating effect of SCH23390 on the apomorphine-elicited

increases in adrenal enzyme activity may be attributed to the binding of this compound to presynaptic D-2 sites with the doses used. Finally, El Mestikawy and Hamon (1986) proposed that DA autoreceptors, although clearly not of the D-1 type, do not correspond completely to the D-2 type either. The DA autoreceptor may actually be a "hybrid" receptor, with specificities intermediate between the D-1 and D-2 subtypes. This may then explain the similar efficacies of sulpiride and SCH23390 in blocking the enzyme induction evoked by apomorphine.

In summary, the "presynaptic hypothesis" for the induction of adrenal enzymes as mediated by DA agonists is not necessarily inconsistent with the findings obtained using D-1 and D-2-specific receptor blockers. The results obtained here merely serve to illustrate further the complexity of dopamine receptor subtypes and their interactions. It also suggests that the DA autoreceptor may indeed by biochemically distinct from the D-1 and D-2 receptors.

E. SUMMARY AND CONCLUSIONS

As is well-known, the adrenal glands serve as a site of catecholamine biosynthesis and they contain the biosynthetic enzymes necessary for this process. The release of these catecholamines is an important constituent of an organism's response to stressful stimuli, sometimes referred to as the "flight or fight" response. Thus, the regulation of adrenal enzymes may serve as a useful paradigm in studying the physiology of the stress response; monitoring these enzyme activities provides an "index" of the animal's reaction to an "anti-homeostatic" stimulus. Various stressors and drugs cause an increase in the levels of adrenal enzymes. The drugs concerned act at sites in the brain which influence the adrenals and thereby affect the activity of the catecholamine-producing enzymes. It is thought that by understanding how the brain controls the organism's response to stressful situations, presumably through its influence on adrenal function, one might then be able to develop therapeutic agents to help counter the many pathophysiological manifestations of stress, gastric ulceration and cardiovascular disease to name but a couple. To this end, this laboratory has studied the neural pathways governing the function of the adrenal gland.

Hitherto, it has been determined that the dopaminergic and cholinergic neurotransmitter systems play an excitatory role, whereas 5HT contributes inhibitorily to the induction of amODC and TH. The work in this thesis has uncovered the existence of a GABAergic pathway which exerts a net, central excitatory influence on both of these adrenal enzymes. In retrospect, this finding is not unreasonable, especially in view of the ubiquitous nature of this neurotransmitter in the CNS. Interestingly, not all of the GABA analogues tested were able to elicit an increase in the activity of these adrenal enzymes. This inconsistency may, to some extent, be attributed to the differences in specificity of the GABAergic drugs used, the different

metabolic routes associated with each of them as well as to the multiplicity of mechanisms by which these substances affect the GABA system. Indeed, at present, many of this neurotransmitter's properties and effects are still unknown. Future work in this area may include the use of other GABA analogues such as gamma-vinyl GABA (GVG, Jung et al., 1977), baclofen (Hill and Bowery, 1981) or perhaps the metabolites of progabide (see Introduction, 2 (a) iii). It may be anticipated that by broadening the spectrum of drugs used, one will be provided with greater insight as to the GABAergic mechanisms involved in the regulation of these enzymes.

Once the central excitatory GABAergic effect on amODC and TH had been established, it was of interest to determine whether GABA interacts with other neurotransmitters in bringing about increases in adrenal enzyme activity. Despite an extensive series of experiments, involving a combination of GABA analogues and serotonergic, cholinergic or dopaminergic substances, no such interaction was demonstrated. The fact that GABA's influence on these adrenal enzymes is independent of the effects of other central neurotransmitters testifies to the versatility of the organism in its ability to respond to a stressful stimulus. In the event that a given nervous pathway is inaccessible other pathways exist to transmit these all-important messages to the periphery.

The similarity of the inductive effects of apomorphine and GABA analogues such as GOBA and HA-966, combined with the fact that GABA affects DA transmission in the striatum inhibitorily, led to speculation that the DA-elicited increase in adrenal enzyme activity is a presynaptically-mediated phenomenon, not a postsynaptic one, as was previously thought (see Discussion, 3 (a)). Moreover, although the similar abilities of D-1 and D-2-specific antagonists in attenuating the apomorphine-elicited increase in TH and amODC may mean that these two receptor subtypes interact in this

regulatory process and that they do so at postsynaptic sites, these findings are not necessarily contradictory with a presynaptic effect. If the mechanism is indeed a presynaptic one, then these D-1/D-2 findings lend support to the notion that autoreceptors on dopaminergic neurons may not necessarily be strictly categorized as D-1 or D-2 subtypes (El Mestikawy et al., 1986).

While the "presynaptic hypothesis" serves to provide some insight into the inductive process, at least insofar as DA agonists are concerned, it also lends support to the "feed-forward model" describing the role of GABA in the A9 region (Scheel-Krüger, 1986). According to this model, it seems that the modulatory influence of GABA on dopamine neurons in the pars compacta of the substantia nigra (i.e. the traditional "feed-back loop") is merely an auxiliary function of the striatonigral GABAergic pathway. The main function of this neurotransmitter is to convey information to efferent pathways in the basal ganglia and from thence, to more distal output stations in the CNS. Conceivably, the GABA-elicited induction of adrenal enzymes may be a result of the activation of such efferent pathways, which ultimately lead to the adrenal medulla.

As with most scientific endeavors, this thesis has raised more questions than it has answered. The role of GABA in the striatonigral region in general and its function in adrenal enzyme regulation in particular remains open for future research. The putative "presynaptic hypothesis" in DA-elicited enzyme induction also requires further confirmation. Future work may involve the administration of DA agonists to rats subjected to lesions (either chemically-induced with 6-OH-DA or electrolytically) of the nigrostriatal pathway. The absence of enzyme induction under these conditions would corroborate a presynaptic mechanism. The advent of DA autoreceptor-selective compounds such as (+)3-PPP and

BHT90 (Andén et al., 1982) also provides useful tools in studying this hypothesis. With the development of more and more such site-specific compounds, the prospects for elucidating the detailed mechanisms involved in adrenal enzyme regulation, and in turn, the neural pathways of stress, are good.

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