MECHANISM OF INTERACTION OF STRYCHNINE AND THEBAINE

Ph.D. Pharmacology and Therapeutics

A STUDY ON THE MECHANISM OF THE INTERACTION BETWEEN STRYCHNINE AND THEBAINE

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Abstract

Although strychnine and thebaine have very similar convulsant actions, mixtures of the two drugs were found to interact infra-additively in intact mice. It was established that this interaction also occurs at the postsynaptic inhibitory synapses on motoneurons in the cat spinal cord. Thebaine diminishes reflex inhibition, just as strychnine does. The drug also blocks direct, recurrent, and polysynaptic IPSP without altering membrane resting potential, resistance or potassium permeability. No effect of thebaine on spinal synaptic transmission was discovered which could produce a physiological antagonism of the anti-inhibitory action of strychnine. It was concluded that the mechanism of the anti-inhibitory action of thebaine and strychnine is very similar and their interaction is probably a reflection of the competition of the two drugs for the same receptor site in the inhibitory synapse.

A STUDY ON THE MECHANISM OF THE INTERACTION BETWEEN STRYCHNINE AND THEBAINE

by

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TABLE OF CONTENTS

INDEX OF TABLES

INTRODUCTION

Aim of Project

Twenty years after the general acceptance of chemical transmission in the central nervous system, the identification of central synaptic transmitters remains a major problem. The successful search for neurotransmitters at peripheral synapses established several criteria whose conditions should be met by any substance for which a transmitter function is postulated. They require that the proposed transmitter be present at the site of transmission, be released by nerve stimulation, and mimic the actions of the natural transmitter. Also, a mechanism for the termination of its action must be demonstrated. Werman (1966) has suggested that the failure to identify transmitters within the central nervous system (CNS) can be attributed to the naive application of these criteria in a system of much greater complexity than that in which they were developed. He argues that it would be more realistic to place greater emphasis on the ability of a substance to mimic the effect of nerve stimulation.

An important part of the criterion of identical action is the requirement for pharmacological identity. Drugs which can be shown to alter transmission by the natural mediator must have a similar effect on responses evoked by the transmitter candidate. In this respect, agents that selectively block the response of the postsynaptic membrane to transmitter are particularly useful tools. The possibility of pharmacological blockade of synapses has been recognized since Claude Bernard (1850) determined the site of action of curare. The

potential of such drugs did not escape his notice for he described them as follows:

> "Comme des éspèces d'instruments physiologiques plus délicates que nos moyens méchanique et destiné a dissequer, pour ainsi dire, une a une les propriétés des éléments anatomique de l'organisme vivant."
>
> (Bernard, 1857)

Several blocking drugs are available for central inhibitory synapses. In the spinal cord, strychnine, picrotoxin, and tetanus toxin block inhibition without affecting excitatory transmission (Curtis, 1963). However, the site of action of these drugs within the synapse is still unknown, and their ability to distinguish between various transmitter candidates is therefore questionable (e.g., Roper et al., 1969). This is a circular dilemma: as long as the identity of the transmitter is unknown, it is extremely difficult to directly determine the synaptic site of action of a blocking drug. A new experimental approach has recently been used to investigate the action of strychnine on the postsynaptic inhibition of spinal motoneurons. By performing a statistical analysis of the postsynaptic potentials evoked by stimulation of a single inhibitory interneuron, evidence has been obtained for a postsynaptic action of strychnine at this synapse (Weakly and Kuno, in press). Eventually, it will probably be possible to obtain the information on the synaptic site of action of drugs which block central synapses. The criterion of pharmacological identity can then be applied to differentiate between various transmitter candidates, at those synapses for which several well investigated blocking drugs are available.

A classical, if inconclusive, test to determine whether two drugs have a similar mechanism of action is the demonstration of an

interaction between the two drugs when they are administered together (e.g., Ahlquist, 1947). The anti-inhibitory drugs pilocarpine and coniine lower the threshold for strychnine seizures in mice (Zablocka and Esplin, 1963; Sampson et al., 1966), but preliminary experiments in this laboratory indicated that thebaine and strychnine are actually infra-additive when tested on mice and spinal cats (Laskey and Zablocka-Esplin, 1971). For many years neuropharmacologists have regarded thebaine as one of the few anti-inhibitory drugs (Pinto-Corrado and Longo, 1961; Longo and Chiavarelli, 1962), so this type of interaction was unexpected. However, it is an interaction between two drugs with similar effects, and suggests that they share the same mechanism of action. A possible explanation is that thebaine is a partial agonist at the strychnine sensitive receptor (see Collins et al., 1967). If thebaine could be shown to exert a specific action at the spinal inhibitory synapses, it might be possible to exploit its peculiar interaction with strychnine to obtain an indication of the synaptic site of action of the drugs. Therefore, it was decided to see if a thorough comparison of strychnine and thebaine, in several test systems, could reveal some fundamental difference between the actions of these drugs at inhibitory synapses. A profile of the anti-inhibitory effects of thebaine in the spinal cord will be established by studying the effect of the drug on:

1. Direct, recurrent, polysynaptic, and presynaptic inhibition of the monosynaptic reflex (MSR).
2. Monosynaptic and polysynaptic excitatory reflexes.
3. Synaptic potentials and membrane properties of motoneurons.

4. Repetitive discharge of Renshaw cells.

The interaction of thebaine and strychnine will also be studied in greater detail.

Many anti-inhibitory drugs, including thebaine, block ganglionic and neuromuscular transmission (see Ferguson et al., 1970), and a "cholinergic link" in spinal inhibitory transmission has been proposed (Esplin and Zablocka, 1964; Koelle, 1969). If anti-inhibitory drugs are acting on a cholinergic mechanism, the blockade they produce should be attenuated by anticholinesterases. The interaction of thebaine and strychnine with eserine will therefore be investigated.

During the preliminary experiments on mice, an unexpected antagonism of thebaine convulsions by nalorphine was noticed. A few experiments will be done to see if this interaction also occurs in spinal cats.

Historical Background

Central to the interpretation of the action of drugs on the nervous system are the concepts of "synapse" and "chemical transmission". Both concepts grew out of the intensive investigation of reflex function in the seventeenth, eighteenth, and nineteenth centuries, which culminated in the early nineteen-hundreds with the acceptance of the neuron theory, the characterization of synaptic function by Sherrington, and the proposal by Elliott that adrenaline mediates the effects of stimulation of the sympathetic nerves.

Descartes is usually credited with the first attempt to describe and explain reflex behaviour. He had a clear idea that sen-

sory information entered the CNS and, by some unspecified mechanism, was "reflected" out to the muscles in such a way that they performed appropriately.

> "For in certain persons that fear disposes the brain in such a way that the spirits reflected from the image thus formed on the gland, proceed thence to take their places partly in the nerves which serve in turn the back and dispose the legs for flight ... i.e., which are adapted to the holding open, or at least re-opening, of the pores of the brain which conduct them into the same nerves."
>
> (Descartes, 1650)

While many of Descartes' observations, especially those on reciprocal innervation (see Sherrington, 1906), were extremely acute, his conceptual basis of "man as a machine" did not have the scope required to explain the complex integrative actions for which the central nervous system is so highly specialized.

The main contribution of the eighteenth century to reflex physiology was the definition and characterization of reflex responses by Whytt, Unzer, and Prochaska. Whytt has been called the foremost neurologist of his time (Garrison, 1929). He confirmed Stephen Hales's original observation that reflex function depends on an intact spinal cord. Experimenting with spinal and decorticate animals, he classified reflex actions into three groups: voluntary, involuntary, and mixed, and extended the list of reflexes to the autonomic system. He emphasized that, even when reflex activity did not rise to the level of consciousness, it was nevertheless mediated by the central nervous system. In addition, from his studies on spinal animals, he concluded that the integration responsible for the coordination of movements

involving the activity of many muscles, must take place within the spinal cord, since, in spinal animals, it is the only site at which the nerves to several parts of the body are in communication (Whytt, 1768). Whytt had a penetrating mind and made many important and original contributions to neurophysiology. Unzer and Prochaska, on the other hand, systematized existing knowledge rather than adding to it (see Fearing, 1930).

In the early 19th century Marshall Hall repeated much of the previous work done on reflexes and, in spite of being a rather controversial figure, he deserves credit for making the first applications of reflex theory to therapy (see Sherrington, 1900). At this time Sir Charles Bell and François Magendie finally made the observation that the dorsal roots are specialized to carry sensory information while the ventral roots are motor in function.

Meanwhile, an era of technical advances was beginning in histology. With the introduction of the achromatic microscope, and the development of techniques for fixing, sectioning, and staining, the structure of the delicate tissues of the central nervous system became more clearly defined (see Liddel, 1960). The first accurate description of cells in the CNS was made by Purkinje in 1837 (Garrison, 1929). The cerebellar "Purkinje" cells in his diagrams have a characteristic shape, a nucleus, and several short processes. In the following years information on nerve cells and peripheral nerve fibers accumulated rapidly, but the relation between these fundamental units remained obscure until the eighteen-fifties when Waller (1850) published his famous degeneration experiments, and established that nerve

fibers are the long processes of nerve cells. The dendritic processes were studied by Gerlach (see Barker, 1899). He developed a gold chloride stain which displayed the amazing complexities of the dendritic networks in the central grey matter and was the founder of the "reticular theory" of nervous action. This theory assumed protoplasmic continuity between nerve cells, and proposed that a nerve net or reticulum was the means by which one nerve cell is connected to another. This view of central connections was not supported by the existing knowledge of nervous function, and it was soon challenged by the careful histologists, His, Forel, and Cajal. Their ingenious work with embryonic tissue and secondary degeneration led them to think of nerve cells as separate entities, which communicate at points of contact rather than by protoplasmic continuity (see Barker, 1899). This concept was enunciated by Waldeyer as follows:

> "Each nerve cell, inclusive of the nucleated body and all its processes, irrespective of their number, length, complexity, character, and position, forms a distinct unit, which is believed ... to have no structural continuity with any other unit."
>
> (Waldeyer, 1891; cited by Schäfer, 1900)

It became known as 'Waldeyers' Neuron Theory" and has formed the basis for all modern interpretation of nervous function.

With the acceptance of the neuron theory, the attention of physiologists was directed to the point of contact between nerve cells at which communication takes place. Writing in Foster's Textbook of Physiology, Sherrington (1897) described and named these contacts as follows:

> "So far as our present knowledge goes, we are led to think that the tip of a twig of the arborescence is not continuous with but merely in contact with the substance of the dendrite or cell body on which it impinges. Such special connections of the nerve cell with another might be called a synapsis."

Sherrington's careful studies on spinal animals showed that reflex transmission differed from conduction in nerve fibers in the following respects:

a) It is always associated with a characteristic delay.

b) It exhibits afterdischarge.

c) Its response can be graded.

d) It is capable of marked temporal summation.

e) It is unidirectional.

f) It is particularly sensitive to fatigue, anoxia, drugs.

g) It can bring about the inhibition of ongoing activity.

He attributed these differences to the presence of intercellular conduction in the reflex pathway, and was thus able to describe those special functional properties of synapses which are the physical basis for the integrative activities of the nervous system (Sherrington, 1906). Sherrington did not attempt to describe the mechanism by which synaptic transmission is effected, although this problem was still unresolved.

In 1877 du Bois-Reymond, on detecting the characteristic delay in neuromuscular transmission, had proposed that some chemical or electrical event must intervene between the excitation of the nerve and the contraction of the muscle (see Brooks, 1959). Electrical

theories proved more popular, and even when Elliott (1904) noted that adrenaline might be the chemical stimulant released by sympathetic nerves, the possibility of chemical transmission was generally ignored. It was not until 1921 that Otto Loewi demonstrated the release of "Vagusstoff" and - in his own words - "unequivocally demonstrated that transmission was humoral" (Loewi, 1960).

The chemical theory states that transmission of excitation or inhibition across the synapse is effected by a chemical agent which is released by the presynaptic terminal, diffuses across the synaptic cleft, and interacts with specific receptors of the postsynaptic membrane. It was rapidly extended to include the synapses of autonomic ganglia, voluntary muscle, and the central nervous system (Adrian, 1924; Feldberg and Gaddum, 1934; Dale et al., 1936). The special properties of synapses, so different from the characteristics of conduction in nerve, were easily explained by the humoral theory. As early as 1906, an extensive study of synaptic fatigue had led Scott (1906) to conclude that transmission through the spinal cord must be chemical. Sherrington (1925) proposed that a localized, nonpropagated "central excitatory state or agent", whose main features were a prolonged duration and a lack of absolute refractory phase, could account for the synaptic properties of temporal and spatial summation, and afterdischarge.

The proponents of electrical transmission were not convinced. With the introduction of the vacuum tube amplifier and the cathode ray oscilloscope (see Adrian, 1932), the first recordings of synaptic potentials were made (Eccles et al., 1941; Gasser and Graham, 1933).

These slowly decrementing potentials permitted an electrical explanation of synaptic transmission. In a classic paper, Eccles (1946) showed that excitatory synaptic potentials of spinal motoneurons fulfilled all the requirements of the central excitatory state. The active phase of transmission had a duration compatible with electrical excitation, and the prolonged decay of the synaptic potential was a consequence of the electrical properties of the postsynaptic membrane. He concluded that synaptic transmission in the spinal cord was electrical.

Until the introduction of intracellular recording, there was no direct evidence which could decisively exclude either theory (see Fessard and Posternak, 1950). Fatt and Katz (1951) made the first measurement of the amount of charge transferred at the muscle endplate during synaptic excitation and found that it was too large to be the result of presynaptic electrical activity. They concluded that transmission at the neuromuscular junction was chemical. The following year Brock and colleagues (1952) detected the inhibitory postsynaptic potential of spinal motoneurons. They recognized that no electrical theory could explain this hyperpolarization, and accepted chemical transmission for both excitatory and inhibitory synapses of the central nervous system.

Identification of Central Transmitters

Acceptance of chemical mediation for the majority of central synapses implies the existence, within the central nervous system, of chemical transmitters. The search for these mediators is a contemporary

endeavour whose main approach is an attempt to show that transmitter candidates fulfill a set of conditions which a substance must meet in order to be accepted as a transmitter. These criteria were empirically established during the identification of peripheral transmitters, but their effectiveness is reduced by the complexity of the central nervous system. Even the apparently reasonable requirement for the presence of the supposed transmitter at the site of transmission has been confounded. For example, the amino acid candidates are definitely present in the brain, but their ubiquitous distribution has cast doubt on their possible role as transmitters. Since other evidence for a transmitter function of these compounds is extensive, they have not been disqualified on the basis of their nonspecific distribution (see Curtis, 1969; Krnjevic, 1970). Similarly, inability to demonstrate the release of proposed transmitter by physiological stimuli can be attributed to any number of factors (see Werman, 1966). For instance, it has been suggested that the failure of glycine to fulfill this requirement is due to the operation of a mechanism for the uptake of glycine which has recently been demonstrated in the spinal cord (Neal, 1971).

Even when release of the suspected transmitter is shown, the specificity of the release mechanism must be demonstrated or the value of the evidence can be challenged. For example, although the release of noradrenaline by physiological stimuli has been demonstrated in the olfactory bulb, it has been shown that under the same experimental conditions, these stimuli will also release the inert substance, inulin (Chase and Kopin, 1968).

Establishing identity of action requires comparison of the response of the postsynaptic membrane to naturally released transmitter, and to appropriately applied transmitter candidates. Since many central pathways are polysynaptic and anatomically diffuse, it is often difficult to demonstrate the true functional response of neurons to naturally released transmitters. For example, there is reasonable evidence for the existence of descending aminergic pathways in the spinal cord, but a complete analysis is impeded by the difficulty of obtaining specific responses by supraspinal stimulation (Curtis, 1968). Although effective methods have been developed for the highly localized delivery of putative transmitters (Curtis, 1964; Krnjevic, 1964), it is still difficult to ensure that these substances are being applied at the appropriate sites. Bloom and Giarman (1968) have pointed out that in certain regions of the brain, abundance of neurons responsive to electrophetically applied amines is not correlated with the presence of aminergic pathways. Another complicating factor is the distribution of synapses on the soma and dendrites of neurons (see Colonnier, 1968). Because conductance changes caused by synaptic activity on the dendrites cannot be detected at the soma (Rall et al., 1967), it is difficult to make a close comparison of the actions of natural and suspected transmitter for such synapses. A final problem is the necessity of excluding the possibility that the substance being tested duplicates the effect of nerve stimulation indirectly, by releasing the natural transmitter from presynaptic terminals (see Werman, 1966).

Although drugs which interfere with central synaptic trans-

mission are used as aids in transmitter identification, the complications mentioned above often limit their usefulness. Before a drug can be an effective tool, a selective action at a particular type of synapse must be demonstrated and the site of action within the synapse should be known. If these conditions are not fulfilled, even results obtained with a drug as specific as strychnine, in a synaptic system as well understood as spinal inhibition, can become ambiguous (Roper et al., 1969; Davidoff et al., 1969; Curtis et al., 1969). The lack of specific blocking drugs for many synapses and the lack of detailed information about those drugs which are available have hindered the search for central neurotransmitters.

Inhibition

Inhibition can be defined as a process, the temporary operation of which can arrest or attenuate an action in progress, without damaging the inhibited tissue. The necessity for postulating such a process was recognized by Descartes in the seventeenth century (cited in Sherrington, 1906). Since then, many speculative theories of its mechanism of action have been developed, and even today, there are important aspects of central inhibition that are not clearly understood.

A period of renewed interest in inhibition was initiated by the Weber brothers' striking demonstration, in 1845, of vagal inhibition of the frog heart (see Garrison, 1929). Other instances of peripheral inhibition were reported, and in 1863 Setschenow published his experiments on inhibition in the brain (see Fearing, 1930). He

showed that localized stimulation of certain areas of the brain could suppress reflex activity of the spinal cord, and elaborated the "nervous center" theory of inhibition to explain his observations. Although this theory was disputed by Herzen, who showed that intense stimulation of almost any part of the CNS could produce a generalized depression of its activity, Setschenow made an important contribution by recognizing that inhibition of reflexes need not be exerted peripherally.

By 1880, inhibition was well established as a fundamental property of nervous tissue (see Foster, 1880), and many attempts were being made to explain its operation (see Howell, 1925). A widely accepted theory was that of Wedensky (1892), who proposed that inhibition was a form of post-activity depression. Lucas and Adrian extended this theory, suggesting that inhibition was due to the extinction of excitatory impulses by rapid stimulation. Impulses falling within the relative refractory phase of the nerve would be diminished and would not invade the synaptic region, where decremental conduction was assumed to prevail (Adrian, 1918). As late as 1937 Gasser proposed another version of this idea, and tried to correlate inhibition with the subnormal period of the postsynaptic element (Gasser, 1937). These theories were variations of the central idea that inhibition is a consequence of excitatory activity, and not an independent phenomenon.

In direct opposition to this view was Sherrington's concept of inhibition as an active process (Sherrington, 1906). In his studies on reciprocal inhibition and the flexor reflex, he characterized the "central inhibitory state" without attempting to explain the mechanism by which inhibition is achieved, but he did indicate the postsynaptic

membrane as the site of interaction between inhibition and excitation.

Sherrington was continually drawing attention to the properties of reflex inhibition which could not be explained if inhibition was a passive consequence of previous activity. For example, he noted that strong responses were harder to inhibit than weak ones, the opposite of what would be predicted by the Wedensky theory (Eccles and Sherrington, 1931).

However, the active and independent nature of central inhibition was not generally accepted until the experiments of Lloyd and Renshaw in 1941. Renshaw (1941) showed that stimulation of the ventral roots could inhibit motoneurons which were not themselves activated by the antidromic impulse, and Lloyd (1941) found that activation of a certain type of primary afferent pathway could inhibit motoneurons which it was incapable of exciting.

Inhibitory Mechanisms

The inhibition of neural activity can be achieved by three distinct synaptic mechanisms, known as postsynaptic, presynaptic, and electrogenic pump inhibition. Postsynaptic inhibition is the term used to describe a reduction in the responsiveness of the postsynaptic membrane by the interaction of this membrane with an inhibitory transmitter. Intracellular recordings from inhibited cells of many different tissues have confirmed the original observation of Gaskell (1887) that this type of inhibition is accompanied by a hyperpolarization of the postsynaptic membrane. The detectable hyperpolarization is a secondary manifestation of the primary response. The reaction of the

inhibitory transmitter with the postsynaptic membrane results in an increase in the permeability of this membrane to certain ions. The electrochemical gradients of these ions are such that they are distributed at equilibrium when a potential of about -80 mV is maintained across the neuron membrane. During the action of the inhibitory transmitter, the unimpeded fluxes of these ions counterbalance any depolarizing currents, and the motoneuron membrane is "clamped" at the equilibrium potential of the inhibitory process.

The potential change accompanying the change in ionic conductance is called the inhibitory postsynaptic potential or IPSP. Its amplitude and direction depend on the level of the resting polarization of the postsynaptic membrane. Increasing the membrane potential by injecting current into the motoneuron reduces the amplitude of the IPSP and, eventually, a potential is reached at which the IPSP disappears. At this potential the ions which carry the inhibitory current are distributed at equilibrium with respect to the membrane potential; it is called the equilibrium potential of the inhibitory process (see Coombs et al., 1955 a and b). Any further hyperpolarization of the membrane reverses the sign of the IPSP, but does not alter its inhibitory character. Conversely, depolarization enhances the IPSP amplitude.

In an effort to determine the identity of the ion species responsible for the inhibitory current, the effects of intracellular injection of many ions have been studied. In spinal motoneurons, it has been shown that under the influence of the inhibitory transmitter, the postsynaptic membrane becomes permeable to all ions with a dia-

meter less than twice that of the hydrated potassium ion (Araki et al., 1961). Injections of chloride ions cause a reduction in IPSP amplitude and can even reverse the IPSP. Since this effect is due to a change in the equilibrium potential of the ions involved, these reversed IPSP do not retain their inhibitory character, and can even initiate action potentials. Because it was always assumed that chloride is distributed at equilibrium in the resting state (see Adrian, 1960), Eccles (1961) suggested that at least part of the inhibitory current must be carried by potassium ions, whose equilibrium potential was known to be higher than the resting membrane potential. However, it has recently been shown that, in motoneurons, ammonium ions block a mechanism for extrusion of chloride ions which maintains the normal equilibrium potential of postsynaptic inhibition (Lux, 1971). Investigations of the ionic mechanisms at other inhibitory synapses indicate that both potassium and chloride are usually involved, their relative contributions varying from one extreme to the other. The IPSP of heart muscle is produced by potassium redistribution alone (Trautwein et al., 1956), and chloride is the only ion involved in the inhibition of crustacean muscle (Boistel and Fatt, 1958).

Inhibition can be achieved by a process which reduces the excitatory input to the postsynaptic element. This type of inhibition has been termed presynaptic and is a prominent feature of neuromuscular transmission in some invertebrates (Dudel and Kuffler, 1961; Tauc, 1965; Epstein and Grundfest, 1970) and at the Mauthner cells of the goldfish (Furukawa et al., 1963).

The operation of presynaptic inhibition has been inferred,

from indirect evidence, for all primary afferent synapses in mammals and for the recurrent inhibition of motoneurons in the frog spinal cord (Meij and Holemans, 1969).

The mechanism by which presynaptic inhibition is effected remains obscure. Eccles' suggestion (Eccles et al., 1961 a) that inhibition is the result of a reduction of the amount of transmitter released by the afferent terminal has been confirmed at the neuromuscular junction of the crayfish (Dudel and Kuffler, 1961). Since it has been shown that the amount of transmitter released per volley is a function of the potential change produced by activation of the presynaptic terminals (Hagiwara and Tasaki, 1958; Takeuchi and Takeuchi, 1962), a depolarization of these terminals by inhibitory synaptic activity would be expected to decrease the amount of transmitter that they release. As early as 1934 it was noted that a long lasting inhibition of the reflex responses of spinal motoneurons can be produced by conditioning volleys in the dorsal roots. The time course of inhibition was identical to that of certain negative field potentials recorded from the dorsal surface of the cord (Hughes and Gasser, 1934) and from the cut ends of the dorsal roots (Barron and Matthews, 1938). These potentials reflect the depolarization of the activated primary afferent terminals. They accompany normal reflex transmission and increase in amplitude when presynaptic inhibitory pathways are activated (Eccles et al., 1962).

Eccles and his colleagues tried to show that the primary afferent depolarization (PAD) is the cause of presynaptic inhibition in the mammalian CNS (see Eccles, 1964). Changes in depolarization,

however, are not always accompanied by changes in the amount of transmitter released. It was formerly held that posttetanic potentiation was the result of hyperpolarization of the presynaptic terminals produced by repetitive activity. Increased membrane potential was thought to result in increased transmitter release. The two phenomena, however, can be dissociated, and it appears that they are not causally related (Krnjevic and Eccles, 1959 a and b; Martin and Pilar, 1964; Gage and Hubbard, 1966). Manipulation of the primary afferent depolarization and presynaptic inhibition, by physiological and pharmacological means, has shown that they also can be dissociated (Miyahara, 1966). From an intensive investigation in the cat spinal cord, Miyahara concluded that the primary afferent depolarization, like the IPSP, is a secondary change, reflecting a primary action of the presynaptic inhibitory transmitter on the membrane conductance of the terminals. The change in conductance causes a "short circuit" of the excitatory impulse that leads to a reduction in the amount of excitatory transmitter released.

The operation of an electrogenic sodium pump was first investigated in the crayfish stretch receptor (Nakajima and Takahashi, 1966). In this preparation, tetanic activity is followed by a prolonged hyperpolarization (the posttetanic hyperpolarization or PTH) which is not accompanied by a change in the conductance of the postsynaptic membrane. It was shown that the intracellular accumulation of large amounts of sodium during repetitive activity activates an electrogenic sodium pump which generates the prolonged hyperpolarization. The pump is sensitive to the intracellular concentration of

sodium and the external concentration of potassium. It is blocked by ouabain. There is now good evidence that the PTH of snail neurons, mammalian C fibers, and dorsal spinocerebellar tract cells, are due to the operation of an electrogenic sodium pump (Rang and Ritchie, 1968; Gorman and Marmor, 1970; Kuno et al., 1970).

That the electrogenic pump, like changes in membrane conductance, could be synaptically activated, was first suggested by some studies on the bullfrog sympathetic ganglion (Koketsu and Nishi, 1967; Nishi and Koketsu, 1968). The P wave of curarized ganglia is now considered to be a slow IPSP (SIPSP), generated by a sodium pump, and pharmacological evidence suggests that it is elicited by a dopaminergic synapse (see Libet, 1970). Pinsker and Kandel (1969) have described a similar prolonged inhibitory response to synaptic activation of abdominal ganglion cells of Aplysia californica. They showed that its characteristics are those usually attributed to the operation of an electrogenic sodium pump. However, Kehoe and Ascher (1970), on reexamining the evidence, concluded that the SIPSP is due to a conductance change occurring at a site remote from the soma of the postsynaptic cell. Anatomical evidence supports this conclusion. These authors suggest that the criteria used to indicate the presence of synaptically activated sodium pumps are insufficient to exclude the possibility of remote conductance changes.

Inhibition due to the operation of a synaptically activated electrogenic pump has not been demonstrated in the central nervous system.

Inhibition in the Spinal Cord

Animals are equipped to detect and interpret changes in their environment, and they respond to these changes by appropriately modifying their motor activity. The spinal motoneurons receive a complex inhibitory and excitatory input from supraspinal centers which achieves coordinated movement through subtly changing the level of motoneuron excitability. But the characteristic patterns of reflex responses are inherent in the spinal circuits themselves, and the operation of inhibition at the spinal level provides a rudimentary integration of reflex activity.

Sherrington (1906) described reciprocal inhibition as follows: "where two muscles would antagonize each other's action the reflex arc, instead of activating merely one of the two, when it activates the one causes depression of the activity of the other." An example of the operation of this simple principle in the spinal cord is the inhibitory action exerted by spindle afferents on motoneurons of antagonistic muscle groups (Lloyd, 1941, 1946 a and b). Because of its brief central latency, Lloyd called the inhibition "direct" and assumed a monosynaptic pathway. However, accurate measurements indicate that the delay for direct inhibition is consistently longer than that for monosynaptic excitation (Eccles et al., 1956 b), and Eccles has suggested that the inhibitory pathway is disynaptic. A strong argument in favour of the interpolation of an interneuron in the direct inhibitory pathway is provided by Dale's principle, which states that all terminals of a single neuron release the same transmitter. Since the Ia afferent terminals are known to release an excitatory trans-

mitter at the motoneuron, the inhibitory interneuron is postulated to act as a commutator, achieving the transfer from excitatory to inhibitory transmitter. This argument ignores the possibility of specialization of the postsynaptic membrane, and there has been some controversy about the presence of the interpolated interneuron (see Hunt and Perl, 1960). However, Eccles and colleagues (1960) located interneurons with the required characteristics, and recent work has provided convincing physiological evidence for their existence (Hultborn et al., 1971; Ryall and Piercey, 1971).

Bradley and colleagues (1953) have shown that suppression of the reflex response is the result of a brief postsynaptic inhibition of motoneurons. Although inhibition lasts about ten milliseconds, Curtis and Eccles (1959), by resolving the direct IPSP into its active and passive components, have obtained an estimate of two milliseconds for the duration of inhibitory current flow. Inhibition is due to a dual action. The flow of inhibitory current clamps the motoneuron membrane, and during the first two milliseconds inhibition is very intense. The decay of the IPSP, however, is determined by the passive electrical properties of the postsynaptic membrane and inhibits only by virtue of its hyperpolarization. At the equilibrium potential for the inhibitory process, only the inhibitory conductance change itself is effective, and the time course of inhibition becomes very brief (Araki et al., 1960).

Direct inhibition suppresses the response of the motoneuron to any excitatory stimulus, regardless of its origin. Inhibition of this type would be most effectively achieved by inhibitory synapses

at or near the site of impulse generation. Because direct IPSP are always associated with conductance changes detectable at the soma (Smith et al., 1967), and interact with EPSP in a nonlinear way (Burke et al., 1971), the direct inhibitory synapses are probably located exclusively on the soma of motoneurons. However, Kuno and Llinas (1970) have obtained some evidence for a distribution of direct inhibitory synapses on the proximal dendrites. It has been suggested that direct inhibition is a process which normally works at its maximum, because it cannot be increased by tetanization, lowered temperature, or any known drug (Weakly et al., 1968). However, Kruglov (1964) has reported that morphine (5-10 mg/kg) increases direct inhibition of the monosynaptic reflex, and his results have recently been confirmed (Satoh and Takagi, 1971).

Inhibition of the monosynaptic reflex by antidromic impulses in the ventral roots was first demonstrated by Renshaw (1941). He speculated that the motor axons exerted inhibitory effects, via their recurrent collaterals, either directly on motoneurons or through an interneuron. Renshaw (1946 a) obtained extracellular recordings from interneurons in the ventral horn which responded to antidromic volleys with prolonged, high frequency bursts of firing. The experiments of Eccles and his colleagues (1954) have established that motor axon collaterals activate these interneurons, which, in turn, inhibit the motoneurons. This negative feedback loop is known as the recurrent inhibitory pathway and the interneurons have been named "Renshaw cells" in honour of their discoverer.

Intracellular recordings from spinal motoneurons have shown

that recurrent inhibition is of the postsynaptic type. It is usually manifested by the appearance of an IPSP, which is sensitive to the intracellular concentration of chloride ions and has the same reversal potential as the direct IPSP (Coombs et al., 1955 b; Araki et al., 1961). The time course, and shape of the recurrent IPSP is a reflection of the prolonged, repetitive synaptic activity that evokes it. Recently Burke and coworkers (1971) have suggested that the recurrent inhibitory synapses are situated on the proximal dendrites because recurrent IPSP are less sensitive than direct IPSP to changes in internal chloride concentration, and interact with EPSP in a more linear way. This small difference in location does not confer the ability to selectively inhibit different excitatory inputs.

Recurrent inhibition probably achieves several types of motor integration. Its prominence between the motoneurons supplying a single muscle suggests a role in the focusing of motor output (see Wilson, 1964). Because recurrent control is more developed in tonic than phasic motor systems, Eccles and colleagues (1961 b) proposed that it stops the activity of slow muscles when rapid movement is necessary, and Granit and Rutledge (1960) have investigated the interaction of recurrent inhibition with the "surplus excitation" that maintains the repetitive firing of tonic motoneurons.

Another level of spinal integrative activity has been disclosed by studies on the inhibitory interneuron of the recurrent pathway. In addition to their main excitatory innervation from the recurrent collaterals, the activity of Renshaw cells is regulated by a variety of inhibitory inputs (see Wilson, 1966). Renshaw cells can be

inhibited by cutaneous or muscle stimulation and recurrent facilitation has been shown to be a "disinhibition" of motoneurons, due to the inhibitory effects of Renshaw cells on other Renshaw cells (Wilson et al., 1962, 1964).

Motoneurons are also postsynaptically inhibited when the tendon organs of the muscles they innervate are activated (Eccles et al., 1957). Autogenic inhibition functions to protect the muscle from excessive tension. In addition, the excitability of all motoneurons is constantly influenced by polysynaptic propriospinal and supraspinal inhibitory systems.

The presence of presynaptic inhibition in the spinal cord was demonstrated by Eccles and Sherrington (1931) who showed that stimulation of nerves from ipsilateral flexors could inhibit orthodromic responses of extensor motoneurons, without influencing their excitability. Their results have been verified, with increasing technical sophistication, by Renshaw (1946 b) and Frank and Fuortes (1957). Because the morphology of motoneurons allows two possible interpretations of the observed depression of EPSP without a concomitant change in membrane conductance or excitability, detectable at the soma, the existence of presynaptic inhibition was controversial. Kellerth (1968) was able to elicit dendritic IPSP with the required time course by stimulation of the presynaptic inhibitory pathway. He suggested that presynaptic inhibition was an artifact, due to the inability of recording electrodes in the motoneuron soma to detect "remote" conductance changes occurring on the dendrites. However, Cook and Cangiano (1970), using Kellerth's technique to detect remote IPSP, showed that

EPSP can indeed be depressed without modifying motoneuron excitability. The long-lasting inhibition elicited by flexor dendritic afferents seems to be exerted by a combination of presynaptic and postsynaptic actions. The term "remote" will be used to describe this mixed inhibitory input in order to distinguish it from true presynaptic inhibition. There is indirect evidence that presynaptic inhibition also operates at the primary afferent synapses on Clarke's column cells, and on relay cells of the dorsal column nuclei (Eccles et al., 1963 a; Andersen et al., 1964).

Remote inhibition is characterized by a long central latency, a slow increase to maximum, and a prolonged decay. It is therefore assumed to be exerted by repetitive activity of the final interneuron of a polysynaptic pathway. Remote inhibition displays spatial and temporal summation and, unlike direct postsynaptic inhibition, can be potentiated by tetanization and reduced temperature (see Eccles, 1964; Miyahara, 1966).

The operation of presynaptic and dendritic postsynaptic inhibitions provides a mechanism by which excitation from different sources can be selectively controlled. In this way the central nervous system can edit the information it receives, and it is significant that there is evidence for the operation of cortically induced presynaptic inhibition at every primary afferent synapse that has been studied.

Pharmacology of spinal inhibition

The relatively simple organization of the spinal cord has

allowed the demonstration of the amazing degree of specificity with which certain drugs can block spinal inhibitions. Since the experiments of Magendie in 1819, and Marshall Hall in 1833, it has been known that the striking effects of these agents are due to their action on the spinal cord, and more recent studies have shown that the unique convulsion pattern they produce can be explained on the basis of their action at certain well defined central synapses (see Esplin and Zablocka-Esplin, 1969). This type of information is not available for any other class of centrally acting drugs.

The prototype of all drugs which block spinal postsynaptic inhibition is strychnine. Its dramatic convulsant action attracted the attention of early reflexologists, and by the beginning of the nineteenth century the spinal cord was identified as its site of action. Michael Ferrier was the first to note that during strychnine tetanus, all the skeletal muscles are thrown into contraction, the peculiar pattern of the convulsion being due to the dominance of the extensor muscles over the flexors (see Lauder-Brunton, 1888). Sherrington (1905) suggested that the drug blocked the reciprocal inhibition which maintains the proper relation between antagonistic muscles, and Owen and Sherrington (1911) showed that this type of inhibition is reversed by strychnine. Although the anti-inhibitory effect of the drug was known to physiologists working at the beginning of the twentieth century, the review by Dusser de Barenne (1933) clearly shows that they were inclined to emphasize a direct effect of strychnine on the excitability of central and peripheral nervous tissues, as the mechanism of its convulsant action.

The advent of intracellular recording permitted the demonstration of the selective action of strychnine on inhibitory processes. Anti-inhibitory doses of strychnine have no effect on the membrane potential, after hyperpolarization, or critical firing level of spinal motoneurons (Kuno, 1957; Fuortes and Nelson, 1963). Although strychnine enhances polysynaptic responses, and sensitivity to sensory stimuli, its lack of effect on the amplitude of the monosynaptic reflex (Bernhard et al., 1951), the monosynaptic EPSP (Curtis, 1962), the activity of inhibitory interneurons excited by primary afferent fibers (Curtis, 1959), and on presynaptic inhibition (Eccles et al., 1963 b), make an action of the drug on primary afferent terminals unlikely.

Bradley and colleagues (1953), when they demonstrated the selective action of strychnine on direct inhibition of the spinal monosynaptic reflex, proposed that the drug was acting as a competitive antagonist of the inhibitory transmitter. This suggestion was widely accepted and is the basis for the use of strychnine as an aid in the identification of spinal inhibitory transmitters. However, it is now recognized that the available evidence cannot exclude an action of strychnine on release of the inhibitory transmitter. Recently, Kuno and Weakly (in press) have demonstrated quantal release of the inhibitory transmitter, and their experiments on the effect of strychnine on the release process indicate that the drug acts predominantly at a postsynaptic site. Several attempts have been made to determine if strychnine could be acting directly on the membrane conductance change responsible for inhibition, instead of competing with the in-

hibitory transmitter (see Araki, 1965; Pollen and Ajmone-Marsan, 1965). Considering the presence of strychnine resistant postsynaptic inhibitions in the brain and spinal cord (Kellerth and Szumski, 1965; Krnjevic et al., 1966 a and b), and assuming that all postsynaptic inhibitions share the same ionic mechanism, this possibility seems unlikely.

Several drugs with actions similar to strychnine have been described. Brucein, thebaine (Longo and Chiavarelli, 1962), tetanus toxin (Brooks et al., 1957), pilocarpine (Zablocka and Esplin, 1963; Esplin and Zablocka, 1964), and the alkaloid coniine (Sampson et al., 1966) have been shown to block spinal inhibition. Longo and Chiavarelli (1962) have studied the structure-activity relationship of a series of synthetic and naturally occurring agents which show anti-inhibitory activity in their test system. Several potent anti-inhibitory drugs had two features in common: they contained a piperidine nucleus and were structurally related to certain analgesics (e.g., thebaine differs from morphine in only two methyl groups). Differences in the mechanism of action of these drugs can only be revealed by detailed comparison with the known actions of strychnine. The only drug for which such a comparison has been made is coniine. In contrast to strychnine, coniine has a direct action on the electrical properties of the postsynaptic membrane (Sampson, 1966). It is significant that pretreatment with coniine lowers the threshold for strychnine-induced convulsions in mice and cats (Sampson et al., 1966). In similar tests, thebaine and strychnine are less than additive, although the actions of thebaine itself resemble those of strychnine very

closely (Laskey and Zablocka-Esplin, 1971). One other drug, 2-phenyl-imidazo- [1,2-a] pyrimidine hydrobromide, has been reported to antagonize strychnine seizures in low doses, but to possess strychnine-like activity at high doses (Collins et al., 1967). The drug also elevates the threshold for electroshock seizures, a type of convulsion which does not involve a selective block of inhibition, which indicates the possibility of a non-specific interaction between it and strychnine.

Spinal inhibitions are readily diminished by a variety of drugs, but attempts to increase them, either pharmacologically or physiologically, have been surprisingly unsuccessful. Eserine has been shown to prolong the evoked responses of Renshaw cells and the duration of the recurrent IPSP is concomitantly increased, but there is no convincing evidence that the maximum level of recurrent inhibition is altered (Eccles et al., 1954). Kruglov (1964) has reported that morphine (5-10 mg/kg) enhances direct inhibition in decerebrate cats, and his results were recently confirmed (Satoh and Takagi, 1971). Both eserine and morphine, in the doses used in these studies, can also depress the spinal monosynaptic reflex, and the effect of morphine on inhibition depends on the degree of integrity of the neuraxis.

The postsynaptic inhibition exerted by various spinal pathways, and the remote inhibition exerted by flexor afferents, are pharmacologically distinct. For this reason, it has been suggested that they are mediated by different transmitters, but the possibility of receptor differentiation cannot be dismissed. Remote inhibition, both pre and postsynaptic, is strychnine resistant (Eccles et al.,

1963 b; Kellerth and Szumski, 1965). It is reduced by picrotoxin, a drug which is inactive at strychnine sensitive synapses. The only drug which has been found to block both postsynaptic and remote inhibition is tetanus toxin (Sverdlov and Alekseeva, 1966). The mechanism and site of action of this bacterial toxin is unknown, and a recent study on the Mauthner cell of the goldfish indicates that the strychnine sensitive inhibitions of this preparation are resistant to tetanus toxin (Diamond and Mellanby, 1971). In contrast to postsynaptic inhibition, several depressant drugs have been shown to increase presynaptic inhibition (Miyahara et al., 1966).

Since the study of Lanari and Luco (1939), it has been known that strychnine blocks ganglionic and neuromuscular transmission. Neuromuscular blockade is due to a "curare-like" action of the drug (Alving, 1961). A blocking action of tetanus toxin at cholinergic synapses has also been demonstrated (Ambache et al., 1948 a and b; Kaeser and Saner, 1969), and a short investigation by Ferguson and colleagues (1970) showed that the major anti-inhibitory drugs all interfere with ganglionic and neuromuscular transmission. In addition, Esplin and Zablocka (1964) have shown that the well-known muscarinic agent, pilocarpine, blocks spinal inhibition, and these authors have suggested that the spinal inhibitory pathways contain a "cholinergic link". Recently, Koelle (1969) has summarized the evidence for a cholinergic link in several central inhibitory pathways. Most of the central cholinergic actions which have been described are of the muscarinic type, and atropine effectively blocks the effects of physostigmine and neostigmine in the spinal cord (Bülbring and Burn, 1941).

Although the excitatory effects of pilocarpine are blocked by atropine (Krnjevic and Phyllis, 1963), the anti-inhibitory effects of much larger doses of this muscarinic agent are unaffected, suggesting that the drug is not acting at a typical muscarinic receptor. The effects of anticholinergic agents have not been studied on the direct inhibitory pathway of the spinal cord.

METHODS

A. Intact Mice.

Median effective doses were determined in male Swiss mice, weighing 18-28 grams. All drugs were administered intraperitoneally in volumes up to 1 ml/20g mouse. When two drugs were administered simultaneously, they were mixed in the syringe and the total volume was kept within the same limit. Each animal was kept in a separate compartment and background noises were minimized to avoid triggering convulsions. General observations on the condition of the animal, and the pattern of convulsion were made without handling the mice because they were hypersensitive to tactile stimuli. The time to convulsion was recorded for each mouse.

Experiments were performed on two different groups of mice; the first group being used several months before the second. The median effective doses of thebaine and strychnine were determined in both groups. With the exception of thebaine, all drugs were dissolved in 0.9% saline. Mice in the first group were injected with a suspension of thebaine in 0.9% saline. The second group received the hydrochloride salt of the drug, neutralized with Trizma buffer (see p.47). The effective dose of thebaine was the same in both groups, but solubilizing the drug reduced the latency of convulsions.

When the interaction of thebaine and strychnine was studied, the administration of the drugs was timed so that their peak effects coincided. Thebaine as a suspension was injected five minutes before

strychnine, and the hydrochloride was administered simultaneously.

Nalorphine (25 mg/kg) produced little overt effect on the behaviour of Swiss mice. When combined with 50 mg/kg thebaine, however, the mice exhibited catatonia, their spontaneous movements were entirely suppressed, and they vocalized when touched lightly. Reducing the dose of nalorphine to 10 mg/kg alleviated the catatonia and vocalizing but spontaneous movements were still absent. Piloerection, depressed respiration, and ptosis were pronounced. These symptoms were qualitatively similar to those observed after lower doses of thebaine, and were probably not due to the small amount of nalorphine. Pretreatment of mice receiving strychnine with this dose of nalorphine failed to alter their behaviour. Both nalorphine and naloxone were administered five minutes before injecting strychnine and thebaine.

The method and apparatus described by Woodbury and Davenport (1952) were used to determine the threshold for electroshock seizures (EST) in mice. Clonus of the jaw and forelimbs was taken as the endpoint, and the "staircase method" (Finney, 1952) was used to determine the current delivered to each animal. One half of the CD_{50} of strychnine or thebaine was administered five minutes before the determination of the EST.

All data on effective doses in mice were analyzed by the method of Litchfield and Wilcoxon (1949).

B. Spinal Cats.

The Spinal Preparation

The animals used in these experiments were adult cats (2.5-3.5 kg) of either sex. Initially, ether was the only anesthetic used, but in the majority of experiments, anesthesia was induced with ethylchloride and maintained with ether while the trachea was cannulated, both carotid arteries were ligated, and the spinal cord was severed at the atlanto-occipital junction. The anesthetic was discontinued and the spinal animal was artificially respired. Anemic decerebration was achieved by occluding the vertebral arteries with a clamp. The absence of pinna, corneal, and pupillary reflexes, at a time when the action of the anesthetic must have been terminated, indicated that this method of decerebration was effective. The left brachial vein was cannulated to provide a route for drug injection, and a cannula was placed in the right carotid artery.

A laminectomy was then performed to expose the spinal cord from the insertion of the fifth lumbar dorsal root (DR-L_5) to the entry zone of the first sacral root (S_1). Two metal pins and a clamp were used to fix the animal to a rigid frame by the hips and the spinous process of the most cephalad exposed vertebra. The skin of the back was reflected to form a "pool" which was filled with warm mineral oil, and an incision, extending for the length of the laminectomy, was made in the dura mater above the midline of the spinal cord. When intracellular recordings were to be made, respiratory movements were reduced by performing a bilateral pneumothorax, and the spinal

cord was firmly held in position by four metal bars which were pressed against the remaining vertebral fragments on both sides of the cord, and fixed to the rigid frame. The small ligaments attaching the spinal cord to the dura mater were severed, and a section of the dura was isolated by transverse cuts above the insertion of the L_6 dorsal root and the entry zone of the L_7 root. The spinal cord was then rotated by gently pulling away this section of dura and pinning it to the back muscles. Rotation of the spinal cord changes the angle at which microelectrodes approach the ventral horn, and reduces the depth of tissue which must be penetrated before reaching the region where the motoneurons are located. Before the microelectrodes were inserted into the cord, the pia mater was also removed. With the aid of a dissecting microscope, small holes were cut in this membrane, using a Dumont forceps and the tip of a very fine, curved, surgical needle, held by a hemostat. These holes were made between the surface blood vessels to avoid bleeding.

Arterial blood pressure was monitored via a cannula in the carotid artery, which was connected to a Tycos direct reading pressure gauge. The average blood pressure of these spinal animals was 50-70 mm Hg. Occasionally, dextran (6% in isotonic saline) was administered to maintain the blood pressure above 40 mm Hg. Once the preparation had stabilized, spontaneous variations in the blood pressure were normally within 5 mm Hg, but the injection of drugs often caused large, transient changes in blood pressure.

Temperature was monitored by a tele-thermometer, whose probe was placed under the back muscles. The metal board, on which the ani-

mal was mounted, was heated by a strip of heating tape placed under it, and the temperature of the preparation was adjusted with a heat lamp. Temperature was maintained at 35° C for reflex studies and 37° C for intracellular experiments. Fluctuations were kept within ± 0.5° C during the recording period.

All animals were immobilized with gallamine triethiodide (2-5 mg/kg) and paralysis was maintained with additional injections when necessary.

Stimulating and Recording Procedures

Stimuli were delivered to spinal roots or peripheral nerves through bipolar platinum wire electrodes, which were mounted on vertical supports so that they could be easily positioned. The quadriceps nerve, because of its ventral position, was stimulated by a buried electrode. The nerve was dissected away from the surrounding muscle, it was severed, and its central end was drawn through a plastic sleeve electrode. The nerve was firmly tied to the electrode, the site of the dissection was covered with warm mineral oil, and the electrode was fixed in the appropriate position by suturing it to the muscles surrounding the nerve. The stimuli were square, monophasic pulses of 50 microseconds duration, delivered singly or in trains, by a pair of synchronized stimulators, through two isolation transformers.

Motoneurons were stimulated intracellularly by using a Wheatstone bridge circuit similar to the one used by Araki and Otani (1955) and Frank and Fuortes (1956), to pass current through the re-

cording micropipette. A schematic diagram of the bridge circuit used appears in Figure 1. The current which was passed through the microelectrode was measured across the 500 megohm resistor and the unity-gain preamplifier, and monitored on one beam of the oscilloscope.

For recording reflex responses, the whole preparation was grounded through the metal animal board. Reflex responses were recorded monophasically with platinum wire electrodes. Input recordings were made with one arm of the electrode in contact with the surface of the dorsal roots, and the other in contact with the exposed muscles of the back. The recorded signals were fed directly into a Tektronix type 2A61, AC coupled amplifier, and displayed on one beam of a Tektronix model RM565 dual beam oscilloscope.

For intracellular recording, the preparation was isolated from ground and an indifferent silver-silver chloride electrode was buried in the neck muscles. Glass microelectrodes were inserted into a White Plains Instruments model EH-IR electrode holder, filled with the appropriate concentrated salt solution. The holder was attached to the cylinder of a hydraulic microdrive, and the cylinder itself was mounted on a micromanipulator, which was firmly fixed to the metal animal board. The manipulator was used to position the microelectrode in the horizontal plane, and the microdrive was used for vertical displacement. Potential changes detected by the microelectrode were fed through a unity-gain, negative-capacity preamplifier, to the two Tektronix type 3A3 differential amplifiers of the oscilloscope. A DC coupled amplifier was used to drive the oscilloscope beam on which the action potential and resting potential of the motoneurons were

displayed. An AC coupled amplifier was used to drive the other beam of the oscilloscope, on which the synaptic potentials or after potentials of the motoneurons were displayed. In those experiments in which the resistance or critical firing level of motoneurons was being measured, this beam was used to display the current pulse being passed through the microelectrode, which was recorded as a DC signal.

The resistance of the microelectrodes could be tested at any time during the experiment. Square current pulses (obtained from the calibration signal of the oscilloscope) were applied across the electrode resistance, to produce a voltage change which was displayed on the oscilloscope. The electrode resistance could be determined directly from this deflection by the use of a known calibration factor. A variable DC voltage (supplied by a 1.4 V mercury battery) was used to cancel out the electrochemical potentials which developed at the various junctions between dissimilar chemical substances in the recording system. An additional calibrated potentiometer was used to measure the membrane resting potential by equalizing the DC shift that occurred on penetration of the motoneuron.

Extracellular recordings of the activity of Renshaw cells were made with tungsten wire electrodes (mounted in a jeweller's chuck) or with glass micropipettes, filled with sodium chloride.

The main oscilloscope was wired in parallel with a "slave" cathode-ray tube. Permanent records were made on Kodak photographic paper, by photographing the responses displayed on the screen of the "slave" cathode-ray tube with a Grass kymograph camera. In most experiments, the responses were also relayed to a Mnemotron model 400B

computer of average transients (CAT), which was used with accessory unit 600. The computer summed a preset number of consecutive oscilloscope sweeps, and the sum was recorded by a Clevite mark 250 X-Y plotter (see Figures 2 and 3). In addition, all responses were relayed to a speaker so that they could be monitored aurally as well as visually.

Glass microelectrodes were made on a Kopff model 700C vertical pipette puller. Because the motoneurons were approached from the dorsal surface of the cord, it was necessary to penetrate several millimeters of tissue before reaching them. To reduce tissue damage, the electrodes were made with the tapered portion or shank at least 15 mm long. Very stable impalements were obtained with these electrodes. However, the conditions used to produce this long taper resulted in electrodes with very fine tips, and electrical resistances greater than 50 megohms. The electrodes were boiled in the appropriate concentrated salt solution, under vacuum, at a temperature of 80^{o} C. Potassium citrate, potassium chloride, and sodium chloride electrodes were used. The citrate electrodes were filled with 1.7 M potassium citrate, adjusted to pH 8 with hydrochloric acid. Boiling in citrate etched the tips of the microelectrodes, and if they were boiled for a suitable length of time (one hour), their resistance was reduced to 8-12 megohms, and no further manipulations were necessary. Potassium chloride (3 M), and sodium chloride (4 M) electrodes had very high resistances even when filled, and their tips were broken by gently abrading them with very fine sandpaper. All electrodes were examined under a microscope after this procedure, to make certain that the

reading obtained for the electrical resistance was not an artifact, resulting from the occlusion of an unsuitably large tip with debris. The sodium chloride electrodes used for extracellular recordings were broken to a resistance of 5 megohms or less, while the potassium chloride electrodes which were used had resistances of 5-10 megohms.

In a few experiments, the activity of single Renshaw cells was recorded extracellularly with tungsten microelectrodes, made by the method of Hubel (1957).

Experimental Design

1. Afferent Drive.

The relation of afferent drive to inhibition was determined as follows. Afferent drive was reduced by stepwise decreases in the intensity of the testing stimulus, and ten to twenty test responses were recorded at each level of afferent drive. The average amplitude of these responses was expressed as a percentage of the response to a stimulus of twice the intensity required to elicit the maximum MSR. The average amplitude of the test response, conditioned by a stimulus of constant intensity to the appropriate inhibitory pathway, was also determined at each level of afferent drive. The amplitude of the inhibited response, expressed as a percentage of the corresponding test response, was used to indicate the degree of inhibition. A control relation (in the absence of drug) was constructed by plotting the degree of inhibition against the level of afferent drive at which it was obtained (see Figure 5). All lines in these graphs represent regression lines computed from the experimental values.

To establish a control relation for increases in afferent drive, a tetanus (500/sec for 20 sec) was used to induce posttetanic potentiation (PTP) in the test pathway. The decline of PTP from its maximum was followed by eliciting test and inhibited responses alternately at a rate of one every two seconds. The amplitudes of the five test responses, recorded during consecutive 20 second time periods, were averaged and expressed as a percentage of the average value of the test response at the maximum of PTP. The degree of inhibition was obtained by comparing the average values of test and conditioned responses for corresponding 20 second periods, and a control relation was constructed as described above. Control relations were determined in several experiments and the average slope of the relation was computed from the slopes of the individual regression lines.

This value of the slope was used to correct for the effect of changes in afferent drive on the degree of inhibition observed after drug administration. The amplitude of the test response was determined before and after drug administration. The larger value was arbitrarily designated 100% and the smaller value was expressed as a percentage of the larger. The difference between these two numbers represented the change in afferent drive caused by the drug. The change in the degree of inhibition that could be expected from such a change in afferent drive is given by the product of the slope of the control relation and the change in drive (see sample calculation on p. 49). The corrected change in the degree of inhibition is given by the observed level minus the level predicted by the above calculation. This method of correcting for changes in afferent drive was used for the

direct and recurrent inhibitory pathways. It is only valid if the relation between inhibition and drive remains the same after drug administration. A less arbitrary approach to the problem would be to determine the whole relation between inhibition and drive before and after the administration of the drug. The studies on remote inhibition were designed in this manner, and drug induced changes in the degree of inhibition were determined at the same level of afferent drive in all four experiments.

2. Inhibition.

After the appropriate surgical preparation, reflex responses were elicited and the parameters of stimulation adjusted as follows: stimuli used to elicit the test MSR were twice the intensity required to produce the maximum monosynaptic response, and stimuli to the inhibitory pathway were of an intensity which gave the maximum inhibition without causing too much facilitation at the longer intervals between conditioning and test stimuli. The motoneuron pool was conditioned by a stimulus to the inhibitory pathway, and its response to a test stimulus, delivered at various intervals after the conditioning stimulus, was recorded. In this way the entire time course of the inhibitory process was explored. Ten to twenty consecutive responses, elicited every two seconds, were averaged at each interval tested, and the amplitude of the conditioned response was expressed as a percentage of the unconditioned value. These values were plotted against the interval between the conditioning and test stimuli, to obtain an "inhibitory curve" (see Figure 8). When necessary, the test

MSR was prorated to correct for any changes in its amplitude observed during the testing period, and the inhibited responses were expressed as a percentage of this prorated value.

The following pathways were used to display the various types of inhibition studied. Direct inhibition of the hamstring motornucleus was produced by stimuli to the quadriceps nerve (Q). The hamstring nerve, which supplies the synergistic muscle group of anterior and posterior biceps (AB and PB), semimembranosus (SM), and semitendinosus (ST), was stimulated and the monosynaptic response of its motornucleus was recorded at the ventral roots of the first sacral (S_1) and seventh lumbar (L_7) segments. Direct inhibition of the MSR of the first sacral segment (DR-VR S_1) was produced by stimulation of the sixth lumbar dorsal root.

Recurrent inhibition was displayed in the following manner. The test response was elicited by a stimulus to the cut L_7 and S_1 dorsal roots, and recorded from the peripheral nerve to one member of a synergistic muscle group. The inhibitory stimulus was delivered to the peripheral nerves of the remaining members of the muscle group. The hamstring or triceps surae (TS) muscle groups were used. TS is an ankle extensor consisting of the median gastrocnemius (MG), and the combined lateral gastrocnemius and soleus (LGS).

Polysynaptic inhibition of the test MSR, elicited by stimulation of the TS nerve, and recorded at the L_7 and S_1 ventral roots, was produced by stimuli to cutaneous afferents of the sural (S) or peroneal (P) nerves.

Remote inhibition of the same test reflex was elicited by

stimuli to the hamstring nerve. Single stimuli were used instead of trains of pulses, because thebaine facilitated and prolonged the initial polysynaptic excitatory effect of the conditioning stimulus. When trains of stimuli were used, this effect of the drug caused a change in the level of excitation on which the inhibitory effects exerted at shorter conditioning intervals were superimposed. This problem could be avoided by using a single stimulus to elicit inhibition. The rather short time course of remote inhibition seen in these experiments (see Figure 15) is probably due to the use of this method of eliciting inhibition.

3. Intracellular studies.

Stimulation of a peripheral nerve will only give rise to synaptic potentials in a certain population of motoneurons. For example, stimuli to the Ia afferents of a particular muscle produce monosynaptic EPSP in its own motoneurons, and direct IPSP in the motoneurons of its antagonists. For this reason only those motoneurons innervating identified muscles were studied in these experiments. All spinal roots were left intact, and the motoneurons supplying a given muscle nerve (or synergistic muscle group) were fired by a stimulus to the appropriate muscle nerve. Microelectrodes, filled with potassium citrate, were advanced slowly into the spinal cord, and extracellular field potentials were used to locate the firing motoneurons. Penetration of the desired cells was signalled by the sudden appearance of the membrane resting potential and the antidromic action potential. Reducing the intensity of the stimulus to the peripheral

nerve usually revealed a monosynaptic EPSP, and activation of the appropriate inhibitory pathway produced an IPSP. Penetrated motoneurons were allowed to stabilize for at least five minutes before control recordings of synaptic, action, and resting potentials commenced. Synaptic potentials were recorded on film and by the computer of average transients.

In those experiments in which the properties of the motoneuron membrane were studied, potassium chloride electrodes with a resistance of 5-10 megohms were used. Both dorsal and ventral roots of the L_7 and S_1 segments were severed, and stimuli to the ventral roots produced the antidromic action potentials which identified the motoneurons. Membrane resistance and excitability were measured by the indirect methods described by Frank and Fuortes (1956). Current pulses of 8.5 milliseconds duration were applied intracellularly through the recording electrode, by using the Wheatstone bridge circuit shown in Figure 1.

4. Drug interactions.

The actions of strychnine and thebaine resemble each other closely, and their dose response lines are parallel when determined in intact mice. Accordingly, the method developed by Finney (1952) for planning a test of similar action, was used to design an experiment to investigate the interaction of the two drugs. Finney defines similar action as follows: "In a mixture whose constituents act similarly any constituent can be replaced by a proportionate amount of any other, without disturbing the potency...." In other words, when

the two drugs are mixed, their joint action is exactly equal to the sum of the actions they would exert if given separately (with appropriate corrections for differences in potency). Because "additive" is a less ambiguous way of describing this type of interaction, it has been substituted for Finney's term "similar". The planned test for additive action requires that the effect of a constant ratio mixture of the two drugs be experimentally determined. The ratio in which the drugs are mixed is chosen to produce a shift of the dose response line of the mixture, whose extent can be predicted on the assumption that the drugs are additive. The experimentally determined shift is compared to the predicted value, and the drugs can be assumed to act additively if the two values are not significantly different. Shifts of less than the predicted extent indicate that the drugs interact infra-additively. The predicted shift is determined from the following equation:

$$\log(\pi + \rho[1 - \pi]) = \theta \log \rho$$

The two drugs are mixed in the ratio of $\pi:(1 - \pi)$, ρ is their potency ratio, and θ the expected shift of the dose response line of the mixture.

Drugs Used

All drugs were administered intravenously, through a cannula in the left brachial vein. Thebaine alkaloid (S.B. Penick and Company) was dissolved in 0.9% saline acidified with hydrochloric acid (0.03 meq HCl/ml). The pH of the drug solution was adjusted to

7.4 with 400 mM Tris (Hydroxymethyl) Aminomethane (Fisher Scientific Company). A thebaine solution of 1 mg/ml was used in all experiments.

The duration of action of thebaine in spinal cats was determined by using a modification of the method described by Toman and colleagues (1948) and Borys and Esplin (1969). It was assumed that the concentration of thebaine at its site of action declines as a first order process, and the half-life of the drug can be calculated from a semilogarithmic plot of the amount of drug present against time. Spontaneous activity of spinal motoneurons was recorded at a ventral root, and a dose of thebaine, just sufficient to produce sustained convulsive activity, was administered. The preparation was left until the convulsive activity ceased, and sufficient thebaine was re-administered to attain the initial endpoint. This quantity of drug represented the amount which had disappeared from the site of action in the time which had elapsed since the first dose was administered. Values of 1.75 hours and 1.08 hours were obtained for the half-life of thebaine in two preparations. It was therefore assumed that doses of the drug administered within half an hour were cumulative.

Strychnine sulphate (Mallinckrodt), eserine sulphate (Nutritional Biochemicals), naloxone hydrochloride (Endo Laboratories) and nalorphine hydrochloride (Merck and Company) were all dissolved in 0.9% saline solution. Gallamine triethiodide (Poulenc Limited), supplied as a 20 mg/ml solution, was used in every experiment to immobilize the spinal animals.

Sample Calculations

A. Correction of the level of inhibition for changes in amplitude of the MSR.

1. Raw data:

	Amplitude in arbitrary units	
	MSR	Conditioned MSR
Control	183	37
Thebaine 1.45 mg/kg	292	189

2. Relative values:

The largest value of the test MSR is taken as 100% and the smaller test MSR is expressed as a percentage of the larger (183/292 x 100%). Both conditioned MSR are expressed as percentages of the corresponding test MSR.

	Relative test MSR (%)	Conditioned MSR as (%) test MSR
Control	63	20
Thebaine 1.45 mg/kg	100	65

3. Change in the relative MSR = 100-63 = + 37% (the + sign indicates that the drug increased the MSR).

4. Decrease in degree of inhibition expected from the change in MSR = slope of control relation x change in relative MSR

= 0.717 x 37%

= 26.5%

5. Expected level of inhibition after thebaine

= initial level + expected change

= 20% + 26.5%

= 46.5%

6. Observed degree of inhibition

= 65%

7. Actual change in inhibition

= observed degree - expected degree

= 65% - 46.5%

= 18.5%

B. Calculation of the observed shift of the dose response line by Finney's method.

1. Distance that dose response line actually moves

= log CD_{50} of drug A - log CD_{50} mixture

= log 1.08 - log 0.52 mg/kg

2. Total distance between the dose response lines of the two components of the mixture

   = log of the potency ratio

   = log 30

3. Shift (θ) expressed as a fraction of the distance between the two dose response lines

   =(log 1.08 mg/kg - log 0.52 mg/kg) / log 30

   = 0.21

RESULTS

A. Experiments in Intact Mice.

Convulsant doses of thebaine have a pronounced effect on the behaviour of intact mice. Their spontaneous exploratory movements cease, they huddle in a corner of the cage, their respiration becomes slow and deep, and they exhibit piloerection and ptosis. Paradoxically, these animals are also extraordinarily sensitive to auditory and tactile stimuli, responding to them with a short period of running and violent trembling. Animals which do not convulse are visibly affected for about 30 minutes and appear to have recovered completely within an hour. Those mice which do convulse often "hop" and make jerking movements, seemingly caused by brief, uncontrollable extension of the limbs. Pure extensor convulsions develop about five minutes after injection of the drug. The convulsions are less explosive than those caused by strychnine, and a few mice survive them. The LD_{50} of thebaine is therefore slightly higher than the CD_{50} (see Table 1) and was found to be 20.0 (18.2-22.0) mg/kg in the first group of mice and 21.0 (19.6-22.5) mg/kg in the second group. The dose-response curve of thebaine is parallel to that of strychnine and the potency ratio of the two drugs is 20:1.

The CD_{50} of three combinations of strychnine and thebaine are presented in Table 1. Assuming that two drugs, A and B, are additive if $0.5\ CD_{50}A + 0.5\ CD_{50}B = CD_{50}(A+B)$, it can be seen that thebaine and strychnine are less than additive in two of the combinations

TABLE 1: Determination of median convulsant dose (CD_{50}) in mice.

PRETREATMENT (mg/kg)		CD_{50} (mg/kg) AND 95% CONFIDENCE LIMITS	
		THEBAINE	STRYCHNINE
GROUP I:			
None		19.5 (18.8-20.3)	1.00 (0.96-1.05)
Thebaine	10.0	-	0.86 (0.80-0.92)
Nalorphine	10.0	44.0 (40.4-48.0)	1.20 (1.14-1.26)
GROUP II:			
None		19.5 (16.8-21.5)	1.05 (1.01-1.09)
Thebaine	14.0	-	0.32 (0.28-0.36)
Strychnine	1.0	8.00 (7.34-8.72)	-
Naloxone	20.0	22.0 (20.8-23.3)	-

TABLE 2: Analysis of the interaction of thebaine and strychnine in mice.

A.

FRACTION OF CD_{50} OF EACH COMPONENT PRESENT IN MIXTURE		SUM OF FRACTIONS OF CD_{50} PRESENT AT CD_{50} OF MIXTURE
STRYCHNINE	THEBAINE	
0.95	0.40	1.35
0.86	0.50	1.36
0.32	0.72	1.04

B. As analysed by Finney's method.

RATIO OF STRYCHNINE TO THEBAINE	EXPECTED CD_{50} (mg/kg)	ACTUAL CD_{50} OF MIXTURE (mg/kg)
11:89	5.9	9.0
8:92	8.0	10.9
3:97	14.3	14.3

tested, and just additive in the third (Table 2A). If Finney's method is used to analyse for additive action (see p. 46) the same results are obtained (Table 2B).

Because thebaine is chemically related to morphine, its interaction with the narcotic antagonists nalorphine and naloxone was studied (see Table 1). Pretreatment with 10 mg/kg of nalorphine caused a parallel shift to the right of the dose response curve. This dose of nalorphine also antagonized strychnine convulsions, but to a lesser degree. Although the pretreatment had no noticeable effect on the behaviour of the mice, it enhanced the effects of thebaine on their activity. Naloxone, a narcotic antagonist whose action is minimally complicated by agonist effects, did not antagonize the convulsant action of thebaine, nor did it enhance thebaine's effects on the behaviour of the mice.

Thebaine (10 mg/kg) had no effect on electroshock threshold (EST) in mice; control and treated groups having thresholds of 7.4 (6.98-7.84) mA and 7.0(6.73-7.28) mA respectively (95% confidence limits in brackets).

B. Experiments in Spinal Cats.

Afferent Drive

In the course of the experiments on inhibition, it was noticed that thebaine generally increased the fraction of the motoneuron pool firing in response to the testing volley (see Figure 4). Activation of an inhibitory input confines a fraction of the motoneurons

TABLE 3: Control relations between afferent drive and inhibition for direct, recurrent, and remote inhibition.

TYPE OF INHIBITION	METHOD	NO. OF EXPTS.	SLOPE OF CONTROL RELATION (±SE OF MEAN)
Direct	PTP	4	0.72(±0.09)
	Decreasing test stimulus	6	0.44(±0.06)*
Recurrent	PTP	6	0.44(±0.08)
	Decreasing test stimulus	6	0.46(±0.08)
Remote	PTP	4	0.40(±0.09)
	Decreasing test stimulus	4	0.40 - **

* From Weakly et al., 1968.

** From Miyahara, 1966.

in the discharge zone to the subliminal fringe. The size of this fraction is inversely proportional to the level of excitation in the pool, and, in reflex studies, an increase in the response of the motoneuron pool to the test stimulus results in an apparent decrease in the level of inhibition (Weakly et al., 1968). The change in the level of inhibition caused by an anti-inhibitory drug that increases the amplitude of the test response will therefore have two components, and this factor must be considered when the effect of such a drug on inhibition is studied. In order to correct for changes in afferent drive caused by thebaine, a control relation between afferent drive and the degree of inhibition of the MSR was determined when large fractions of the motoneuron pool were firing in response to the test stimulus. Posttetanic potentiation (PTP) in response to a tetanus (20 sec at 500/sec) was used to increase the amplitude of the MSR, and a control relation was constructed as described on p. 42). The average slopes of the regression lines obtained for direct, recurrent, and remote inhibitory pathways, are presented in Table 3.

Afferent drive can also be altered by decreasing the strength of the testing stimulus. This approach was used in six experiments on the recurrent pathway. Direct and presynaptic pathways have been studied in this way by other investigators, and the values they obtained are included in Table 3. It is apparent that only in the case of direct inhibition is there a difference in the values obtained with the two techniques. Since thebaine increases the MSR, the control relation obtained by following the decay of PTP was used to calculate the actual

changes it produced in direct and recurrent inhibition. The average value of the slope of the regression line representing the relation between afferent drive and inhibition, for the particular pathway being studied, was used to determine the degree of inhibition that would be expected for the level of afferent drive that was observed after the administration of thebaine (see sample calculation on p. 49). The corrected change in inhibition represents the difference between the expected degree of inhibition, and the degree of inhibition actually observed after the administration of thebaine.

In a few experiments, the relation between afferent drive and degree of inhibition was redetermined in the presence of a drug. Figure 5 shows the effects of three drugs on direct inhibition and its relation to afferent drive, in two different experiments. Eserine is without effect on either parameter, while both thebaine and strychnine (after eserine) diminish inhibition at all levels of afferent drive tested. Similar information for the recurrent pathway is presented in Figure 6. Eserine and strychnine were studied on both recurrent and direct inhibition in the same animal. For both pathways, some change in the slope of the relation between afferent drive and degree of inhibition was noted, but the number of experiments of this type was too small to determine if these differences were statistically significant. If treatment with a drug alters the relation between afferent drive and inhibition, as well as the effectiveness of inhibition, the actual change in inhibition cannot be determined by measuring the level of inhibition before and after the treatment and using the slope of the control relation to correct for changes in drive. Instead, the

whole relation must be determined in both control and treated situations, and changes in inhibition assessed at a specified level of afferent drive. Presynaptic inhibition can be expected to have a complex relation with alterations in afferent drive produced by PTP, because it is exerted on the transmitter release mechanism of the afferent terminals. However, in all four experiments on remote inhibition, the control relation showed no sign of nonlinearity between 100% and 30% pool firing (see Figure 7), and the average slope was identical to the slope Miyahara obtained by reducing the response of the motoneuron population (see Table 3). The effect of thebaine on the degree of inhibition was assessed at levels of afferent drive between 100% and 30% pool firing in four experiments. A typical experiment is presented in Figure 7. The average slope and standard error of the regression lines determined in the presence of the drug was 0.20 ($\pm$0.08). This value is not significantly different from the control slope of 0.40 ($\pm$0.09) ($P = 0.3$ using a t-test for paired data), but a definite statement cannot be made on the basis of this small sample.

For this reason, corrections for the effect of changes in afferent drive on the degree of remote inhibition were not made by multiplying by the slope of the control relation, as described for the direct and recurrent pathways. Instead, the entire relation between afferent drive and inhibition was redetermined in the presence of an anti-inhibitory dose of thebaine, as shown in Figure 7, and the change in inhibition was assessed at the same level of afferent drive in all four experiments. This level was chosen in the following manner: the amplitude of the control MSR was expressed as a percentage

of the maximum amplitude attained during PTP, and this value was averaged for the four experiments. The change in the degree of inhibition caused by thebaine was then determined from the regression lines of the two relations, at this average level of drive (which was 34% of the PTP maximum).

Complex interactions with afferent drive may also be predicted for the recurrent pathway, because it exerts a negative feedback on the motoneurons which are responsible for its activation. Such a complex relation has been reported (Kuno, 1959; Granit et al., 1960), but no evidence for it was found in this study. Control relations obtained by both methods had similar average slopes and displayed no sign of nonlinearity.

The direct inhibitory pathway was the simplest used in this study and the different value of the control relation obtained by the PTP method was unexpected. It was not possible to assess the significance of this difference statistically, as the sample size was small in both determinations (see Table 3).

Inhibition

The effect of thebaine on direct inhibition of the spinal monosynaptic reflex (MSR) was studied in 6 unanesthetized spinal cats. In each of these experiments, the time course of inhibition was followed by varying the interval between conditioning and testing stimuli. Figure 8 presents a typical inhibitory curve obtained in this manner. Thebaine (0.78 and 1.45 mg/kg) reduced the maximum level of inhibition and uncovered the delayed facilitatory effect of Ib affer-

TABLE 4: The effect of thebaine on direct inhibition of the monosynaptic reflex.

PATHWAY	THEBAINE (mg/kg)	COND./TEST (%) CONTROL	DRUG	CORRECTED CHANGE IN INHIBITION (%)
Q-BST	1.25	14	53	-48
"	1.10	29	60	-16
"	0.80	2	54	-27
"	1.45	20	65	-18
DR-VR	1.00	3.3	81	-37
"	0.87	24	106	-82

ents, a result which was obtained consistently. In no experiment was it possible to completely block inhibition with a subconvulsive dose of thebaine.

The results of the six experiments are summarized in Table 4. Inhibition was measured at its maximum, and, in this and all other tables, the size of the conditioned response as a percent of the test response is used to express the degree of inhibition. Changes in the level of inhibition are computed from the relative size of the conditioned response before and after drug administration, and are therefore expressed as percents (see column 5, Table 4). These numbers represent differences only, and must not be interpreted as "percent change". Because thebaine generally increases the amplitude of the test response, the values in column 5 of Table 4 represent the change in inhibition, corrected for any changes in afferent drive that occurred in the course of the experiment (see sample calculation on p. 49).

The suppression of the monosynaptic reflex described above is a result of the postsynaptic inhibition of spinal motoneurons (Bradley et al., 1953). Activation of inhibitory synapses gives rise to inhibitory postsynaptic potentials (IPSP) in these neurons. Figure 9 shows the direct IPSP of a biceps motoneuron, produced by a stimulus to the quadriceps nerve. Intravenous administration of incremental doses of thebaine successively reduced the amplitude of this IPSP to 45% of its control value. Table 5 summarizes the effect of thebaine on the four direct IPSP studied. The reduction in IPSP amplitude is not accompanied by a consistent change in membrane potential.

Motoneurons are also postsynaptically inhibited when Renshaw

TABLE 5: The effect of thebaine on direct IPSP of hamstring motoneurons, elicited by stimulation of the quadriceps nerve. Increases in membrane potential (V_m) are indicated by +.

CELL	THEBAINE (mg/kg)	IPSP AMPLITUDE AS % CONTROL	CHANGE IN V_m (mV)
1	0.80	35	-7
2	0.50	70	+2
3	0.50	84	+4
	1.00	29	
4	0.25	91	0
	0.50	69	
	0.75	51	
	1.00	45	

cells are activated by recurrent collaterals of motor axons (Eccles et al., 1954). The action of thebaine on recurrent inhibition of the spinal monosynaptic reflex was studied in six cats. Figure 10 presents a typical inhibitory curve, and shows that thebaine (1.03 mg/kg) reduced the maximum level of inhibition. However, as shown in Table 6, when all six experiments are considered, relatively large doses of thebaine produced surprisingly small changes in inhibition. The table also shows that the maximum depression of the monosynaptic test response produced by antidromic conditioning is less than that which can be obtained by direct inhibition. The maximum possible reduction in inhibition, measured as the difference between control and treated values, is therefore limited. Even if it were possible to administer a subconvulsive dose of thebaine which would completely block recurrent inhibition, the maximum possible change in inhibition would be no more than 30% in most experiments.

Figure 11 shows the recurrent IPSP of an SM motoneuron produced by antidromic stimulation of the BST nerve. Intravenous administration of incremental doses of thebaine successively reduced the amplitude of this IPSP without changing the membrane potential of the cell. A delayed recurrent facilitatory potential (RFP) was also present, and was unaffected by thebaine (see values for SM cell in Table 7).

Because an increase in resting membrane potential will reduce the size of the recurrent IPSP, the hyperpolarization of the two PB motoneurons (see Table 7) which occurred during the course of these experiments probably contributed to the observed depression of the

TABLE 6: The effect of thebaine on recurrent inhibition of the monosynaptic reflex.

PATHWAY	THEBAINE (mg/kg)	COND./TEST (%) CONTROL	COND./TEST (%) DRUG	CORRECTED CHANGE IN INHIBITION (%)
MG-LG	1.6	60	77	+15
"	1.03	32	82	-29
"	2.00	45	82	-26
"	1.83	62	81	-12
"	1.0	72	87	-3

IPSP. However, significant hyperpolarizations did not occur until pentobarbital was administered, and it can be seen that thebaine (0.80 mg/kg) reduced both the recurrent IPSP and RFP in the second cell, at a time when the motoneuron was depolarized by 5 mV.

As noted in Table 7, small quantities of pentobarbital were administered intravenously in two of the experiments, to reduce the high level of background synaptic excitation resulting from the initial dose of thebaine. These doses of the barbiturate did not alter the amplitude of the recurrent IPSP in these two cells.

Since thebaine has been shown to block neuromuscular and ganglionic transmission, the possibility that it depresses recurrent inhibition by an action at the cholinergic synapses on Renshaw cells was investigated. Extracellular recordings of the repetitive response of single Renshaw cells to stimulation of a ventral root were made with metal or glass microelectrodes. The inset in Figure 10 shows the response of a Renshaw cell, recorded in the presence of an amount of thebaine which was sufficient to block recurrent inhibition in the same cat (see inhibitory curves of Figure 10). The results of six experiments, presented in Table 8, show that an anti-inhibitory dose of thebaine (1 mg/kg) was without effect on responses of Renshaw cells evoked by stimulation of the ventral roots. The response of these cells to stimulation of the dorsal roots was too inconsistent to be useful in determining the effect of drugs on firing rate or latency of the first response. Thebaine (2 mg/kg) was without effect on either orthodromic or antidromic responses in the single experiment in which they were both studied. Figure 12 shows the results obtained in a

TABLE 7: The effect of thebaine on recurrent IPSP and recurrent facilitatory potentials (RFP) of hamstring motoneurons. Increases in membrane potential (V_m) are indicated by +.

TYPE OF CELL	THEBAINE (mg/kg)	PENTOBARB. (mg/kg)	IPSP AMPL. AS % CONTROL	RFP AMPL. AS % CONTROL	CHANGE IN V_m (mV)
PB	0.87	-	68	-64	+4
	0.87	3.26	75	-	-
	2.17	3.26	20	68	-
	2.17	5.40	39	18	+13
PB	0.80	-	46	68	-5
	0.80	2.0	46	66	-
	1.20	2.0	44	45	-
	1.20	6.0	50	45	+3
SM	0.50	-	65	114	+1
	1.00	-	54	117	-
	1.50	-	46	107	-
	2.00	-	46	86	-
	2.50	-	41	96	-

typical experiment. Thebaine (1.5 mg/kg) did not affect the initial rate of firing or the total number of action potentials in the response of a Renshaw cell, firing in response to stimulation of the L_7 ventral root.

In these unanesthetized preparations, higher doses of thebaine always elicited intermittent bursts of spontaneous activity, during which the Renshaw cells failed to respond to the ventral root stimulus. This "silent period" usually terminated a few seconds after the convulsive discharges had stopped. It appeared that these interruptions in the responses of the Renshaw cells were due to the increased neuronal activity during the convulsive burst, rather than a direct action of thebaine on the excitation of Renshaw cells. To test this hypothesis, small doses of pentobarbital (less than 10 mg/kg) were administered intravenously, when enough thebaine had been given to increase the spontaneous activity. In each preparation, only enough barbiturate was given to suppress the convulsive discharges. When the bursts were eliminated, so were the silent periods in the responses of the Renshaw cells. Because pentobarbital itself depresses Renshaw cells, if given in sufficiently high doses (Eccles et al., 1956), it is unlikely that any action of the barbiturate was responsible for abolishing the silent period. The small quantities of pentobarbital used to suppress convulsive discharges did not affect the amplitude of recurrent IPSP (see Table 7). When the convulsive discharges were suppressed, doses of thebaine up to 3 mg/kg had no significant effect on the evoked responses of the six Renshaw cells studied (see Table 8).

TABLE 8: The effect of thebaine on the responses of Renshaw cells, evoked by stimuli to the ventral roots of L_7 and S_1.

	NO. OF ACTION POTENTIALS IN THE EVOKED RESPONSE MEASURED IN THE FIRST 45 msec. AFTER THE STIMULUS			
THEBAINE (mg/kg)	0	1.0	2.0	3.0
PENTOBARBITAL	0	0	+	+
CELL 1	10	9.4	10	8.8
2	18	18.8	18	17.4
3	8.6	7.7	9.5	8.3
4	13.6	12.8	12.2	12.8
5	9.0	8.7	7.6	-
6	19	19	17.2	18.8

These experiments on the recurrent pathway indicate that the postsynaptic inhibition of motoneurons by Renshaw cells is blocked by thebaine. However, the data obtained on recurrent facilitation are inconclusive.

The effect of thebaine on polysynaptic inhibition of the monosynaptic reflex by impulses in cutaneous afferents was studied on three motoneuron pools in two cats. Test responses of the triceps surae motoneuron pool were conditioned by impulses to either sural or peroneal nerves. Figure 13 shows that, at short conditioning intervals, the drug converts inhibition of the monosynaptic response to facilitation. The intracellular recordings in Figure 14 show similar changes in the polysynaptic IPSP of two unidentified motoneurons, evoked by impulses in the Q and BST nerves (two different experiments).

Excitation of low threshold flexor afferents elicits a "remote" inhibition of motoneurons, composed of a mixture of pre and postsynaptic effects. Both components of this inhibitory input have been shown to be strychnine insensitive. Figure 15 shows the action of thebaine on remote inhibition of the MSR. The drug reduced the maximum inhibition attained, but its effects were not uniform at the various intervals tested, probably because of the polysynaptic nature of this pathway. Similar results were obtained in five experiments. Table 9 summarizes the results of four experiments on remote inhibition. The values for the degree of inhibition before and after the administration of thebaine were determined at the same level of afferent drive, by the method described on p. 43. It can be seen that, in every experiment, subconvulsive doses of thebaine reduced the maximum

TABLE 9: The effect of thebaine on remote inhibition of the monosynaptic reflex. The degree of inhibition before and after drug is expressed at the same level of afferent drive.

PATHWAY	THEBAINE (mg/kg)	COND./TEST (%)	
		CONTROL	DRUG
BST-TS	1.00	20	42
"	1.52	11	22
"	1.50	20	43
"	2.00	7	20

degree of remote inhibition.

Properties of the Motoneuron Membrane

The observed effects of thebaine on synaptic potentials could be due to an action of the drug on membrane resting potential, resistance, or ionic permeability. Alterations in the excitability of the postsynaptic membrane could increase synaptic efficacy. Fortunately, these parameters can be easily measured in spinal motoneurons and their modification by thebaine was studied with the following results. From the data in Table 10 it is apparent that the drug produced no significant change in the membrane resting potential (Vm) of the motoneurons studied.

When direct information on the permeability of the motoneuron membrane to a certain ionic species is not available, the amplitude of the after hyperpolarization (AHP) can be used as an indicator of the permeability of the membrane to potassium ions (Coombs et al., 1955a; Eccles et al., 1966). It is apparent from the values in Table 10 that thebaine had no significant depressant action on the amplitude of the AHP in the seven motoneurons that were studied. Therefore, it is unlikely that the drug reduces the amplitude of the IPSP by an action on the permeability of the postsynaptic membrane to potassium ions.

Because the AHP of motoneurons is often contaminated by recurrent IPSP, a small reduction of its amplitude by thebaine can be expected. The effect of thebaine on the AHP of an SM motoneuron is shown in Figure 16. The recurrent IPSP produced in this neuron by

TABLE 10: The effect of thebaine on the properties of the postsynaptic membrane: resting membrane potential (V_m), action potential, and afterhyperpolarization (AHP).

THEBAINE (mg/kg)	V_m (mV)		ACTION POTENTIAL (mV)		AHP AS %
	CONTROL	DRUG	CONTROL	DRUG	CONTROL
0.80	73	60	116	48	
0.50	82	84	100	73	112
1.00	68	72	112	98	
1.00	74	74	108	82	
2.00	78	79	78	86	117
0.87	53	57	86	79	
0.80	80	75	88	80	98
2.00	75	76	74	44	96
0.77	65	70	-	-	
2.00	78	79	-	-	
2.00	84	86	98	99	88
1.00	77	72	85	70	107
1.20	75	75	-	-	

volleys in the BST nerve was reduced to 46% of control by the doses of thebaine used (see Figure 11), and the membrane resting potential increased by 1 mV. The AHP was reduced to 96% of its control value.

Further information on the permeability of the resting membrane was obtained by studying the effect of thebaine on its resistance. The indirect method of Frank and Fuortes (1956) was used to measure the membrane resistance of the three motoneurons. The average resistance of these motoneurons (0.86 megohms) was altered to 96% of its control value by the administration of thebaine.

Thebaine did reduce the maximum depolarization attained by antidromically evoked action potentials, in eight of the ten motoneurons studied (see Table 10).

The excitability of the motoneuron membrane was measured in three experiments. Motoneurons were stimulated with current pulses delivered through the recording electrode, and a strength latency curve was constructed, as described on p. 46. Figure 17 shows the increase in the excitability of an L_7 motoneuron produced by 2.00 mg/kg of thebaine. This change was not due to depolarization of the motoneuron, whose membrane potential was increased by 2 mV. Similar results were obtained in the three neurons that were studied.

Drug Interactions

Because combinations of strychnine and thebaine were unexpectedly infra-additive in intact mice, an attempt was made to study the interaction of these two drugs on direct inhibition in the spinal cord. Figure 18 shows a preliminary experiment which suggested that

the drugs were infra-additive. When the control inhibitory curve had been established, the animal was pretreated with a sub-effective dose of thebaine (0.44 mg/kg). 0.047 mg/kg of strychnine were then administered intravenously, and the inhibitory curve was redetermined in the presence of both drugs. It can be seen that this dose of strychnine, which should have had an anti-inhibitory effect (see Table 11), was not able to block inhibition in the presence of the small dose of thebaine. This experiment certainly suggests that the two drugs are also infra-additive in the cat spinal cord, but the large variation of the effective dose of both drugs in the spinal preparation (see Tables 4 and 11) made it difficult to obtain any definite information from this type of experiment.

It was necessary to find a method which would detect infra-addition even though strychnine lowered the effective dose of thebaine (as it did in the mice), without requiring the use of a large number of animals. The method of Finney (see p. 46) fulfills these conditions. It requires that the two drugs to be studied have parallel dose response curves and that their potency ratio be known. In order to determine the potency ratio, the anti-inhibitory dose of strychnine was determined on direct inhibition in four experiments (see Table 11), and compared to the anti-inhibitory dose of thebaine obtained from the results in Table 4. Because the individual responses obtained during the course of the experiment must be measured, averaged, expressed as percents, and corrected for changes in afferent drive, before the actual change in inhibition produced by a given dose of drug is known, it is very difficult to choose a particular degree of inhi-

TABLE 11: The effect of strychnine on direct inhibition of the monosynaptic reflex.

PATHWAY	STRYCHNINE (µg/kg)	COND./TEST (%)		CORRECTED CHANGE IN INHIBITION (%)
		CONTROL	DRUG	
Q-BST	37	62	113	-60
"	32	17	47	-8
"	50	0	44	-62
"	25	25	68	-60

bitory blockade as an endpoint for all experiments. An additional complication is the drug induced increase in variability and spontaneous activity, which often makes it impossible to accurately determine the anti-inhibitory effects of higher doses of convulsant drugs. The results of these experiments were therefore expressed as the average dose required to produce an average effect. For example, using the data in Table 11, strychnine, 0.036 mg/kg, produced an average decrease in inhibition of 47.5%. Similarly, using the data in Table 4, thebaine, 1.08 mg/kg, produced an average decrease in inhibition of 38%. The potency ratio of the two drugs is therefore greater than 30.

To study their interaction, thebaine and strychnine were combined in a ratio of 82:12 (by weight), and the average anti-inhibitory dose of the mixture, expressed as the number of milligrams of both components per kilogram, was determined in six animals. From the data in Table 12, an average dose of 0.52 mg/kg of the mixture produced an average change in inhibition of 43.7%, a value which is within 10% of the change produced by strychnine and within 15% of the change produced by thebaine. The observed value of theta, the shift of the dose response line of the mixture, expressed as a fraction of the distance between the log-dose response lines of its components, was 0.21. Because the doses used to calculate this value of theta did not all cause the same degree of effect, it is only an approximation. Assuming that the two drugs were additive, the minimum predicted shift for this combination is 0.55. It was therefore concluded that combinations of strychnine and thebaine have infra-additive effects on direct spinal inhibition.

TABLE 12: The effect of an 18:82 mixture of strychnine and thebaine on direct inhibition of the monosynaptic reflex.

PATHWAY	TOTAL DRUG IN MIXTURE (mg/kg)	COND./TEST (%)		CORRECTED CHANGE IN INHIBITION (%)
		CONTROL	DRUG	
Q-BST	0.60	32	61	-5
"	0.32	17	78	-100
"	0.63	10	81	-53
"	0.58	28	78	-50
"	0.42	9	46	-25
"	0.60	0	44	-29

Because narcotic antagonists do not interfere with the excitatory actions of narcotic analgesics, the antagonism by nalorphine of thebaine convulsions in intact mice was an unexpected finding. To see if this antagonism was exerted at the spinal level, the effect of nalorphine on the anti-inhibitory action of thebaine was investigated in seven spinal cats. In two experiments, nalorphine (5 and 10 mg/kg) was without effect on direct inhibition or the test MSR, and thebaine, administered after nalorphine, exerted its usual anti-inhibitory effects in doses of 1.0 and 0.8 mg/kg. In three other experiments, one of which is illustrated in Figure 19, nalorphine administered after an anti-inhibitory dose of thebaine had no discernable effect on spinal reflex transmission. Similar results were obtained in single experiments on the recurrent and polysynaptic inhibitory pathways. The dose range of nalorphine in these experiments was 2.0 - 10.0 mg/kg.

Because all anti-inhibitory drugs that have been tested block peripheral cholinergic junctions and synapses, the possibility of a cholinergic link in spinal inhibitory transmission has been raised (see p. 31 for references). Agents which block the action of acetylcholinesterase usually have a biphasic effect on transmission across cholinergic synapses. Small doses of these drugs enhance transmission, while larger doses produce an initial enhancement followed by a depolarization blockade. If typical cholinergic transmission is involved in the spinal inhibitory pathways, eserine should have a predictable effect on direct inhibition of the MSR. The recurrent inhibitory pathway containes a well studied cholinergic synapse, so recurrent inhibition was studied at the same time as direct inhibition to

provide an indicator of the effectiveness of the doses of eserine that were used.

The effect of eserine on spinal inhibition was studied in eight experiments. As shown in Figure 5a, eserine (1 mg/kg) was without effect on direct inhibition of the MSR at all levels of afferent drive that were tested. Figure 6a shows that the dose of eserine that was used was sufficient to block recurrent inhibition, recorded in the same animal. In the presence of this high dose of eserine, strychnine exerted its usual anti-inhibitory effect. Similar results were obtained in the other four experiments in which both recurrent and direct inhibition were monitored. Thebaine also effectively blocks direct inhibition in the presence of eserine. Inhibition in either the recurrent or direct pathway, which had been diminished by thebaine or strychnine, could not be restored by doses of eserine as high as 2.00 mg/kg. These data are consistent with the lack of effect of anti-inhibitory doses of strychnine and thebaine on antidromically evoked responses of Renshaw cells. However, 0.33 mg/kg of eserine, a dose within the range reported by Eccles and colleagues (1954) to potentiate the activation of Renshaw cells effectively, blocked recurrent inhibition in most of these preparations, and usually, a further 1 mg/kg of the drug did not augment the blockade. At no time was an increase in inhibition seen, if correction was made for the depressant action of eserine on the MSR.

Excitatory Transmission

Administration of twice the anti-inhibitory dose of thebaine

to spinal cats produces sustained spontaneous activity of spinal motoneurons, detectable at the ventral roots. Convulsive discharges occur in bursts, recurring with a characteristic period of about one every three minutes. The typical "waves" of activity observed during these episodes are shown in Figure 20. Lower doses of the drug cause an increase in the background synaptic activity, and even elicit intermittent bursts of spontaneous firing, but the dose must be increased to obtain sustained discharges. The convulsant dose determined in seven animals was 1.94 (±0.18) mg/kg (S.E. of the mean).

Subconvulsive doses of anti-inhibitory drugs characteristically enhance transmission through the polysynaptic excitatory pathways terminating on motoneurons, without affecting the amplitude of the monosynaptic reflex (see p. 28 for references). In contrast, thebaine consistently enhances transmission through both mono and polysynaptic spinal pathways (see Figure 1). Increases in MSR amplitude ranging from 20 - 300% of control values were noted in 10 of 13 consecutive experiments. Since the increased amplitude reflects recruitment of neurons in the subliminal fringe of the motoneuron pool, the effect of the drug will depend on the fraction of the pool which fires in response to the testing stimulus. A preparation in which the subliminal fringe is large, will show a dramatic increase in MSR for a relatively small change in synaptic efficacy, and conversely, even a pronounced enhancement of transmission will fail to affect the MSR of a preparation in which most of the motoneuron pool fires in response to the test stimulus.

In any spinal monosynaptic pathway, there are a definite

number of motoneurons which receive a synaptic input from the afferents activated by the test stimulus. Some of these motoneurons will fire in response to the stimulus, but a certain proportion of them will only be subliminally excited. Motoneurons in the subliminal fringe can be recruited into the firing zone by increasing the efficacy of synaptic transmission. In these experiments, synaptic efficacy was increased by applying a train of stimuli (500/sec for 20 sec) to the afferent nerves, to induce posttetanic potentiation of the MSR. The number of motoneurons that could be recruited by an increase in synaptic efficacy was estimated by determining the PTP ratio. This parameter expresses the amplitude of the MSR at the maximum of PTP as a multiple of the test MSR amplitude.

The PTP ratio of six motoneuron pools was determined before and after the administration of thebaine. The experiments were performed in four animals. The results presented in Table 13 show that thebaine has a pronounced effect on MSR amplitude in those preparations whose relatively large PTP ratios indicated the presence of a substantial number of motoneurons which could be recruited by increasing the efficacy of synaptic transmission.

A change in the response of the postsynaptic neuron to a stimulus of constant intensity is an indication that the efficacy of synaptic transmission has changed. The increase in MSR amplitude caused by thebaine is not due to a concomitant increase in the intensity of the afferent input, measured at the dorsal root (see Figure 4). Therefore, thebaine must enhance the efficacy of transmission at the primary afferent synapses of motoneurons. The increase in the excita-

TABLE 13: The effect of thebaine on monosynaptic spinal reflexes.

THEBAINE (mg/kg)	MOTONEURON POOL	PTP RATIO		MSR AS % CONTROL	PTP MAXIMUM AS % CONTROL
		CONTROL	DRUG		
1.00	TS	8.9	3.6	214	99
	SMST	1.6	1.2	90	66
1.50	TS	4.0	2.1	192	103
2.50	BSTSM	2.4	2.6	116	92
2.00	SMST	2.5	2.4	91.8	89
	TS	12.7	3.0	660	155

bility of the motoneuron membrane caused by thebaine (see Figure 17) is probably sufficient to account for the observed enhancement of synaptic transmission.

The monosynaptic EPSP of motoneurons was studied because an effect of the drug on the intensity of synaptic input (which would be reflected by the apmlitude of the EPSP) could also be involved in its action on the efficacy of synaptic transmission. In order to correlate the effects of thebaine on excitation and inhibition, both EPSP and IPSP were recorded in the same motoneuron. The results presented in Table 14 show that anti-inhibitory doses of thebaine reduce the amplitude of monosynaptic EPSP. In one cell (not included in Table 14), the EPSP disappeared immediately after thebaine was administered, but could be restored by increasing the intensity of the test stimulus. Figure 21 shows the effect of incremental doses of thebaine on the monosynaptic EPSP of a hamstring motoneuron. After 0.50 mg/kg of thebaine, the EPSP was transiently reduced, but recovered 88% of its initial amplitude, although the dose of thebaine was increased and the IPSP recorded in the same cell (see Figure 9) remained depressed. In three of the four cells in which both types of synaptic potentials were studied, thebaine depressed IPSP to a greater extent than EPSP. The fourth cell was lost a few minutes after the second drug injection and it was not possible to determine if the depression of the EPSP (to 65% of its initial value) was transient. Changes in the amplitude of synaptic potentials were accompanied by reductions in the antidromic action potentials of motoneurons, but their membrane resting potentials were not significantly altered (see Table 14).

TABLE 14: The effect of thebaine on monosynaptic excitatory postsynaptic potentials (EPSP) of motoneurons. In four neurons, inhibitory postsynaptic potentials (IPSP), antidromic action potentials (AP) were also recorded. Increases in membrane resting potential (V_m) are indicated with a +.

TYPE OF MOTONEURON	THEBAINE (mg/kg)	AMPLITUDE AS % CONTROL EPSP	IPSP	AP	CHANGE IN V_m (mV)
Hamstring	0.77	84	-	-	0
* "	0.50	116	70	73	+2
"	0.50	65	84	88	+4
		-	29	-	-
** "	0.50	42	69	76	0
	1.00	89	45		
L_7	2.00	88	43	110	0

* The synaptic potentials of this motoneuron are shown in Figures 2 and 3.

** The synaptic potentials of this motoneuron are shown in Figures 5 and 15.

From these results, it appears that thebaine changes the efficacy of monosynaptic excitatory transmission by increasing the excitability of the motoneuron. This effect on excitability is sufficient to maintain the excitation of cells that were previously in the discharge zone, in spite of a reduction in the amplitude of their EPSP, and even permits the recruitment of cells in the subliminal fringe. In some motoneuron pools the balance between the two effects of the drug results in a decrease in the efficacy of transmission, which is reflected in a reduced MSR amplitude and PTP maximum (see Table 13).

DISCUSSION

The similarity between the actions of thebaine and strychnine was well known to early pharmacologists (see Lauder-Brunton, 1888), who were disappointed by the low therapeutic potential of this opium alkaloid. A great variety of drugs are convulsants in toxic doses, but only those agents which interfere selectively with inhibitory transmission in the spinal cord produce convulsions without an initial component of tonic flexion (see Esplin and Zablocka-Esplin, 1969). Thebaine causes pure extensor seizures when administered to intact mice, and, for this reason, it was considered likely that the mechanism of its convulsant action is similar to that of strychnine. Pinto-Corrado and Longo (1961) have reported a value of 42 mg/kg for the intraperitoneal LD_{50} of thebaine, determined in intact mice. A reproducible LD_{50} of half this value (see Table 1) was obtained in the present study. The discrepancy may be attributable to a difference in the strain of mice used, but this hypothesis could not be verified because Pinto-Corrado and Longo did not indicate the strain of mice used in their experiments. These authors also report that thebaine increased spontaneous activity, induced the Straub reaction, and caused seizures with an initial clonic phase. Of the more than two hundred mice in the present study which received convulsant doses of thebaine, or thebaine and strychnine, not one animal displayed any of the three symptoms described by these authors.

Drugs with similar mechanisms of action might be expected to interact with each other when they are administered together. The

anti-inhibitory drugs coniine and pilocarpine reduce the threshold for strychnine convulsions in mice (Zablocka and Esplin, 1963; Sampson et al., 1966). A similar interaction between thebaine and strychnine was indicated by the reduction of the strychnine CD_{50} to one third of its original value in mice pretreated with 14 mg/kg of thebaine (see Table 1). However, a closer examination of the data revealed that the two drugs are only just additive in this combination, and are actually infra-additive when the effects of other combinations are studied (see Tables 1 and 2). There are two possible explanations for this observation. Thebaine could be interfering with transmission at the spinal inhibitory synapses, but also be exerting some other action which gives rise to a physiological antagonism of the expression of the convulsant action of strychnine. If this physiologically antagonistic action is produced by doses of thebaine lower than those which block inhibitory synapses, thebaine could conceivably hinder the expression of its own convulsant action. An extreme example of such a situation is provided by the anti-inhibitory drug coniine, which produces neuromuscular blockade at a lower dose than that required to cause spinal convulsions. The major symptom of coniine poisoning is a flaccid paralysis, and its anti-inhibitory action was only revealed by electrophysiological studies on reflex transmission in the spinal cord (see Sampson et al., 1966).

An alternative explanation is that strychnine and thebaine have identical mechanisms of action at the spinal inhibitory synapses, and the observed interaction is the logical consequence of a competition between two drugs with different efficacies for the same receptor site.

Several observations made during the studies on intact mice suggested that a physiological type of antagonism might be involved. Convulsant doses of thebaine produced a decrease in the spontaneous activity of mice which was in striking contrast to the hyperexcitability so characteristic of the behaviour of mice injected with strychnine. In addition, thebaine seizures, although pure extensor in pattern, were not as "explosive" as those caused by strychnine; an effect which was reflected by the finding that the LD_{50} of thebaine is slightly greater than its CD_{50}. The suggestion that the physiological antagonism may be dose related receives some support from the observation that lower doses of thebaine interact infra-additively with strychnine, while 14 mg/kg of thebaine was just additive with strychnine.

A nonspecific anticonvulsant action of thebaine should manifest itself by increasing the threshold for electroshock seizures (EST) in mice. Electrically induced convulsions do not involve a selective blockade of inhibitory synapses, and strychnine itself has no effect on EST (Toman and Everett, 1966). The best evidence against the physiological antagonism theory is the lack of effect of thebaine on EST, in a dose which is distinctly infra-additive with strychnine. It is unlikely that any depressant effects on transmission at the spinal level can account for the infra-addition because thebaine is known to enhance reflex transmission in chronic spinal dogs (McClane and Martin, 1967).

The alternative possibility that thebaine and strychnine are competing for the same receptor site seems more attractive. To provide more evidence for this theory, the action of thebaine was

studied at the strychnine sensitive synapses of the cat spinal cord. A sufficiently detailed analysis should reveal any basic differences in the mechanism of action of the two drugs which could account for the infra-addition.

The action of thebaine on postsynaptic inhibition of the spinal monosynaptic reflex is identical to that of strychnine, described by Bradley and colleagues (1953) and Curtis (1959). Thebaine diminished direct, recurrent, and polysynaptic inhibition, but subconvulsive doses of the drug never completely abolished reflex inhibition. Intracellular recordings showed that thebaine, in similar doses, also caused a reduction of comparable magnitude in the amplitude of the IPSP of motoneurons, initiated by stimulation of the three postsynaptic inhibitory pathways that were used in the reflex studies. A drug can alter IPSP amplitude by an action on one, or a combination, of several aspects of the inhibitory process.

Since none of the inhibitory pathways that were studied are monosynaptic, it is possible that an action of thebaine on the excitation of inhibitory interneurons reduced the inhibitory synaptic input to the motoneurons and contributed to the observed depression of their IPSP. This possibility was investigated in the recurrent inhibitory pathway because thebaine is known to block peripheral cholinergic synapses and junctions (Ferguson et al., 1970), and because the inhibitory interneurons of this pathway are readily identified by their characteristic repetitive response to stimulation of the ventral roots. In doses which effectively blocked recurrent inhibition and reduced the amplitude of recurrent IPSP, thebaine did not affect the

activation of Renshaw cells. The action of strychnine on the elements of the recurrent inhibitory pathway is identical to that of thebaine (Larson, 1969).

Because thebaine usually enhances transmission at the primary afferent synapses of motoneurons, it was considered unlikely that the observed depression of direct IPSP was due to an action of the drug on the excitation of inhibitory interneurons in this pathway. However, intracellular studies indicate that both the direct IPSP and monosynaptic EPSP of motoneurons are reduced by thebaine. In the absence of more direct information, it must be assumed that part of the IPSP depression could possibly be due to a reduction in inhibitory synaptic input. Strychnine does not affect the excitation of interneurons in the direct inhibitory pathway (Curtis, 1959). Both thebaine and strychnine enhance the activity of spinal interneurons, as shown by their effect on polysynaptic reflexes, and these drugs would not be expected to reduce the activity of inhibitory interneurons selectively.

Both thebaine and strychnine act so rapidly that it is unlikely that they reduce the efficacy of inhibitory transmission by an action on the storage or synthesis of inhibitory transmitter. Techniques for the demonstration of the actions of drugs on transmitter release at central synapses have only recently been developed. Kuno and Weakly (in press) have shown that the major site of action of strychnine is postsynaptic. Although similar information for thebaine was not obtained in the present study, it is likely that the postsynaptic blockade of inhibitory transmission produced by strychnine would be enhanced by a presynaptic action of thebaine on the release of

inhibitory transmitter. For instance, hemicholinium, a drug which reduces the amount of transmitter released by affecting the synthesis of acetylcholine, enhances the blockade of the neuromuscular junction produced by curare (e.g., Capek et al., 1971).

IPSP amplitude is a reflection of the difference between the membrane resting potential and the equilibrium potential of the ions which carry the inhibitory current (E_{IPSP}). A drug induced decrease in this difference will cause a reduction in IPSP amplitude. Neither thebaine nor strychnine have a significant effect on the resting potential of motoneurons (see Fuortes and Nelson, 1963), and they could only reduce the driving force on the inhibitory ions by altering their equilibrium potential. Neither thebaine nor strychnine alter membrane resistance, which is a reflection of the general permeability of the motoneuron. They also have no effect on the equilibrium potential of the potassium ion, estimated by the amplitude of the AHP (see Fuortes and Nelson, 1963; Curtis, 1962). However, it is possible that these drugs could interfere with a recently demonstrated mechanism for the extrusion of chloride ions, which maintains the level of E_{IPSP} in spinal motoneurons (Lux, 1971). Pollen and Ajmone-Marsan (1965) have suggested that the anti-inhibitory action of strychnine is due to an alteration of E_{IPSP} by the drug. The evidence of strychnine resistant postsynaptic inhibition in spinal motoneurons (Kellerth and Szumski, 1965) argues against this possibility because it is unliekly that two different mechanisms for the maintenance of E_{IPSP} would be operating in the same cell. Also, Larson (1969) has shown that strychnine does not alter the reversal potential of recurrent IPSP. Thebaine, however, does reduce remote inhibition of the MSR. It was not determined if the drug has an action on the post-

synaptic component of this strychnine resistant inhibition, so it remains a possibility that thebaine has a direct effect on E_{IPSP} of motoneurons. It is significant that coniine, a drug which has been demonstrated to alter the E_{IPSP} by its effect on membrane permeability, interacts supra-additively with strychnine in spite of its depressant effect on excitatory transmission (Sampson, 1966; Sampson et al., 1966).

The remaining possible mechanism of anti-inhibitory action is a reduction of the conductance change induced by the interaction of the postsynaptic membrane with the inhibitory transmitter. By process of elimination, this seems the most likely mechanism of action of strychnine, and it is usually assumed that this drug affects conductance change because it is a competitive antagonist of the inhibitory transmitter. Thebaine could also be acting in this way, but the physiological evidence obtained in this study is insufficient to eliminate the two other possible mechanisms of action of the drug. Pharmacological evidence, however, indicates that strychnine and thebaine are acting at the same site. It has been established that the two drugs interact infra-additively at spinal inhibitory synapses. The results of these studies were unambiguous, in spite of the technical difficulties involved in assessing the anti-inhibitory effects quantitatively. The effective dose of strychnine, when combined with thebaine (in an 18:82 constant ratio mixture), was approximately three times the dose required to produce the same effect when strychnine was administered alone. As mentioned above, an action of thebaine on the release of inhibitory transmitter or the equilibrium potential of the

IPSP could explain the type of interaction that was observed.

When two drugs which act at the same receptor site are administered together, they compete with one another for occupancy of the receptor site. Their interaction will depend on the difference in their abilities to produce an effect when combined with the receptor. Theoretical models describing competitive interactions at a single receptor site have been developed, and their predictions have been tested experimentally with considerable success (see Ariens et al., 1964). These models are based on the idea that agonists can vary in their ability to produce a response when combined with the receptor. This property is termed "intrinsic activity", and, by definition, agonists have a maximum intrinsic activity of one, partial agonists or competitive dualists have intrinsic activities less than one but greater than zero, and competitive antagonists have intrinsic activities of zero. Because antagonists are considered to have zero intrinsic activity, it has been assumed that combinations of true antagonists would not display the type of interaction described for partial agonists or competitive dualists. However, Collins and colleagues (1967), in a study on the mechanism of action of an "unusual" strychnine antagonist, concluded that a competitive interaction between 2-phenyl-imidazo- [1,2-a] pyrimidine hydrobromide and strychnine was the most logical explanation of their results. A competitive interaction between antagonists has also been described by Stephenson and Ginsborg (1969). These authors suggest that although antagonists have equal intrinsic activities, and produce the same degree of blockade when combined with the receptor, a difference in the rate at which the drug

receptor complex dissociates could lead to a competitive dualist type of interaction between antagonists.

Strychnine and thebaine (within the limitations of the test systems used) produce the same degree of blockade, but twenty molecules of thebaine are required to produce the same effect as a single molecule of strychnine. It is conceivable that, when they are both present, thebaine and strychnine molecules compete for the same receptor site, and infra-addition results because the number of thebaine molecules present, while sufficient to hinder the access of strychnine molecules to the receptor site, are not quite sufficient to produce the equivalent degree of blockade. This proposal is supported by the finding that the mixture containing the highest proportion of thebaine (14 mg/kg) displayed a strictly additive interaction while the mixtures containing lower proportions of thebaine showed a distinctly infra-additive interaction (see Tables 2A and 2B).

Since it is unlikely that the action of thebaine on excitatory transmission or presynaptic inhibition could account for the observed interaction between strychnine and thebaine, it was concluded that they both act at a single receptor site, in a competitive way.

Because the action of thebaine on postsynaptic inhibition is so similar to that of strychnine, the effect of the drug on transmission at the primary afferent synapses of motoneurons was unexpected. The anti-inhibitory and convulsant action of thebaine might be expected to raise the excitability of the spinal reflex centers and indirectly enhance the efficacy of synaptic transmission. However, none of the other anti-inhibitory convulsants, strychnine, pilocarpine, coniine,

or tetanus toxin enhance monosynaptic transmission, and neither does pentylenetetrazol, a drug whose convulsant action is independent of a blockade of inhibition.

Thebaine increases the excitability of spinal motoneurons, an effect which could be due to a release of tonic inhibition rather than a direct action of the drug on the motoneuron membrane. Wilson and colleagues (Wilson and Burgess, 1962; Wilson et al., 1964) have suggested that the increased excitability of motoneurons which is reflected by recurrent facilitation of the MSR, is the result of a release from tonic inhibition. These authors have shown that the excitability increase produced in this way is accompanied by a depolarization of the motoneuron membrane: the recurrent facilitatory potential (RFP). Because strychnine affects neither the excitability nor the resting potential of spinal motoneurons, thebaine would have to act on a strychnine resistant tonic inhibition in order to increase excitability by disinhibition. However, since the thebaine induced increase in motoneuron excitability is not accompanied by depolarization, it is unlikely that it is the result of disinhibition.

The effect of thebaine on strychnine resistant remote inhibition might be expected to contribute to an enhancement of the efficacy of monosynaptic transmission by increasing the excitatory synaptic input to motoneurons. However, tetanus toxin, a drug which also reduces remote inhibition, has no effect on MSR amplitude (Brooks et al., 1957; Sverdlov and Alekseeva, 1966), and monosynaptic EPSP of motoneurons are depressed rather than enhanced by thebaine.

The results of this study show that thebaine has a dual

effect at primary afferent synapses of motoneurons. The drug reduces excitatory synaptic input but increases excitability of the postsynaptic neuron, and its effect on the efficacy of synaptic transmission depends on the balance between these two factors.

The depressant action of thebaine on the amplitude of the monosynaptic EPSP is similar to the action of the drug on the amplitude of antidromic action potentials of motoneurons. The amplitude of the action potential can be reduced by changing the equilibrium potential for sodium ions (E_{Na}) or impairing the permeability of the activated membrane to these ions. E_{Na} can only be altered by reducing the electrochemical gradient for sodium ions across the membrane (Hodgkin and Katz, 1949). Since thebaine does not affect membrane permeability or resting potential, it is unlikely that it acts by changing E_{Na}. Thebaine probably exerts a depressant action on the sodium conductance of the activated membrane. A similar local anesthetic action has been demonstrated for strychnine, on peripheral nerve (Heinbecker and Bartley, 1939). It is possible that the reduction in safety factor resulting from this action of thebaine on the action potential could increase the occurrence of failure of invasion of the presynaptic terminals by the action potential. The observed decrease in EPSP amplitude could be a consequence of a reduction in the number of terminals activated by the afferent stimulus, but no direct evidence for such an action of thebaine was obtained in this study. Alternatively, a reduction in the extent to which afferent terminals are depolarized during the action potential could result in a depression of the amount of transmitter released by a single terminal (see Hagiwara and Tasaki, 1958;

Takeuchi and Takeuchi, 1962).

In the spinal cord, the most extensively investigated strychnine resistant inhibition is that exerted on the MSR of extensors by the large diameter afferents of flexor muscles (see Eccles, 1964). Remote inhibition has been shown to consist of both pre and postsynaptic types of inhibition (Cook and Cangiano, 1970), but only the postsynaptic component has been demonstrated to be strychnine resistant and picrotoxin sensitive (Kellerth and Szumski, 1965). Thebaine diminishes remote inhibition of the MSR, and in this respect it resembles tetanus toxin rather than strychnine. The effect of thebaine on remote inhibition is limited, and appears to have a complex relation with the level of afferent drive (see Table 9 and Figure 7). This type of inhibition was not investigated in sufficient detail to obtain any information on the mechanism of the anti-inhibitory action of thebaine.

Narcotic antagonists do not usually interfere with the convulsant actions of narcotics (see Martin, 1967). Koppanyi and Karczmar (1953) have reported that pretreatment of mice with a wide range of doses of nalorphine does not affect the dose of thebaine required to produce convulsions. Therefore, the antagonism of thebaine convulsions by nalorphine, observed in intact mice, was unexpected. In this study, nalorphine pretreatment also slightly increased the CD_{50} of strychnine. However, nalorphine did not antagonize the effects of thebaine on transmission in the cat spinal cord. The pronounced effect of species differences on the action of narcotics may account for this discrepancy.

Nalorphine is a partial agonist at narcotic receptors, and

it is significant that naloxone, the narcotic antagonist whose action is the least contaminated by agonist effects (see Martin, 1967), was without effect on thebaine convulsions in mice.

In many areas of the central nervous system, there is a tantalizing similarity between the pharmacology of postsynaptic inhibition and that of cholinergic transmission (see Koelle, 1969). In addition, several drugs which effectively block spinal inhibitory synapses also interrupt transmission at peripheral cholinergic junctions and synapses (Ferguson et al., 1970). Because eserine modifies cholinergic transmission indirectly, by prolonging the action of acetylcholine itself, the effect of this drug on direct inhibition was studied. A positive result with eserine would have provided some evidence for the operation of cholinergic transmission at spinal inhibitory synapses, and would also have shown that direct inhibition could indeed be increased. However, the results obtained in this study showed that eserine is without effect on direct inhibition of the MSR, even in doses which are sufficient to block transmission in the recurrent inhibitory pathway (presumably by depolarization blockade of the Renshaw cells). These results do not conclusively eliminate the possibility that cholinergic transmission is operating. Acetylcholinesterase may not play an important role in the inactivation of acetylcholine at this site. It is significant that olivo-cochlear inhibition, which can be reduced by hemicholinium, is also resistant to the action of eserine (Brown et al., 1969).

SUMMARY

1. In preliminary experiments, the convulsant drugs thebaine and strychnine were found to interact infra-additively.
2. This interaction could be the result of a physiological antagonism of the action of strychnine by thebaine. However, the actions of the two drugs are very similar. Therefore, the alternative possibility that they compete for the same receptor site was considered to be more likely.
3. The action of thebaine was studied in detail at the strychnine sensitive inhibitory synapses of the spinal cord, in order to reveal any actions of the drug which may account for the observed interaction on the basis of a physiological antagonism.
4. In spinal cats, subconvulsive doses of thebaine diminish direct, recurrent, and polysynaptic inhibition of the spinal monosynaptic reflex, in the same manner as strychnine does.
5. Intracellular studies in spinal motoneurons showed that thebaine, like strychnine, diminishes direct, recurrent, and polysynaptic IPSP, without affecting the membrane resting potential, resistance, or potassium permeability of these neurons.
6. Although these findings indicate that the two drugs have a similar mechanism of action at the spinal inhibitory synapses, mixtures of strychnine and thebaine were shown to interact infra-additively at this site.
7. Unlike strychnine, thebaine has a complex effect on transmission at the primary afferent synapses of motoneurons, and reduces

remote inhibition of the spinal monosynaptic reflex. Neither of these effects of the drug could produce a physiological antagonism of the anti-inhibitory action of strychnine.

8. It is therefore probable that strychnine and thebaine interact infra-additively because they compete for the same receptor site within the inhibitory synapse.

REFERENCES

Adrian, E. D. Conduction in peripheral nerve and in the central nervous system. Brain, 41:23-47, 1918.

Adrian, E. D. Some recent work on inhibition. Brain, 47: 399-416, 1924.

Adrian, E. D. The Mechanism of Nervous Action. University of Pennsylvania Press, Philadelphia, 1932.

Adrian, R. H. Potassium chloride movements and the membrane potential of frog muscle. J. Physiol. (Lond.), 151:154-185, 1960.

Ahlquist, R. P. The synergism of central nervous system stimulants. J. Am. Pharm. Assoc., 36:414-415, 1947.

Alving, B. O. The action of strychnine at cholinergic junctions. Arch. Int. Pharmacodyn., 131:123-150, 1961.

Ambache, N., Morgan, R. S. and Wright, G. P. The action of tetanus toxin on the rabbit's iris. J. Physiol. (Lond.), 107:45-53, 1948 a.

Ambache, N., Morgan, R. S. and Wright, G. P. The action of tetanus toxin on the acetylcholine and cholinesterase contents of the rabbit's iris. Br. J. exp. Path., 29:408-418, 1948 b.

Andersen, P., Eccles, J. C., Schmidt, R. F. and Yokota, T. Depolarization of presynaptic fibers in the cuneate nucleus. J. Neurophysiol., 27:92-106, 1964.

Araki, T. The effects of strychnine on the postsynaptic inhibitory action. Proceedings of the International Union of Physiological Sciences. Vol. 4, XXIII International Congress. Excerpta Medica Foundation, Mouton and Co., Amsterdam, 1965.

Araki, T. and Otani, T. Response of single motoneurons to direct stimulation in toad's spinal cord. J. Neurophysiol., 18: 472-485, 1955.

Araki, T., Eccles, J. C. and Ito, M. Correlation of the inhibitory postsynaptic potential of motoneurons with the latency and time course of inhibition of monosynaptic reflexes. J. Physiol. (Lond.), 154:354-377, 1960.

Araki, T., Ito, M. and Oscarsson, O. Anion permeability of the synaptic and nonsynaptic motoneurone membrane. J. Physiol. (Lond.), 159:410-435, 1961.

Ariëns, E. J., Simonis, A. M., and van Rossum, J. M. Drug-receptor interaction: interaction of one or more drugs with one receptor system. In, Molecular Pharmacology. (Ariëns, E. J., ed.) Vol. 1, Academic Press, New York and London, 1964.

Barker, L. F. The Nervous System and its Constituent Neurones. D. Appleton and Company, New York, 1899.

Barron, D. H. and Matthews, B. H. C. The interpretation of potential changes in the spinal cord. J. Physiol. (Lond.), 92:276-321, 1938.

Bernard, C. Action du curare et de la nicotine sur le système nerveux et sur le système musculaire. Comptes Rendus de la Société de Biologie, 2:195, 1850.

Bernard, C. Leçons sur les Effets des Substances Toxiques et Médicamenteuses. Baillière, Paris, 1857.

Bernhard, C. G., Taverner, D. and Widen, L. Differences in the actions of tubocurarine and strychnine on the spinal reflex excitability of the cat. Br. J. Pharmacol., 6:551-559, 1951.

Bloom, F. E. and Giarman, N. J. Physiologic and pharmacologic considerations of biogenic amines in the nervous system. Annu. Rev. Pharmacol., 8:229-258, 1968.

Boistel, J. and Fatt, P. Membrane permeability change during inhibitory transmitter action in crustacean muscle. J. Physiol. (Lond.), 144:176-191, 1958.

Borys, H. K. and Esplin, D. W. Pentylenetetrazol and Renshaw cell activity. Int. J. Neuropharmacol., 8:627-630, 1969.

Bradley, K., Easton, D. M. and Eccles, J. C. An investigation of primary or direct inhibition. J. Physiol. (Lond.), 122:474-488, 1953.

Brock, L. G., Coombs, J. S. and Eccles, J. C. The nature of the monosynaptic excitatory and inhibitory processes in the spinal cord. Proc. R. Soc. Lond., 140:170-176, 1952.

Brooks, C. McC. The discovery of the function of chemical mediators in the transmission of excitation and inhibition of effector tissues. In, The Historical Development of Physiological

Thought. (Brooks, C. McC. and Cranefield, P. F., eds.) The Hafner Publishing Co., New York, 1959.

Brooks, V. B., Curtis, D. R. and Eccles, J. C. The action of tetanus toxin on the inhibition of motoneurones. J. Physiol. (Lond.), 135:655-672, 1957.

Brown, R. D., Daigneault, E. A. and Pruett, J. R. The effects of selected cholinergic drugs and strychnine on cochlear responses and olivo-cochlear inhibition. J. Pharmacol. Exp. Ther., 165:300-309, 1969.

Bülbring, E. and Burn, J. H. Observations bearing on synaptic transmission by acetylcholine in the spinal cord. J. Physiol. (Lond.), 100:337-368, 1941.

Burke, R. E., Fedina, L. and Lundberg, A. Spatial synaptic distribution of recurrent and group Ia inhibitory systems in cat spinal motoneurones. J. Physiol. (Lond.), 214:305-326, 1971.

Capek, R., Esplin, D. W. and Salehmoghaddam, S. Rates of transmitter turnover at the frog neuromuscular junction estimated by electrophysiological techniques. J. Neurophysiol., 34:831-841, 1971.

Chase, T. N. and Kopin, I. J. Stimulus induced release of substances from olfactory bulb using a push pull cannula. Nature, 217: 466-467, 1968.

Collins, R. J., Tang, A. H. and Keasling, H. H. Neuropharmacological evaluation of 2-phenylimidazo- [1,2-a] pyrimidine hydrobromide (U-13, 376B), an unusual strychnine antagonist. J. Pharmacol. Exp. Ther., 158:428-436, 1967.

Collonier, M. Synaptic patterns on different cell types in the different laminae of the cat visual cortex. An electron microscope study. Brain Research, 9:268-287, 1968.

Cook, W. A. and Cangiano, A. Presynaptic inhibition of spinal motoneurons. Brain Research, 24:521-524, 1970.

Coombs, J. S., Eccles, J. C. and Fatt, P. The electrical properties of the motoneurone membrane. J. Physiol. (Lond.), 130:291-325, 1955 a.

Coombs, J. S., Eccles, J. C. and Fatt, P. The specific ion conductances and the ionic movements across the motoneurone membrane that produce the inhibitory postsynaptic potential. J. Physiol. (Lond.), 130:326-373, 1955 b.

Curtis, D. R. Pharmacological investigation upon inhibition of spinal motoneurones. J. Physiol. (Lond.), 145:175-192, 1959.

Curtis, D. R. The depression of spinal inhibition by electrophoretically administered strychnine. Int. J. Neuropharmacol., 1: 239-250, 1962.

Curtis, D. R. The pharmacology of central and peripheral inhibition. Pharmacol. Rev., 15:333-364, 1963.

Curtis, D. R. Microelectrophoresis. In, Physical Techniques in Biological Research. (Nastuk, W. L., ed.) Vol. 5, Electrophysiological Methods. Academic Press, New York, 1964.

Curtis, D. R. Pharmacology and neurochemistry of mammalian central inhibitory processes. In, Structure and Function of Inhibitory Neuronal Mechanisms. (von Euler, C., Skoglund, U. and Soderberg, V., eds.) Pergamon Press, Oxford, 1968.

Curtis, D. R. Central synaptic transmitters. In, Basic Mechanisms of the Epilepsies. (Jasper, H. H., Ward, A. A. and Pope, A., eds.) Little, Brown and Co., Boston, 1969.

Curtis, D. R. and Eccles, J. C. The time courses of excitatory and inhibitory synaptic actions. J. Physiol. (Lond.), 145:529-546, 1959.

Curtis, D. R., Duggan, A. W. and Johnston, G. A. R. Glycine, strychnine and picrotoxin and spinal inhibition. Brain Research, 14:759-762, 1969.

Dale, H. H., Feldberg, W. and Vogt, M. Release of acetylcholine at voluntary motor endings. J. Physiol. (Lond.), 86:353-380, 1936.

Davidoff, R. A., Aprison, M. H. and Werman, R. Effects of strychnine on the inhibition of interneurons by glycine and γ-aminobutyric acid. Int. J. Neuropharmacol., 8:191-194, 1969.

Descartes, R. Passions of the Soul, 1650. In, The Philosophical Works of Descartes, Rendered into English. (Haldane, Elizabeth S. and Ross, G. R. T.) Cambridge University Press, Cambridge, 1911-1912.

Diamond, J. and Mellanby, J. The effect of tetanus toxin in the goldfish. J. Physiol. (Lond.), 215:727-741, 1971.

Dudel, J. and Kuffler, S. W. Presynaptic inhibition at the crayfish neuromuscular junction. J. Physiol. (Lond.), 155:543-562, 1961.

Dusser de Barenne, J. G. The mode and site of action of strychnine in the nervous system. Physiol. Rev., 13:325-334, 1933.

Eccles, J. C. The synaptic potentials of motoneurons. J. Neurophysiol., 9:87-120, 1946.

Eccles, J. C. The synaptic mechanism for postsynaptic inhibition. In, Nervous Inhibition. (Florey, E., ed.) Pergamon Press, Oxford, 1961.

Eccles, J. C. Presynaptic inhibition in the spinal cord. Prog. Brain Res., 12:65-91, 1964.

Eccles, J. C. and Sherrington, C. S. Studies on the flexor reflex. Part VI. Proc. R. Soc. Lond. (Biol.), 109:91-113, 1931.

Eccles, J. C., Katz, B. and Kuffler, S. W. Nature of the "endplate potential" in curarized muscle. J. Neurophysiol., 4:362-387, 1941.

Eccles, J. C., Fatt, P. and Koketsu, K. Cholinergic and inhibitory synapses in a pathway from motor axon collaterals to motoneurones. J. Physiol. (Lond.), 126:524-562, 1954.

Eccles, J. C., Eccles, R. M. and Fatt, P. Pharmacological investigations on a central synapse operated by acetylcholine. J. Physiol. (Lond.), 131:154-169, 1956 a.

Eccles, J. C., Fatt, P. and Landgren, S. Central pathway for direct inhibitory action of impulses in largest afferent fibers to muscle. J. Neurophysiol., 19:75-98, 1956 b.

Eccles, J. C., Eccles, R. M. and Lundberg, A. Synaptic actions on motoneurones caused by impulses in Golgi tendon organs. J. Physiol. (Lond.), 138:227-252, 1957.

Eccles, J. C., Eccles, R. M. and Lundberg, A. Types of neurones in and around the intermediate nucleus of the lumbosacral cord. J. Physiol. (Lond.), 154:89-114, 1960.

Eccles, J. C., Eccles, R. M. and Magni, F. Central inhibitory action attributable to presynaptic depolarization produced by muscle afferent volleys. J. Physiol. (Lond.), 159:147-166, 1961 a.

Eccles, J. C., Eccles, R. M., Iggo, A. and Ito, M. Distribution of recurrent inhibition among motoneurones. J. Physiol. (Lond.), 159:479-499, 1961 b.

Eccles, J. C., Magni, F. and Willis, W. D. Depolarization of central terminals of group I afferent fibers from muscle. J. Physiol. (Lond.), 160:62-93, 1962.

Eccles, J. C., Schmidt, R. F. and Willis, W. D. Location and mode of action of the presynaptic inhibitory pathways onto group Ia afferent fibers from muscle. J. Neurophysiol., 26:506-522, 1963 a.

Eccles, J. C., Schmidt, R. F. and Willis, W. D. Pharmacological studies on presynaptic inhibition. J. Physiol. (Lond.), 168:500-530, 1963 b.

Eccles, J. C., Loyning, Y. and Oshima, T. Effects of hypoxia on the monosynaptic reflex pathway in the cat spinal cord. J. Neurophysiol., 29:315-332, 1966.

Elliott, T. R. On the action of adrenalin. J. Physiol. (Lond.), 31: XX-XXI, 1904.

Epstein, R. and Grundfest, H. Desensitization of γ-aminobutyric acid receptors in muscle fibers of the crab. J. Gen. Physiol., 56:33-45, 1970.

Esplin, D. W. and Zablocka, B. Pilocarpine blockade of spinal inhibition in cats. J. Pharmacol. Exp. Ther., 143:174-180, 1964.

Esplin, D. W. and Zablocka-Esplin, B. Mechanism of action of convulsants. In, Basic Mechanisms of the Epilepsies. (Jasper, H. H., Ward, A. A. and Pope, A., eds.) Little, Brown and Co., Boston, 1969.

Fatt, P. and Katz, B. An analysis of the endplate potential recorded with an intracellular electrode. J. Physiol. (Lond.), 115: 320-370, 1951.

Fearing, F. Reflex Action. 1930. Reprinted by Hafner Publishing Co., New York, 1964.

Feldberg, W. and Gaddum, J. H. The chemical transmitter in a sympathetic ganglion. J. Physiol. (Lond.), 81:305-319, 1934.

Ferguson, R. K., Zablocka-Esplin, B. and Esplin, D. W. Peripheral cholinergic blockade by drugs that block postsynaptic central inhibition. Arch. Int. Pharmacodyn., 185:298-307, 1970.

Fessard, A. and Posternak, J. Les méchanismes élémentaires de la transmission synaptique. J. Physiol. (Paris), 42:319-437, 1950.

Finney, D. J. Probit Analysis. Cambridge University Press, Cambridge, 1952.

Foster, M. Textbook of Physiology. (Third Edition) Henry C. Lea's Son and Co., Philadelphia, 1880.

Frank, K. and Fuortes, M. G. F. Stimulation of spinal motoneurones with intracellular electrodes. J. Physiol. (Lond.), 134: 451-470, 1956.

Frank, K. and Fuortes, M. G. F. Presynaptic and postsynaptic inhibition of monosynaptic reflexes. Fed. Proc., 16:39-40, 1957.

Fuortes, M. G. F. and Nelson, P. G. Strychnine; its action on spinal motoneurons of cats. Science, 140:806-808, 1963.

Furukawa, T., Fukami, Y. and Asada, Y. A third type of inhibition in the Mauthner cell of goldfish. J. Neurophysiol., 26:759-774, 1963.

Gage, P. W. and Hubbard, J. I. An investigation of the post-tetanic potentiation of endplate potentials at a mammalian neuromuscular junction. J. Physiol. (Lond.), 184:353-375, 1966.

Garrison, F. H. History of Medicine. (Fourth Edition) W. B. Saunders Co., Philadelphia, 1929.

Gaskell, W. H. On the action of muscarin upon the heart, and on the electrical changes in the nonbeating cardiac muscle brought about by stimulation of the inhibitory and augmentory nerves. J. Physiol. (Lond.), 8:404-415, 1887.

Gasser, H. S. The control of excitation in the nervous system. Harvey Lectures, 32:169-193, 1937.

Gasser, H. S. and Graham, H. T. Potentials produced in the spinal cord by stimulation of the dorsal roots. Am. J. Physiol., 103:303-320, 1933.

Gorman, A. L. F. and Marmor, M. F. Contributions of the sodium pump and ionic gradients to the membrane potential of a molluscan neurone. J. Physiol. (Lond.), 210:897-917, 1970.

Granit, R., Haase, J. and Rutledge, L. T. Recurrent inhibition in relation to frequency of firing and limitation of discharge rate of extensor motoneurons. J. Physiol. (Lond.), 154: 308-328, 1960.

Granit, R. and Rutledge, L. T. Surplus excitation in reflex action of motoneurones as measured by recurrent inhibition. J. Physiol (Lond.), 154:288-307, 1960.

Hagiwara, S. and Tasaki, I. A study of the mechanism of impulse transmission across the giant synapse of the squid. J. Physiol. (Lond.), 143:114-137, 1958.

Heinbecker, P. and Bartley, S. H. Manner of strychnine action on nervous system. Am. J. Physiol., 125:172-187, 1939.

Hodgkin, A. L. and Katz, B. The effect of sodium ions on the electrical activity of the giant axon of the squid. J. Physiol. (Lond.), 108:37-77, 1949.

Howell, W. H. Inhibition. Physiol. Rev., 5:161-181, 1925.

Hubel, D. H. Tungsten microelectrodes for recording from single units. Science, 125:549-550, 1957.

Huges, J. and Gasser, H. S. The response of the spinal cord to two afferent volleys. Am. J. Physiol., 108:307-321, 1934.

Hultborn, H., Jankowska, E. and Lindstrom, S. Recurrent inhibition of interneurones monosynaptically activated from group Ia afferents. J. Physiol. (Lond.), 215:613-636, 1971.

Hunt, C. C. and Perl, E. R. Spinal reflex mechanisms concerned with skeletal muscle. Physiol. Rev., 40:538-579, 1960.

Kaeser, H. E. and Saner, A. Tetanus toxin, a neuromuscular blocking agent. Nature, 223:842, 1969.

Kehoe, J. S. and Ascher, P. Re-evaluation of the synaptic activation of an electrogenic sodium pump. Nature, 225:820-823, 1970.

Kellerth, J. O. Aspects on the relative significance of pre- and postsynaptic inhibition in the spinal cord. In, Structure and Function of Inhibitory Neuronal Mechanisms. (von Euler, C., Skoglund, S. and Soderberg, V., eds.) Pergamon Press, Oxford, 1968.

Kellerth, J. O. and Szumski, A. J. Postsynaptic inhibitions from muscular afferents differentiated by strychnine and picrotoxin. In, Nobel Symposium I: Muscular Afferents and Motor Control. (Granit, R., ed.) John Wiley and Son, New York, 1965.

Koelle, G. B. Pharmacology of synaptic transmitters. In, Basic Mechanisms of the Epilepsies. (Jasper, H. H., Ward, A. A. and Pope, A., eds.) Little, Brown and Co., Boston, 1969.

Koketsu, K. and Nishi, S. Characteristics of the slow inhibitory postsynaptic potential of bullfrog sympathetic ganglia. Life Sci., 6:1827-1836, 1967.

Koppanyi, T. and Karczmar, A. G. Nature of antagonism between N-allylnormorphine (Nalline) and morphine. Fed. Proc., 12: 337, 1953.

Krnjevic, K. Micro-iontophoretic studies on cortical neurons. International Review of Neurobiology, 7:41-98, 1964.

Krnjevic, K. Glutamate and γ-aminobutyric acid in brain. Nature, 288:119-124, 1970.

Krnjevic, K. and Eccles, J. C. Potential changes recorded inside primary afferent fibers within the spinal cord. J. Physiol. (Lond.), 149:250-273, 1959 a.

Krnjevic, K. and Eccles, J. C. Presynaptic changes associated with post-tetanic potentiation. J. Physiol. (Lond.), 149:274-287, 1959 b.

Krnjevic, K. and Phyllis, J. W. Pharmacological properties of acetylcholine sensitive cells in the cerebral cortex. J. Physiol. (Lond.), 166:328-350, 1963.

Krnjevic, K., Randic, M. and Straughan, D. W. Nature of a cortical inhibitory process. J. Physiol. (Lond.), 184:49-77, 1966 a.

Krnjevic, K., Randic, M. and Straughan, D. W. Pharmacology of cortical inhibition. J. Physiol. (Lond.), 184:78-105, 1966 b.

Kruglov, N. A. Effect of the morphine-group analgesics on the central inhibitory mechanisms. Int. J. Neuropharmacol., 3: 197-203, 1964.

Kuno, M. Effects of strychnine on the intracellular potentials of spinal motoneurons of the toad. Japanese Journal of Physiology, 7:42-50, 1957.

Kuno, M. Excitability following antidromic activation in spinal motoneurones supplying red muscles. J. Physiol. (Lond.), 149: 374-393, 1959.

Kuno, M. and Llinas, R. Alterations of synaptic action in chromatolysed motoneurones of the cat. J. Physiol. (Lond.), 210: 823-838, 1970.

Kuno, M., Miyahara, J. T. and Weakly, J. N. Post-tetanic hyperpolarization produced by an electrogenic pump in dorsal spinocerebellar tract neurones of the cat. J. Physiol. (Lond.), 210:839-857, 1970.

Lanari, A. and Luco, J. V. The depressant action of strychnine on the superior cervical sympathetic ganglion and on skeletal muscle. Am. J. Physiol., 126:277-282, 1939.

Larson, M. D. An analysis of the action of strychnine on the recurrent IPSP and amino acid induced inhibitions in the cat spinal cord. Brain Research, 15:185-200, 1969.

Laskey, W., and Zablocka-Esplin, B. Studies on the anticholinergic and anti-inhibitory properties of thebaine. Fed. Proc., 30: 425Abs., 1971.

Lauder-Brunton, T. Pharmacology, Therapeutics and Materia Medica. Lea Brothers and Co., Philadelphia, 1888.

Libet, B. Generation of slow inhibitory and excitatory postsynaptic potentials. Fed. Proc., 29:1945-1956, 1970.

Liddel, E. G. T. The Discovery of Reflexes. Clarendon Press, Oxford, 1960.

Litchfield, J. T. and Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol., 96:99-113, 1949.

Lloyd, D. P. C. A direct central inhibitory action of dromically conducted impulses. J. Neurophysiol., 4:184-190, 1941.

Lloyd, D. P. C. Facilitation and inhibition of spinal motoneurons. J. Neurophysiol., 9:421-438, 1946 a.

Lloyd, D. P. C. Integrative pattern of excitation and inhibition in two-neuron reflex arcs. J. Neurophysiol., 9:439-444,1946 b.

Loewi, O. An autobiographical sketch. Perspectives in Biology and Medicine, 4:3-25, 1960.

Longo, V. G. and Chiavarelli, S. Neuropharmacological analysis of strychnine-like drugs. In, Proceedings of the First International Pharmacological Meeting. Vol. 8, Pharmacological Analysis of Central Nervous Action. (Paton, W. D. M., ed.) Pergamon Press, Oxford, 1962.

Lux, H. D. Ammonium and chloride extrusion: hyperpolarizing synaptic inhibition in spinal motoneurons. Science, 173:555-557, 1971.

Martin, A. R. and Pilar, G. Presynaptic and postsynaptic events during post-tetanic potentiation and facilitation in the avian ciliary ganglion. J. Physiol. (Lond.), 175:17-30, 1964.

Martin, W. R. Opioid antagonists. Pharmacol. Rev., 19:463-521, 1967.

McClane, T. K. and Martin, W. R. Antagonism of the spinal cord effects of morphine and cyclazocine by naloxone and thebaine. Int. J. Neuropharmacol., 6:325-327, 1967.

Meij, H. S. and Holemans, K. C. Inhibitory interaction between motoneurons of adjacent segments in the frog spinal cord. Exp. Neurol., 23:174-186, 1969.

Miyahara, J. T. A Physiological and Pharmacological Analysis of the Mechanism of Presynaptic Inhibition. Doctoral Thesis, University of Utah, 1966.

Miyahara, J. T., Esplin, D. W. and Zablocka, B. Differential effects of depressant drugs on presynaptic inhibition. J. Pharmacol. Exp. Ther., 154:119-127, 1966.

Nakajima, S. and Takahashi, K. Post-tetanic hyperpolarization and electrogenic Na pump in stretch receptor neurone of crayfish. J. Physiol. (Lond.), 187:105-127, 1966.

Neal, M. J. The uptake of glycine by slices of mammalian spinal cord. J. Physiol. (Lond.), 215:103-117, 1971

Nishi, S. and Koketsu, K. Analysis of slow inhibitory postsynaptic potential of bullfrog sympathetic ganglion. J. Neurophysiol., 31:717-728, 1968.

Owen, A. G. W. and Sherrington, C. S. Observations on strychnine reversal. J. Physiol. (Lond.), 43:232-241, 1911.

Pinsker, H. and Kandel, E. Synaptic activation of an electrogenic pump. Science, 163:931-935, 1969.

Pinto-Corrado, A. and Longo, V. G. An electrophysiological analysis of the convulsant action of morphine, codeine and thebaine. Arch. Int. Pharmacodyn., 132:255-268, 1961.

Pollen, D. A. and Ajmone-Marsan, C. Cortical inhibitory postsynaptic potentials and strychninization. J. Neurophysiol., 28:342-358, 1965.

Rall, W., Burke, R. E., Smith, T. G., Nelson, P. G. and Frank, K. Dendritic location of synapses and possible mechanisms for the monosynaptic EPSP in motoneurons. J. Neurophysiol., 30: 1169-1194, 1967.

Rang, H. P. and Ritchie, J. M. On the electrogenic sodium pump in mammalian non-myelinated nerve fibers and its activation by various external cations. J. Physiol. (Lond.), 196:183-221, 1968.

Renshaw, B. Influence of discharge of motoneurons upon excitation of neighbouring motoneurons. J. Neurophysiol., 4:167-183, 1941.

Renshaw, B. Central effects of centripetal impulses in axons of spinal ventral roots. J. Neurophysiol., 9:191-204, 1946 a.

Renshaw, B. Observations on interaction of nerve impulses in grey matter and on the nature of central inhibition. Am. J. Physiol., 146:443-448, 1946 b.

Roper, S., Diamond, J. and Yarsgil, G. M. Does strychnine block inhibition post-synaptically? Nature, 223:1168-1169, 1969.

Ryall, R. W. and Piercy, M. F. Excitation and inhibition of Renshaw cells by impulses in peripheral afferent nerve fibers. J. Neurophysiol., 34:242-251, 1971.

Sampson, S. R. Mechanism of coniine blockade of postsynaptic inhibition in the spinal cord of the cat. Int. J. Neuropharmacol., 5:171-182, 1966.

Sampson, S. R., Esplin, D. W. and Zablocka, B. Effects of coniine on peripheral and central synaptic transmission. J. Pharmacol. Exp. Ther., 152:313-324, 1966.

Satoh, M. and Takagi, H. Effect of morphine on the pre- and postsynaptic inhibitions in the spinal cord. Eu. J. Pharmacol., 14:150-154, 1971.

Schäfer, E. A. The nerve cell. In, Textbook of Physiology. (Schäfer, E. A., ed.) Vol. 2, Young J. Pentland, Edinburgh and London, 1900.

Scott, F. H. On the relation of nerve cells to fatigue of their nerve fibers. J. Physiol. (Lond.), 34:145-162, 1906.

Sherrington, C. S. The spinal cord. In, A Textbook of Physiology: Part III - The Central Nervous System. Foster, M. (assisted by Sherrington, C. S.), McMillan and Co., London, 1897.

Sherrington, C. S. The spinal cord. In, Textbook of Physiology. (Schäfer, E. A., ed.) Vol. 2, Young J. Pentland, Edinburgh and London, 1900.

Sherrington, C. S. On reciprocal innervation of antagonistic muscles - Eighth note. Proc. R. Soc. Lond. (Biol.), 76:269-297, 1905.

Sherrington, C. S. The Integrative Action of the Nervous System. Charles Scribners and Sons, 1906. (Reprinted by Yale University Press, New Haven, 1952.)

Sherrington, C. S. Remarks on some aspects of reflex inhibition. Proc. R. Soc. Lond. (Biol.), 97:519-545, 1925.

Smith, T. G., Weurker, R. B. and Frank, K. Membrane impedance changes during synaptic transmission in cat spinal motoneurons. J. Neurophysiol., 30:1072-1097, 1967.

Stephenson, R. P. and Ginsborg, B. L. Potentiation by an antagonist. Nature, 222:790-791, 1969.

Sverdlov, Y. S. and Alekseeva, V. I. Effect of tetanus toxin on presynaptic inhibition in the spinal cord. Fed. Proc., 25: T931-T936, 1966.

Takeuchi, A. and Takeuchi, N. Electrical changes in pre- and postsynaptic axons of the giant synapse of Loligo. J. Gen. Physiol., 45:1181-1193, 1962.

Tauc, L. Presynaptic inhibition in the abdominal ganglion of Aplysia. J. Physiol. (Lond.), 181:282-307, 1965.

Toman, J. E. P., Swinyard, E. A., Merkin, M. and Goodman, L. S. Studies on the physiology and the therapy of convulsive disorders - II. Some properties of experimental convulsions. J. Neuropath. Exp. Neurol., 7:35-46, 1948.

Toman, J. E. P. and Everett, G. M. Anticonvulsants. In, Evaluation of Drug Activities; Pharmacometrics. (Laurence, D. R. and Bacharach, A. L., eds.) Academic Press, New York, 1966.

Trautwein, W., Kuffler, S. W. and Edwards, C. Changes in the membrane characteristics of heart muscle during inhibition. J. Gen. Physiol., 40:135-145, 1956.

Waller, A. V. Experiments on the section of the glossopharyngeal and hypoglossal nerves, and observations on the alterations produced in the structure of their primitive fibers. Philo-

sophical Transactions of the Royal Society (London), 1850, 423-430.

Weakly, J. N., Esplin, D. W. and Zablocka, B. Criteria for assessing effects of drugs on postsynaptic inhibition. Arch. Int. Pharmacodyn., 171:385-393, 1968.

Wedensky, N. Y. Des rélations entre les processus rhythmiques et l'activité fonctionelle de l'appareil neuro-musculaire excité. Archives de Physiologie, 24:53-59, 1892.

Werman, R. Criteria for identification of a central nervous system transmitter. Comp. Biochem. Physiol., 118:745-766, 1966.

Whytt, R. The Works, Published by his Son. Becket, Edinburgh, 1768.

Wilson, V. J. Inhibition and release of spinal motoneurons by recurrent activity. In, Morphological and Biochemical Correlates of Neural Activity. (Cohen, M. M. and Snider, R. S., eds.) Harper and Row, New York, 1964.

Wilson, V. J. Regulation and function of Renshaw cell discharge. In, Proceedings of the First Nobel Symposium: Muscular Afferents and Motor Control. (Granit, R., ed.) John Wiley and Sons, New York, 1966.

Wilson, V. J. and Burgess, P. R. Disinhibition in the cat spinal cord. J. Neurophysiol., 25:392-404, 1962.

Wilson, V. J., Talbot, W. H. and Kato, M. Inhibitory convergence upon Renshaw cells. J. Neurophysiol., 27:1063-1079, 1964.

Woodbury, L. A. and Davenport, Virginia D. Design and use of a new electroshock seizure apparatus, and analysis of factors altering seizure threshold and pattern. Arch. Int. Pharm-

acodyn., 92:97-107, 1952.

Zablocka, B. and Esplin, D. W. Central excitatory and depressant effects of pilocarpine in rats and mice. J. Pharmacol. Exp. Ther., 140:162-169, 1963.

FIGURE 1: Schematic diagram of bridge circuit used to pass current through the recording electrode.

A. Unity-gain, negative-capacity preamplifier.

B. Variable DC voltage used to cancel junctional potentials.

E. Terminals between which the voltage signals from the preparation were recorded.

I. Terminals between which the current passing through the microelectrode was recorded.

SIU. Stimulus isolation unit.

The circuit was designed by Mr. Harry Fein.

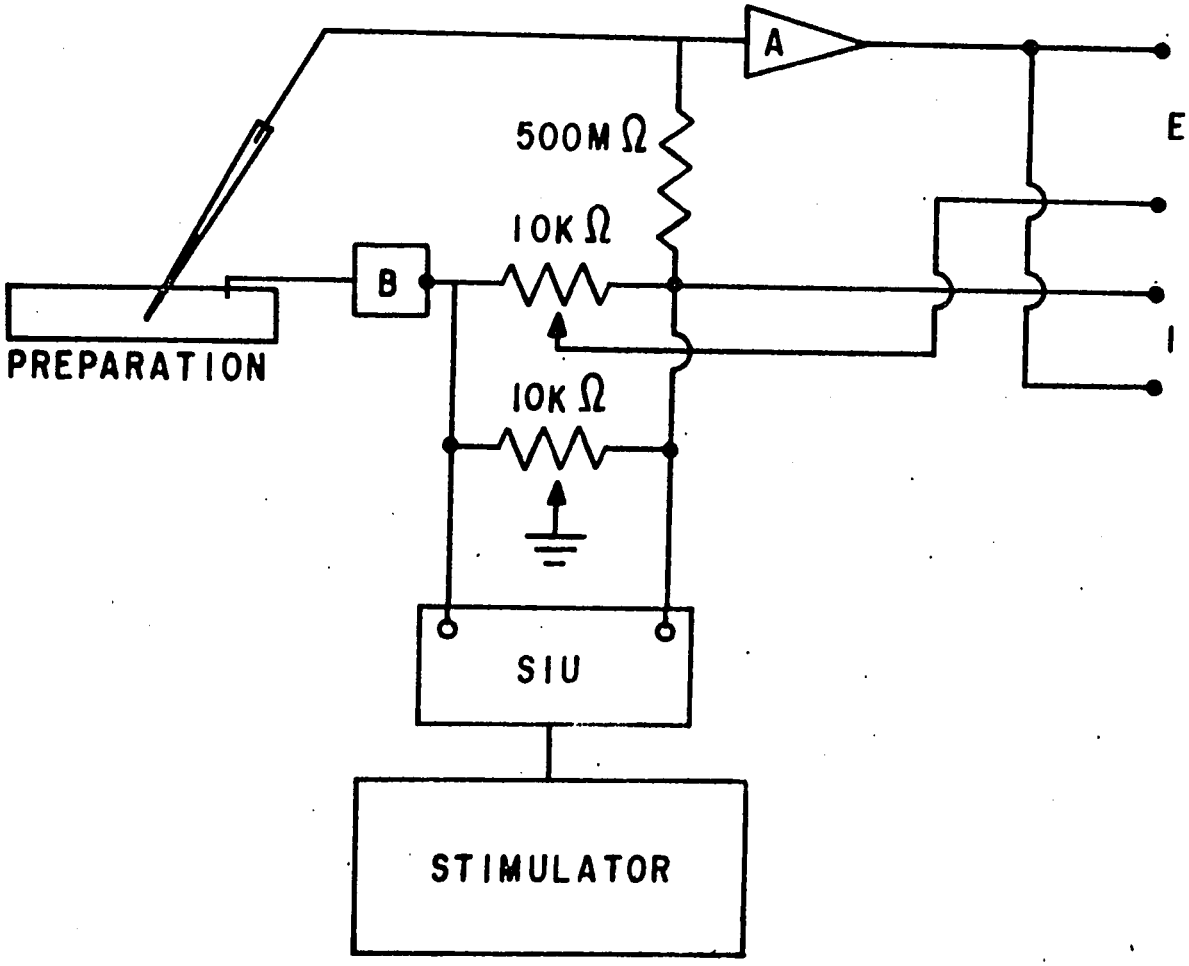

A
500M Ω
E
10K Ω
B
I
PREPARATION
10K Ω
SIU
STIMULATOR

FIGURE 2: Sample recordings of monosynaptic EPSP.

Monosynaptic EPSP were evoked by stimulation of Ia afferents in the hamstring nerve, and recorded intracellularly with a potassium citrate microelectrode. Each of the two large traces represent the sum of 200 consecutive oscilloscope sweeps, as recorded by a computer of average transients. The photographic records of three of the consecutive oscilloscope sweeps are shown to the right of the figure. The upper calibration bar refers to the computer trace and represents 5 msec. The lower calibration pulse refers to the photographic record and represents 5 msec and 1 mV.

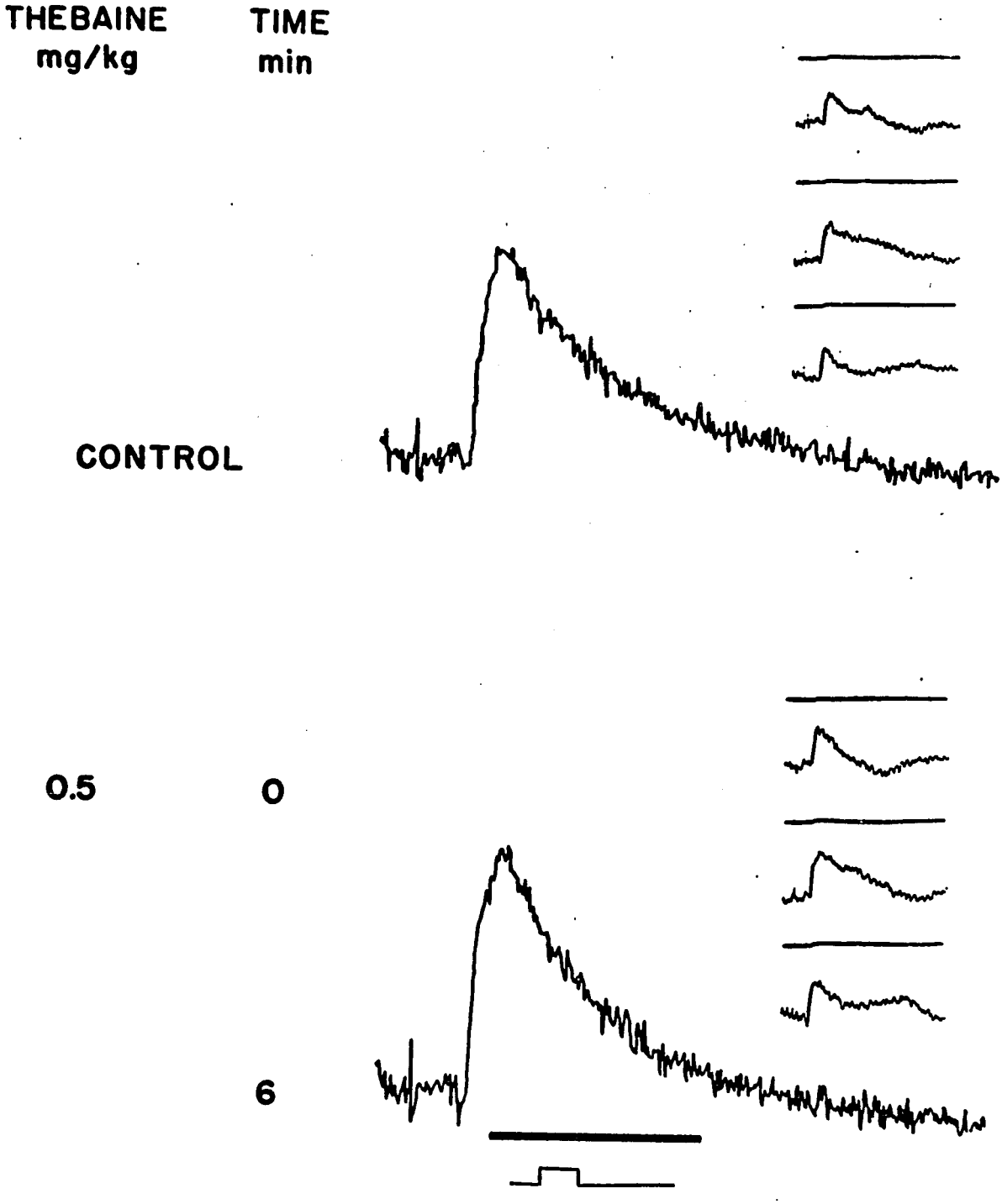

THEBAINE
mg/kg
TIME
min
CONTROL
0.5
0
6

FIGURE 3: Sample recordings of direct IPSP.

Direct IPSP were evoked by stimulation of Ia afferents in the quadriceps nerve and recorded intracellularly in the hamstring motoneuron depicted in Figure 2. In Figure 3, each of the two large traces represents the sum of 200 consecutive oscilloscope sweeps, as recorded by a computer of average transients. The photographic records of three of the consecutive oscilloscope sweeps are shown to the right of the figure. The upper calibration bar refers to the computer trace and represents 5 msec. The lower calibration pulse represents 5 msec and 1 mV with respect to the photographic record.

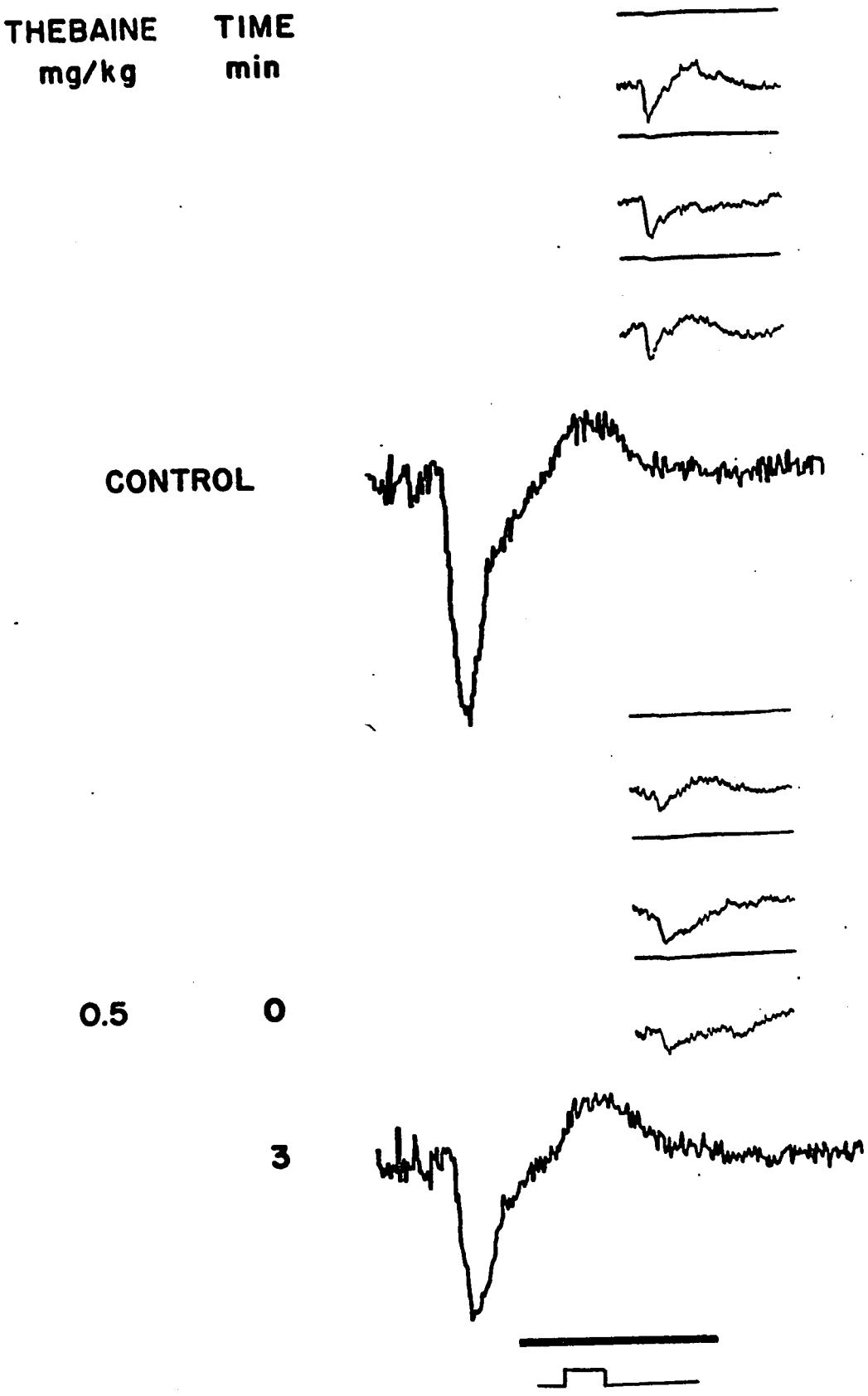

THEBAINE
mg/kg
TIME
min
CONTROL
0.5
0
3

FIGURE 4: Effect of thebaine on the MSR.

The figure presents the photographic record of representative responses obtained in a single experiment. The activity in the stimulated afferents of the hamstring nerve, as recorded by one arm of a bipolar platinum electrode placed in contact with the surface of the L_7 dorsal root (the other arm being in contact with the exposed muscles of the back) is displayed on the upper trace in each sweep. The monosynaptic and polysynaptic reflex responses, recorded bipolarly at the central end of the cut L_7 ventral root, are displayed in the lower trace. The control record was obtained immediately before the intravenous injection of 0.8 mg/kg of thebaine. Records on the right hand side of the figure were obtained 10 mins after drug administration. The calibration pulse represents 5 msec, and 0.2 mV for the lower trace, and 0.1 mV for the upper trace, of each oscilloscope sweep.

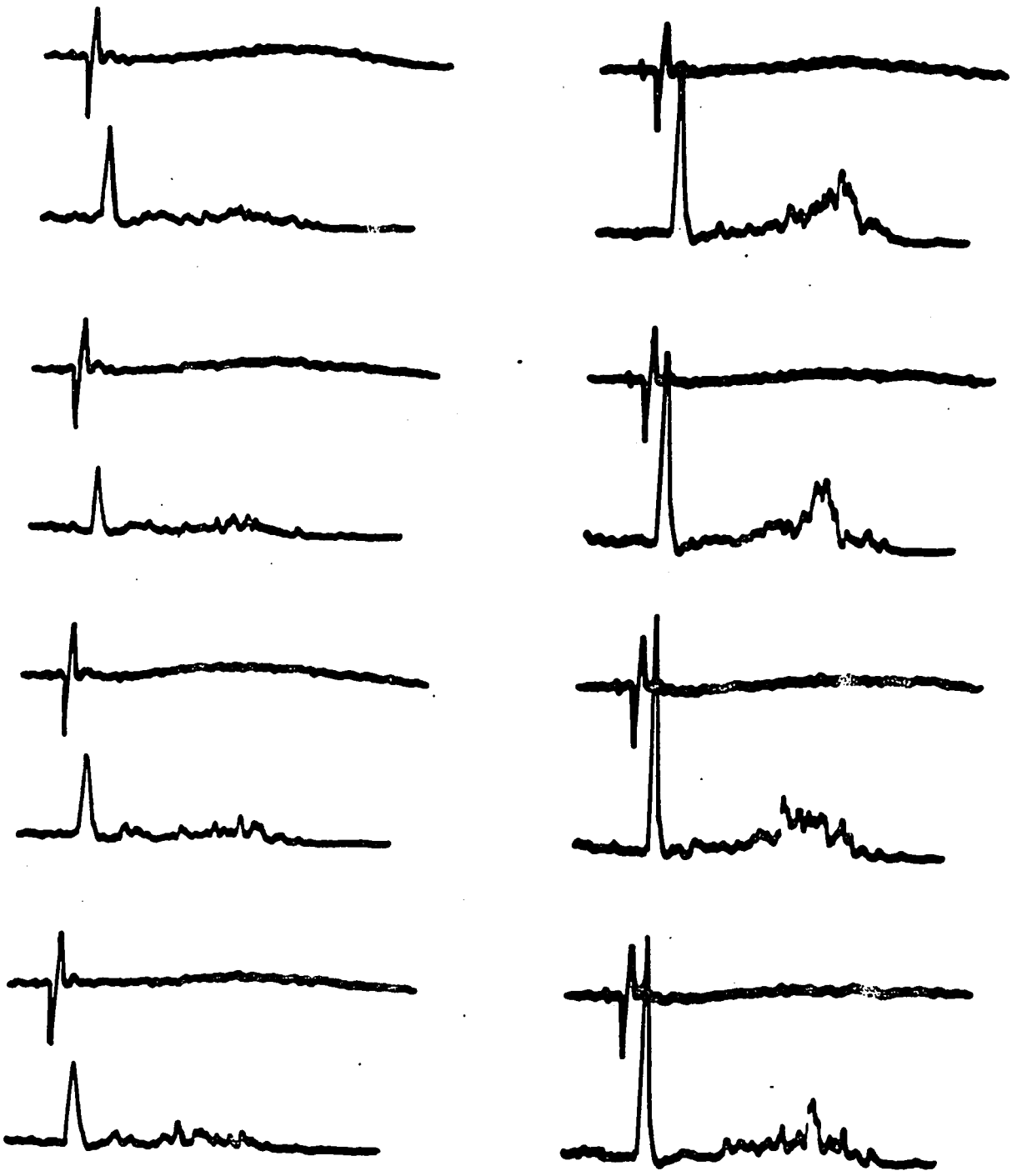

CONTROL
THEBAINE

FIGURE 5: Relation of the level of afferent drive to the degree of direct inhibition of the MSR.

The level of afferent drive was altered by inducing PTP in the test pathway and following its decay (see p. 42). The amplitude of the test MSR, expressed as a percent of the maximum value attained during the peak of PTP, was used to indicate the level of afferent drive. The degree of inhibition was determined by conditioning the test response with a stimulus to the direct inhibitory pathway, at the interval which produced maximum inhibition. The lines in the figure are calculated regression lines through the experimentally determined points.

In each of the two representative experiments presented in this figure, the entire relation between afferent drive and inhibition was determined before and immediately after the administration of the indicated drugs. In the first experiment, an initial dose of 1.00 mg/kg of eserine was administered, and the effect of 0.05 mg/kg of strychnine was determined in its presence. The effect of 2.5 mg/kg of thebaine is shown in the lower part of the figure.

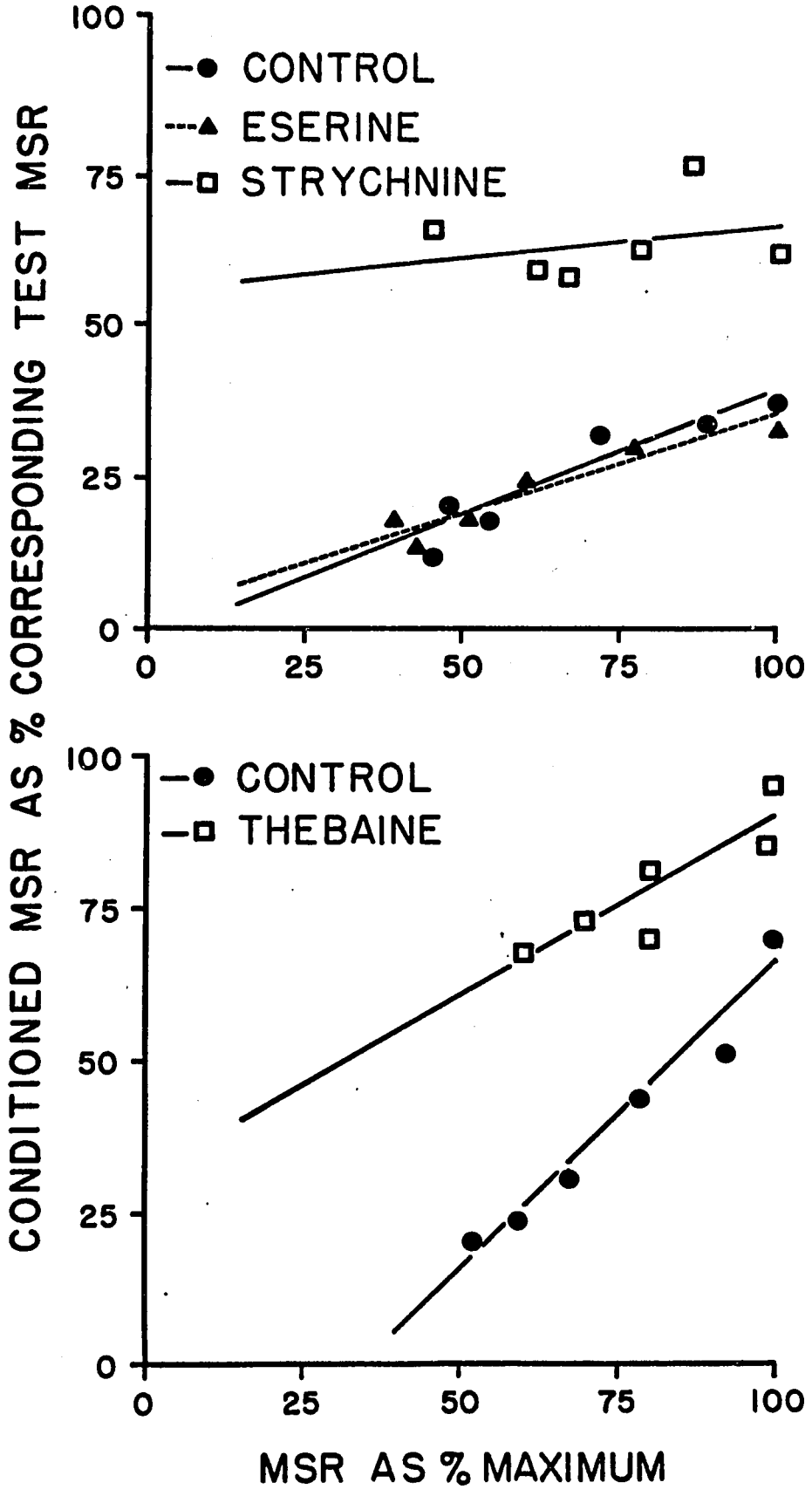

CONTROL
ESERINE
STRYCHNINE
CONTROL
THEBAINE
CONDITIONED MSR AS % CORRESPONDING TEST MSR
MSR AS % MAXIMUM
0
25
50
75
100

FIGURE 6: Relation of the level of afferent drive to the degree of recurrent inhibition of the MSR.

The level of afferent drive was altered by inducing PTP in the test pathway and following its decay. The level of afferent drive and the degree of inhibition were determined and expressed as in Figure 5.

The results presented in the upper part of the figure were obtained in the same experiment whose results are depicted in the upper part of Figure 5. An initial dose of 1.00 mg/kg of eserine was administered initially and the effect of 0.05 mg/kg of strychnine was determined in its presence. The effect of 2.00 mg/kg of thebaine, determined in a separate experiment, is shown in the lower part of the figure.

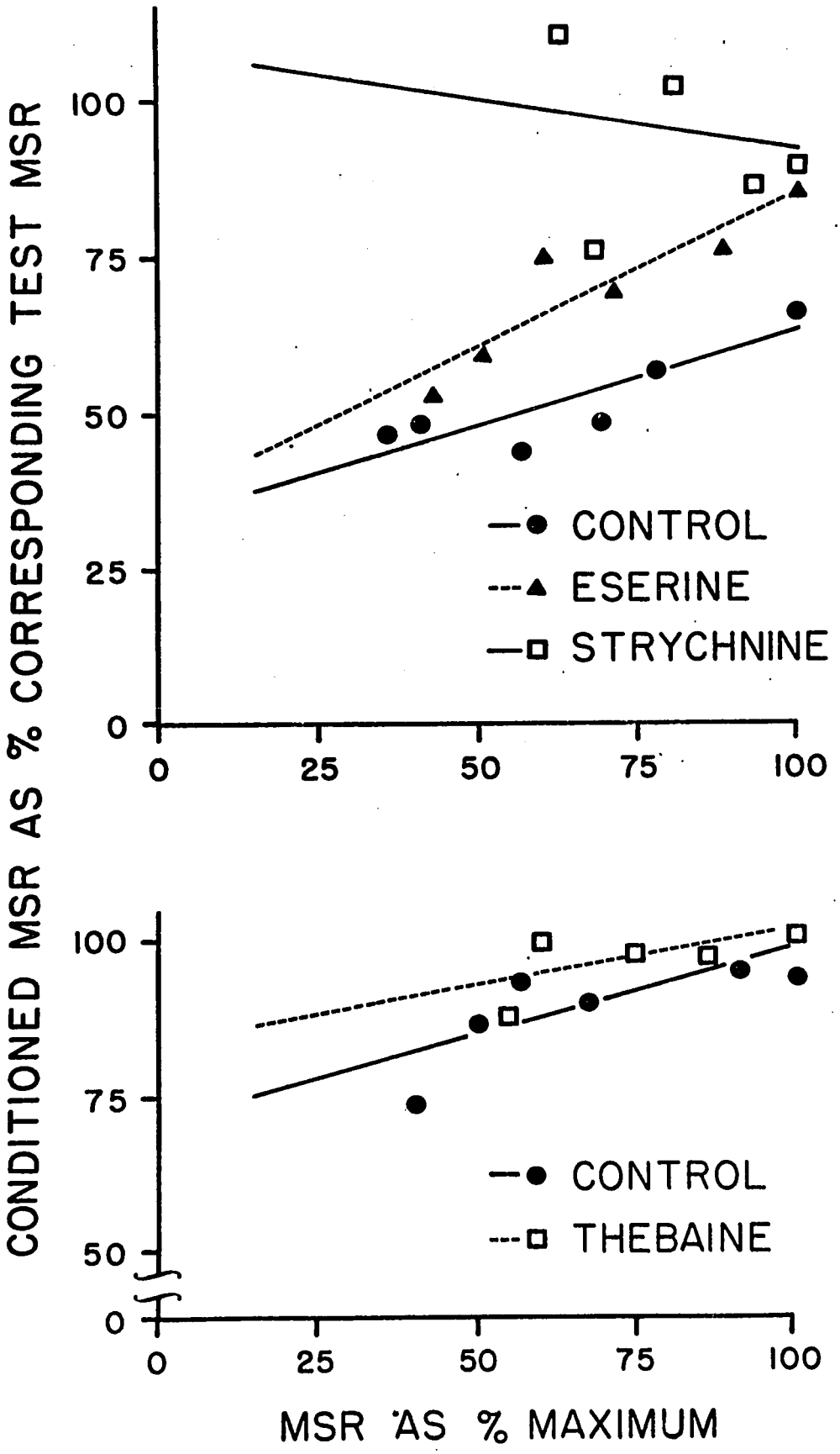

CONDITIONED MSR AS % CORRESPONDING TEST MSR
100
75
50
25
0
0
25
50
75
100
CONTROL
ESERINE
STRYCHNINE
100
75
50
0
0
25
50
75
100
CONTROL
THEBAINE
MSR AS % MAXIMUM

FIGURE 7: Relation of the level of afferent drive to the degree of remote inhibition of the MSR.

The level of afferent drive was altered by inducing PTP in the test pathway and following its decay. The level of afferent drive and the degree of inhibition were determined and expressed as in Figure 5.

The results of a single experiment in which the entire relation between afferent drive and inhibition was determined before, and immediately after, the administration of 1.50 mg/kg of thebaine, are presented in this figure.

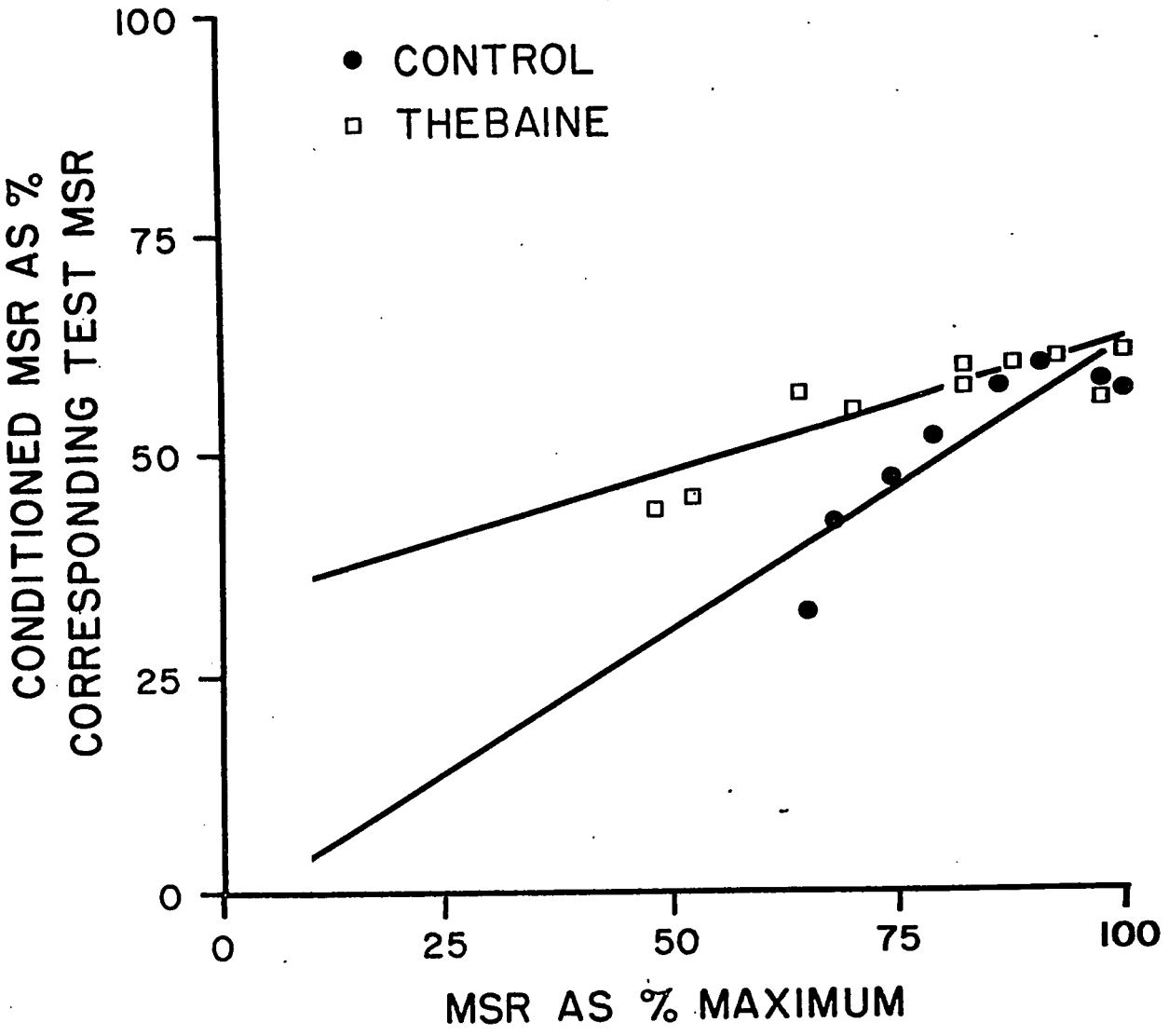

CONTROL
THEBAINE
CONDITIONED MSR AS % CORRESPONDING TEST MSR
MSR AS % MAXIMUM
0
25
50
75
100

FIGURE 8: Effect of thebaine on direct inhibition of the MSR.

This figure presents the results of a single experiment. Test responses elicited by stimulation of the hamstring nerve were recorded at the central ends of the cut L_7 and S_1 ventral roots. These responses were conditioned by a stimulus to the quadriceps nerve, which preceded the test stimulus by a varied interval. The inhibitory curves depicted in the figure were constructed by plotting the average amplitude of 10 consecutive conditioned responses (expressed as a percent of the unconditioned response) against the interval between conditioning and test stimuli. The inhibitory curves (□) and (△) were determined in the presence of 0.78 and 1.45 mg/kg of thebaine respectively.

FIGURE 8: Effect of thebaine on direct inhibition of the MSR.

This figure presents the results of a single experiment. Test responses elicited by stimulation of the hamstring nerve were recorded at the central ends of the cut L_7 and S_1 ventral roots. These responses were conditioned by a stimulus to the quadriceps nerve, which preceded the test stimulus by a varied interval. The inhibitory curves depicted in the figure were constructed by plotting the average amplitude of 10 consecutive conditioned responses (expressed as a percent of the unconditioned response) against the interval between conditioning and test stimuli. The inhibitory curves (□) and (△) were determined in the presence of 0.78 and 1.45 mg/kg of thebaine respectively.

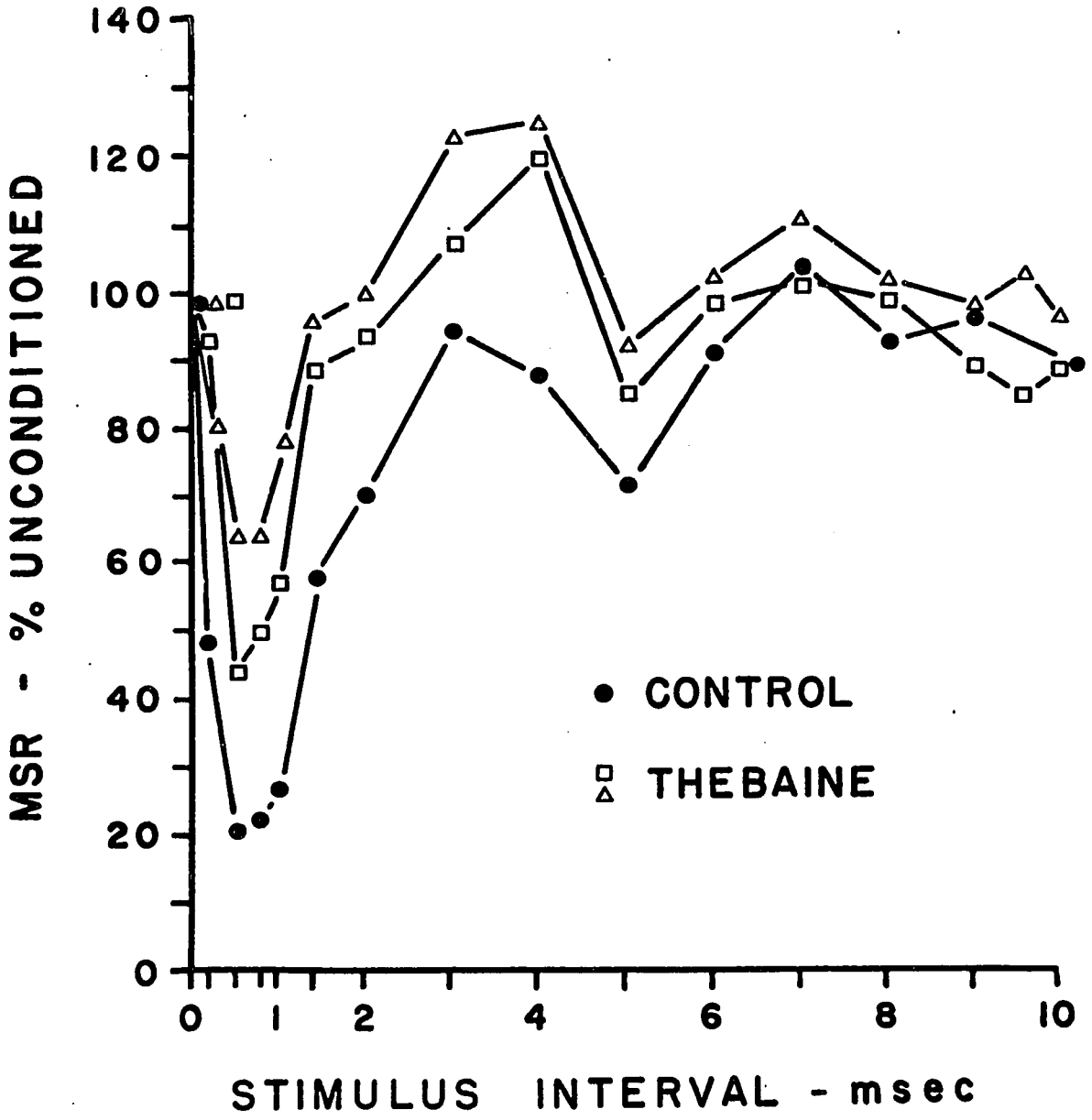

140
120
100
80
60
40
20
0
MSR - % UNCONDITIONED
0
1
2
4
6
8
10
STIMULUS INTERVAL - msec
CONTROL
THEBAINE

FIGURE 9: Effect of thebaine on direct IPSP of spinal motoneurons.

The direct IPSP depicted in the figure was evoked by stimulation of the quadriceps nerve, and recorded intracellularly, in a hamstring motoneuron, with a potassium citrate electrode. The traces represent the sum of 200 consecutive oscilloscope sweeps, recorded by a computer of average transients (calibration bar is 2 msec). The membrane resting potential of this cell was unchanged during the experimental period.

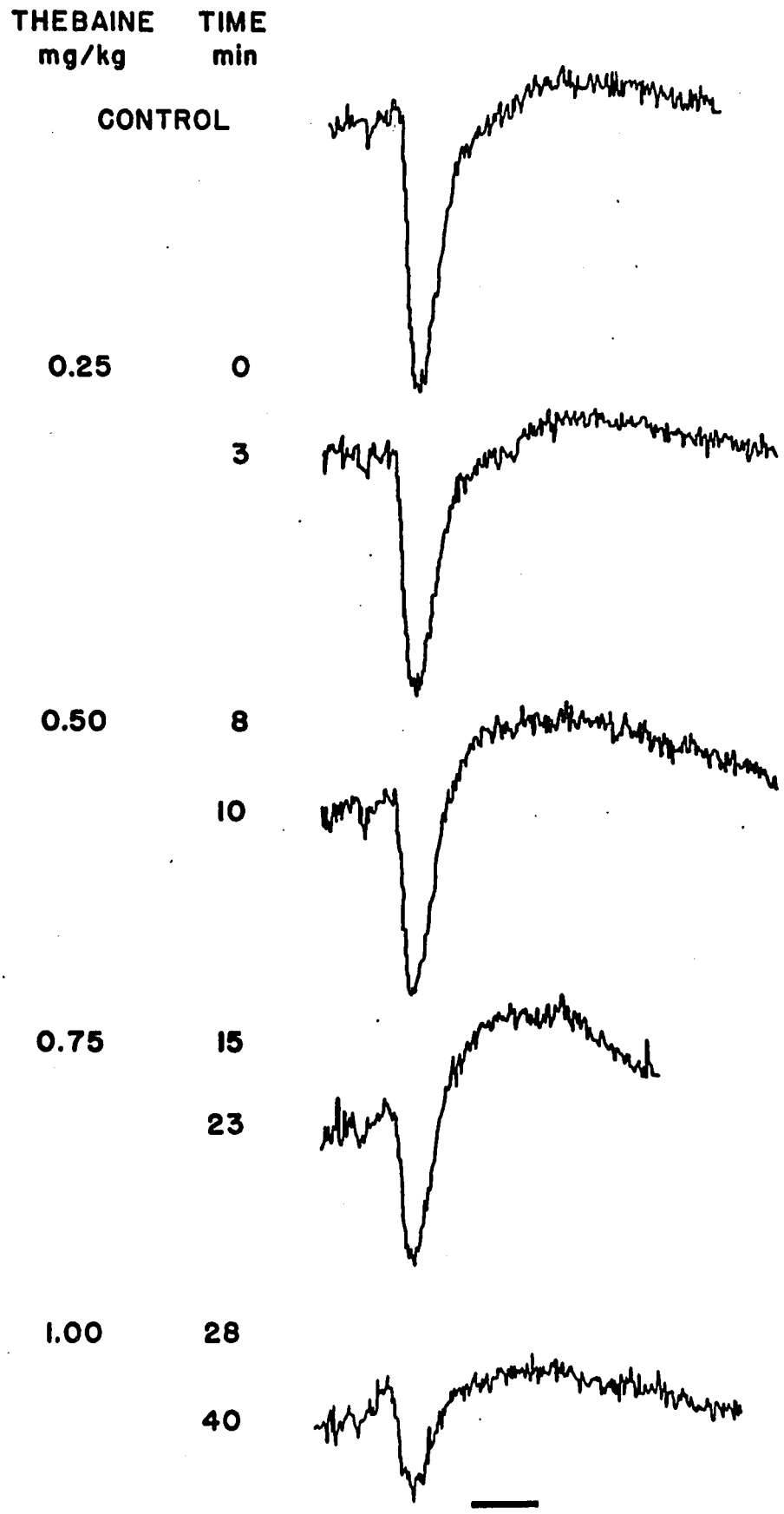

THEBAINE mg/kg
TIME min
CONTROL
0.25
0
3
0.50
8
10
0.75
15
23
1.00
28
40

FIGURE 10: Effect of thebaine on recurrent inhibition of the MSR.

This figure presents the results of a single experiment. Test responses elicited by stimulation of the central ends of the cut L_6 and S_1 dorsal roots were recorded at the lateral gastrocnemius nerve. These responses were conditioned antidromically, by a stimulus to the median gastrocnemius nerve, which preceded the test stimulus by a varied interval. The inhibitory curves were constructed by plotting the average amplitude of 10 consecutive conditioned responses (expressed as a percent of the unconditioned response) against the interval between conditioning and test stimuli at which they were obtained. The inhibitory curve (□) was determined in the presence 1.03 mg/kg of thebaine.

A photographic record of the response of a Renshaw cell in the same animal, to stimulation of the L_7 ventral root in the presence of 1.03 mg/kg of thebaine, appears in the lower right corner of the figure. The calibration pulse represents 5 msec and 1 mV in relation to the photographic record.

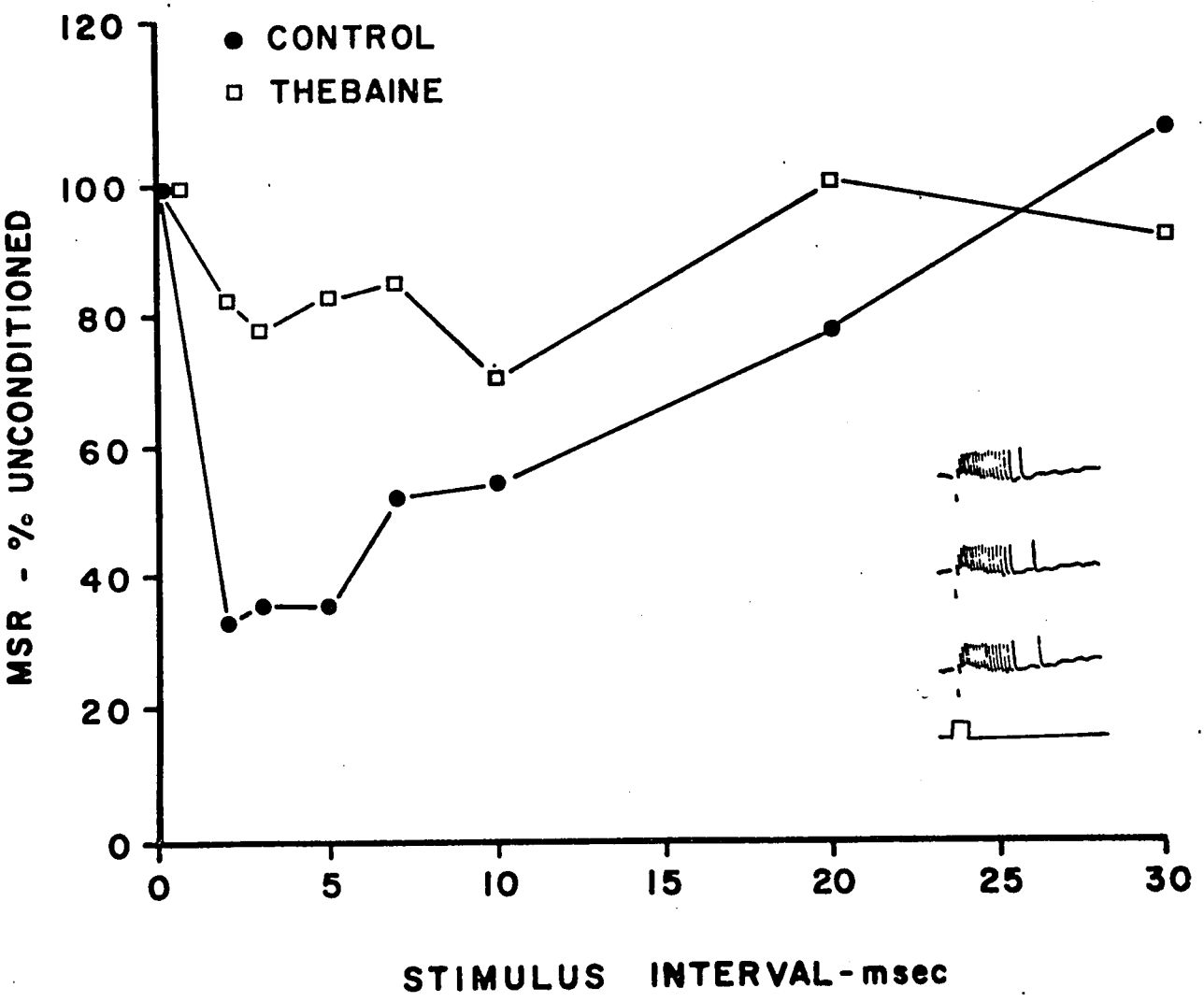

120
100
80
60
40
20
0
MSR - % UNCONDITIONED
CONTROL
THEBAINE
0
5
10
15
20
25
30
STIMULUS INTERVAL - msec

FIGURE 11: Effect of thebaine on recurrent IPSP of spinal motoneurons.

The recurrent IPSP depicted in the figure was evoked by stimulation of the biceps and semitendinosus nerves and recorded intracellularly, in a semimembranosus motoneuron, with a potassium citrate microelectrode. The traces represent the sum of 50 consecutive oscilloscope sweeps recorded by a computer of average transients (calibration bar is 10 msec). The membrane resting potential of this cell increased by 1 mV during the experimental period.

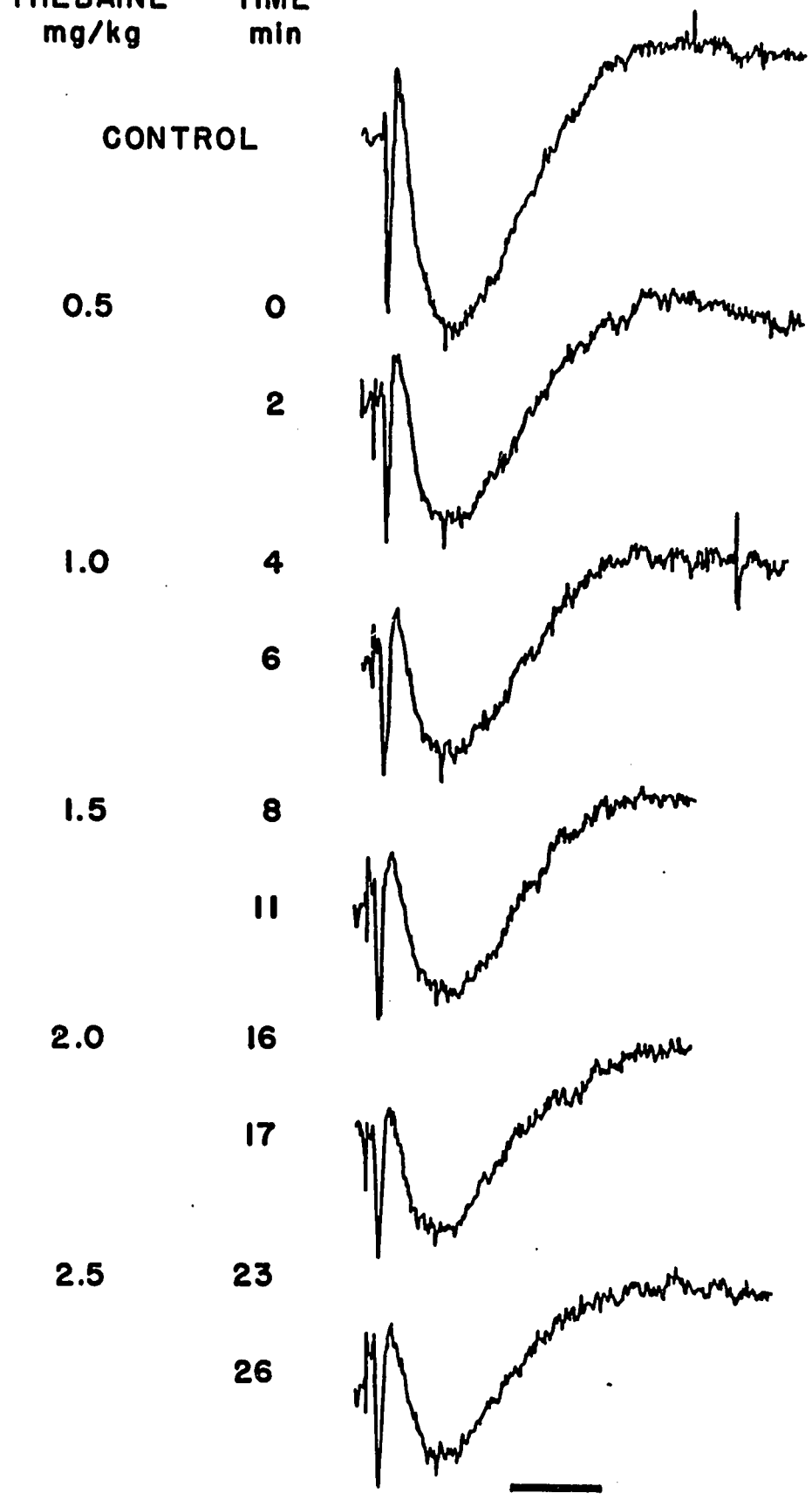

THEBAINE mg/kg
TIME min
CONTROL
0.5
0
2
1.0
4
6
1.5
8
11
2.0
16
17
2.5
23
26

FIGURE 12: Effect of thebaine on Renshaw cell responses evoked by ventral root stimulation.

The figure presents a photographic record of the responses of a single Renshaw cell, recorded extracellularly with a sodium chloride micropipette. The paired traces, representing the response of the cell to a single ventral root stimulus, were photographed at two sweep durations: 20 msec (to the left) and 50 msec (to the right).

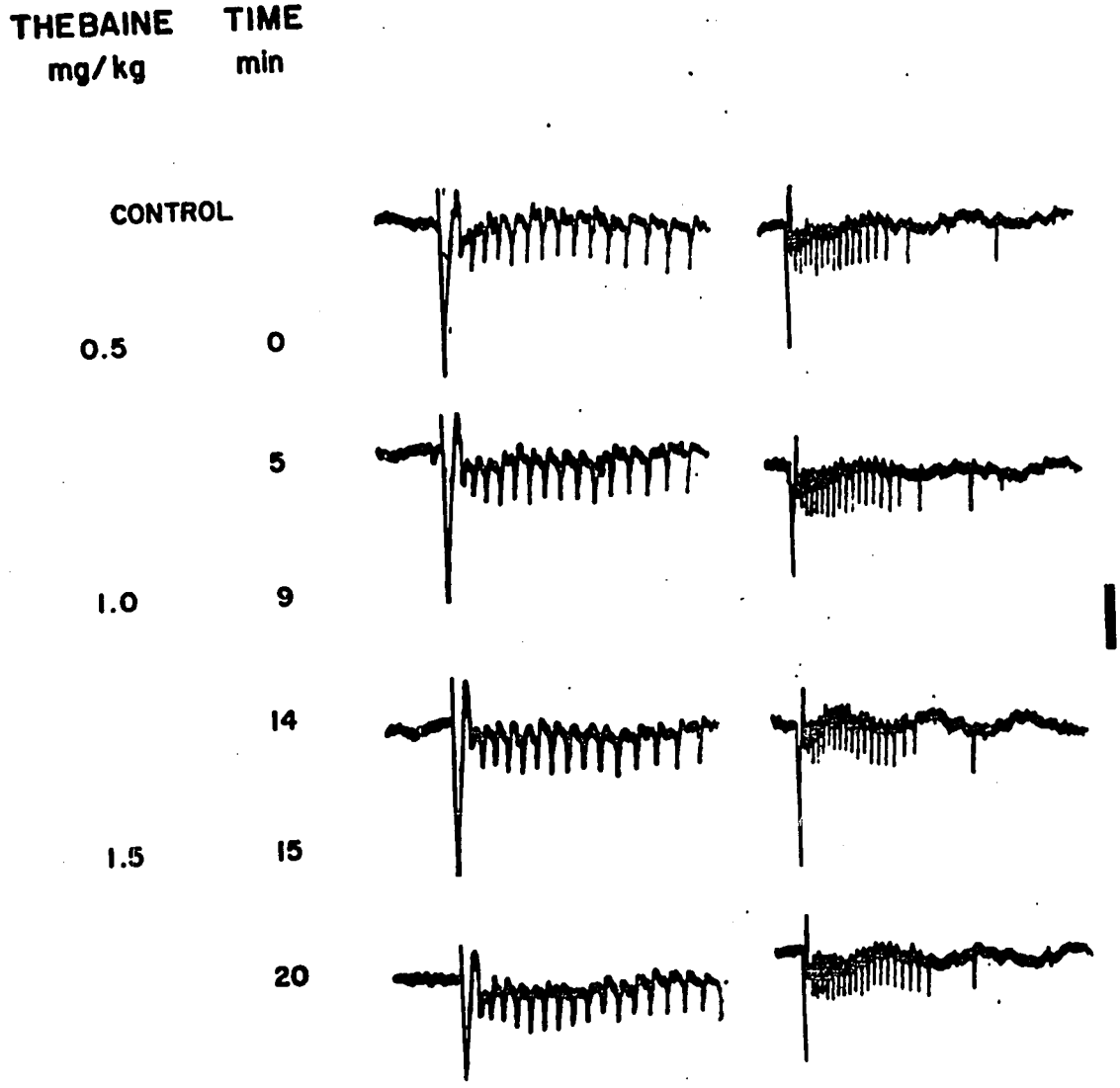

THEBAINE
mg/kg
TIME
min
CONTROL
0.5
1.0
1.5
0
5
9
14
15
20

FIGURE 13: Effect of thebaine on polysynaptic inhibition of the MSR.

This figure presents the results of a single experiment. Test responses elicited by stimuli to the triceps surae nerve were recorded at the central ends of the cut L_7 and S_1 ventral roots. These responses were conditioned by a stimulus to the sural nerve, which preceded the test stimulus by a varied interval. The inhibitory curves depicted in the figure were constructed by plotting the average amplitude of 15 consecutive responses (expressed as a percent of the unconditioned response) against the interval between conditioning and test stimuli at which they were obtained. The inhibitory curve (Δ) was determined in the presence of 0.97 mg/kg of thebaine.

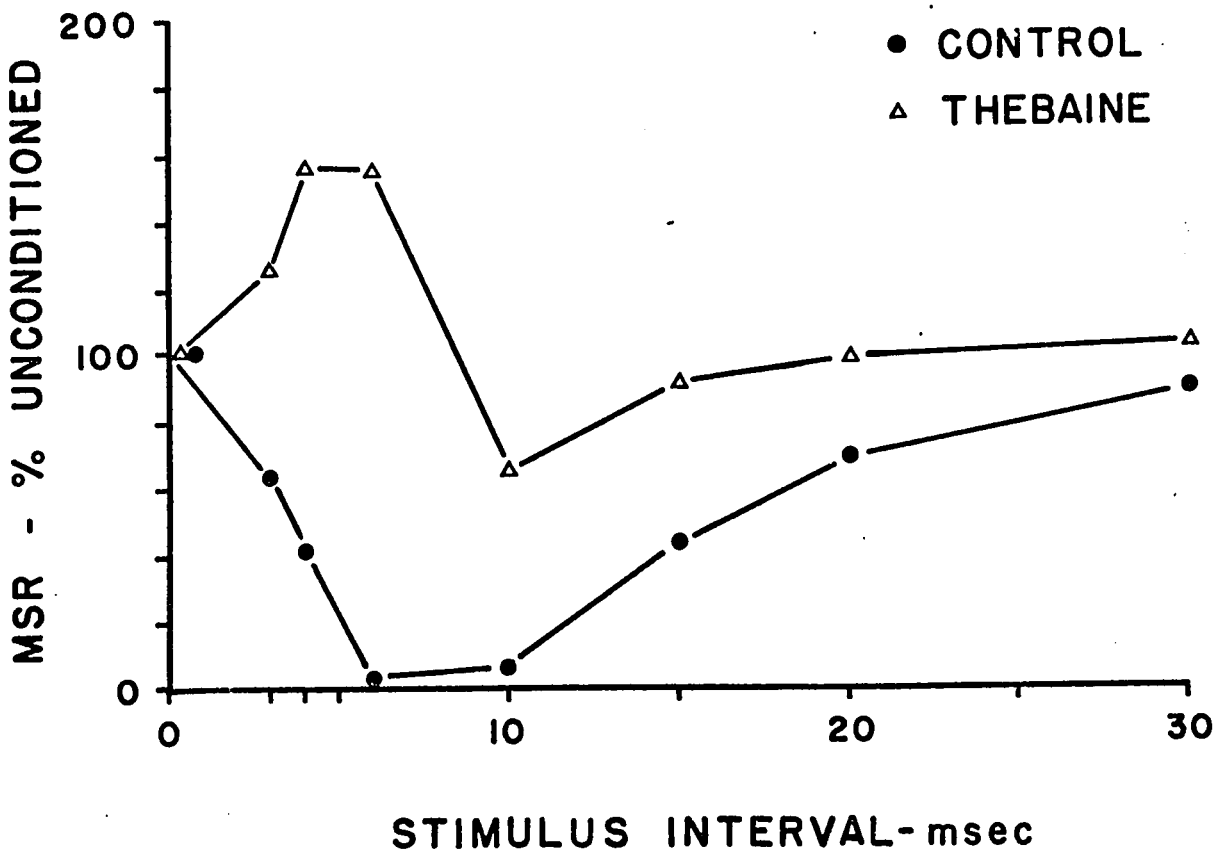

200
100
0
MSR - % UNCONDITIONED
CONTROL
THEBAINE
0
10
20
30
STIMULUS INTERVAL- msec

FIGURE 14: Effect of thebaine on polysynaptic IPSP of spinal motoneurons.

The figure presents the results obtained in two separate experiments. Both polysynaptic IPSP were evoked by stimulation of the hamstring nerve and recorded in a motoneuron, identified by stimulation of the L_7 and S_1 ventral roots. In A, the traces represent the sum of 50 consecutive oscilloscope sweeps recorded by the computer of average transients. In B, the traces represent the sum of 100 consecutive oscilloscope sweeps recorded by the computer of average transients. The calibration bar represents 10 msec.

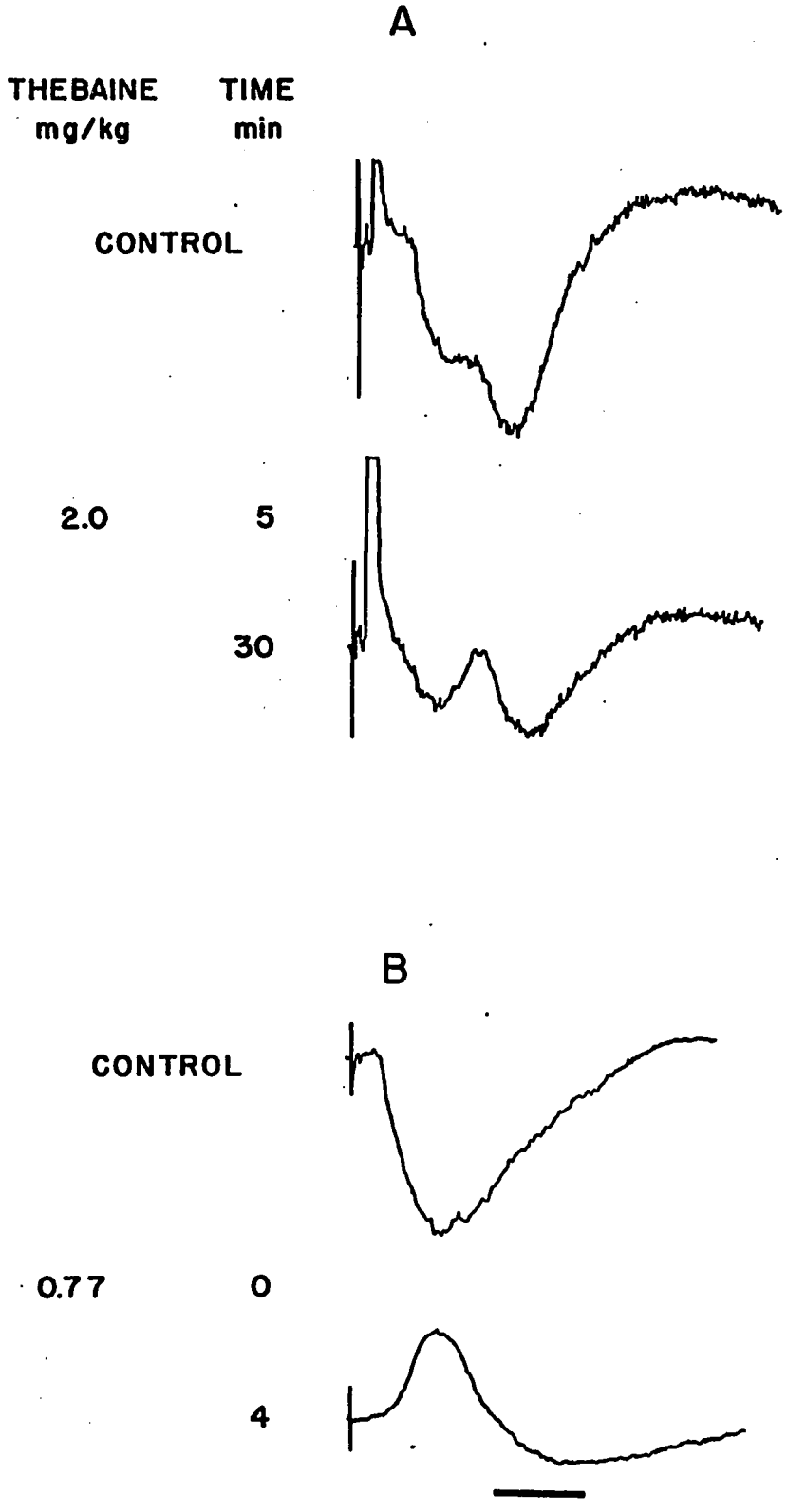

A
THEBAINE
mg/kg
TIME
min
CONTROL
2.0
5
30
B
CONTROL
0.77
0
4

FIGURE 15: Effect of thebaine on remote inhibition of the MSR.

The figure presents the results of a single experiment. Test responses elicited by stimulation of the triceps surae nerve were recorded at the central ends of the cut L_7 and S_1 ventral roots. These responses were conditioned by a stimulus to the hamstring nerve which preceded the test stimulus by a varied interval. The inhibitory curves were constructed by plotting the average amplitude of 20 consecutive conditioned responses (expressed as a percent of the unconditioned response) against the interval between conditioning and test stimuli at which they were obtained. The inhibitory curve (O) was determined in the presence of 2.00 mg/kg of thebaine.

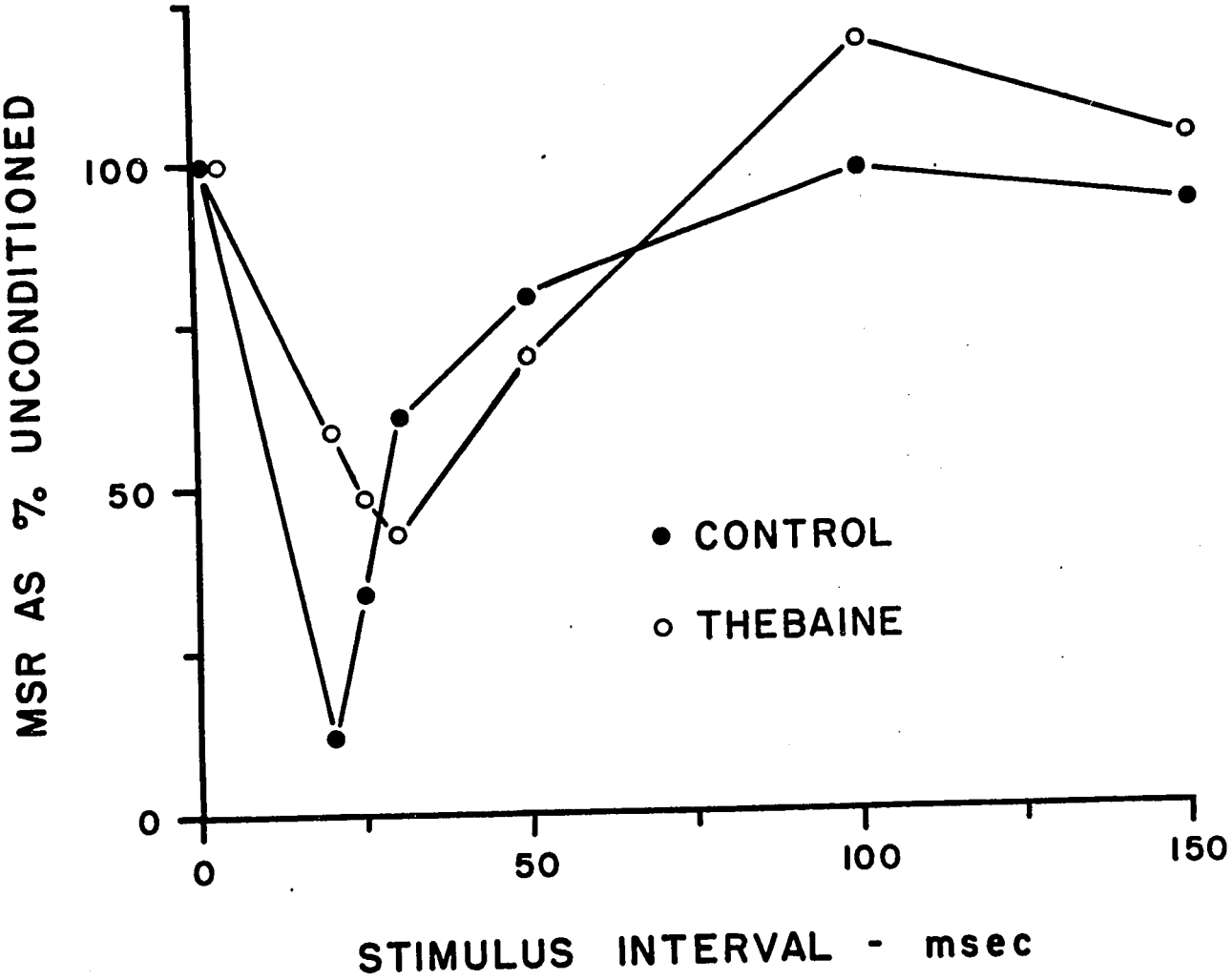

MSR AS % UNCONDITIONED
100
50
0
0
50
100
150
STIMULUS INTERVAL - msec
CONTROL
THEBAINE

FIGURE 16: Effect of thebaine on the afterhyperpolarization of spinal motoneurons.

The figure presents the photographic records of responses obtained in a single experiment. A motoneuron, impaled by a potassium citrate microelectrode, was fired antidromically by stimuli applied to the semimembranosus nerve. The lower of each pair of traces is a record of the antidromic action potential (calibration pulse represents 20 mV and 6 msec). The upper trace is a record of the afterhyperpolarization following the antidromic action potential, obtained at a higher gain (calibration pulse represents 2 mV and 6 msec). The membrane resting potential of this cell increased by 1 mV during the course of the experiment.

THEBAINE	TIME
0	0
0.5	10
1.0	15
1.5	20
2.0	30

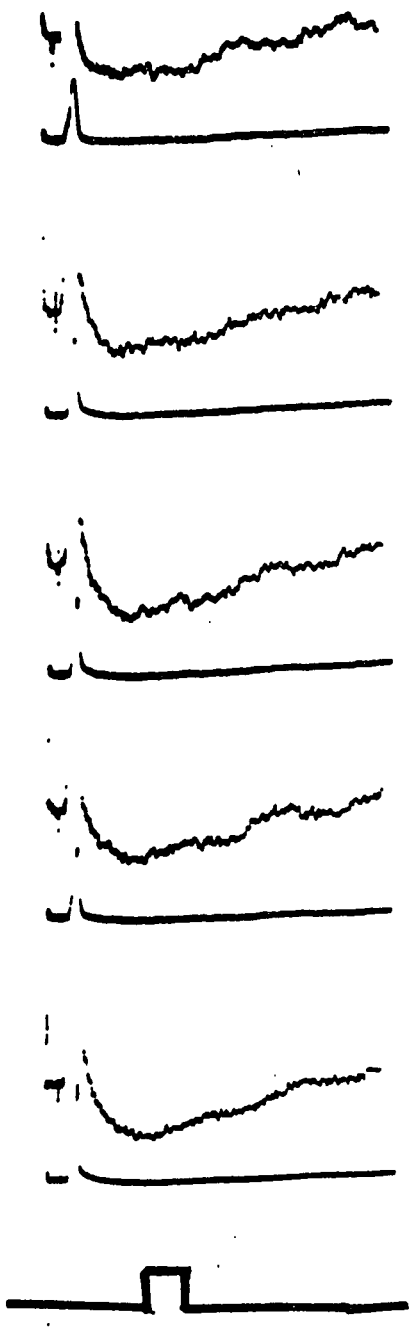


FIGURE 17: Effect of thebaine on the excitability of spinal motoneurons.

The figure presents the strength-duration relation of a single motoneuron determined immediately before and 5 minutes after the intravenous administration of 2.00 mg/kg of thebaine. The ordinate represents the intensity of the depolarizing current pulse (8.5 msec in duration) passed through the recording microelectrode. The abscissa represents the latency of the action potential evoked by the depolarizing current.

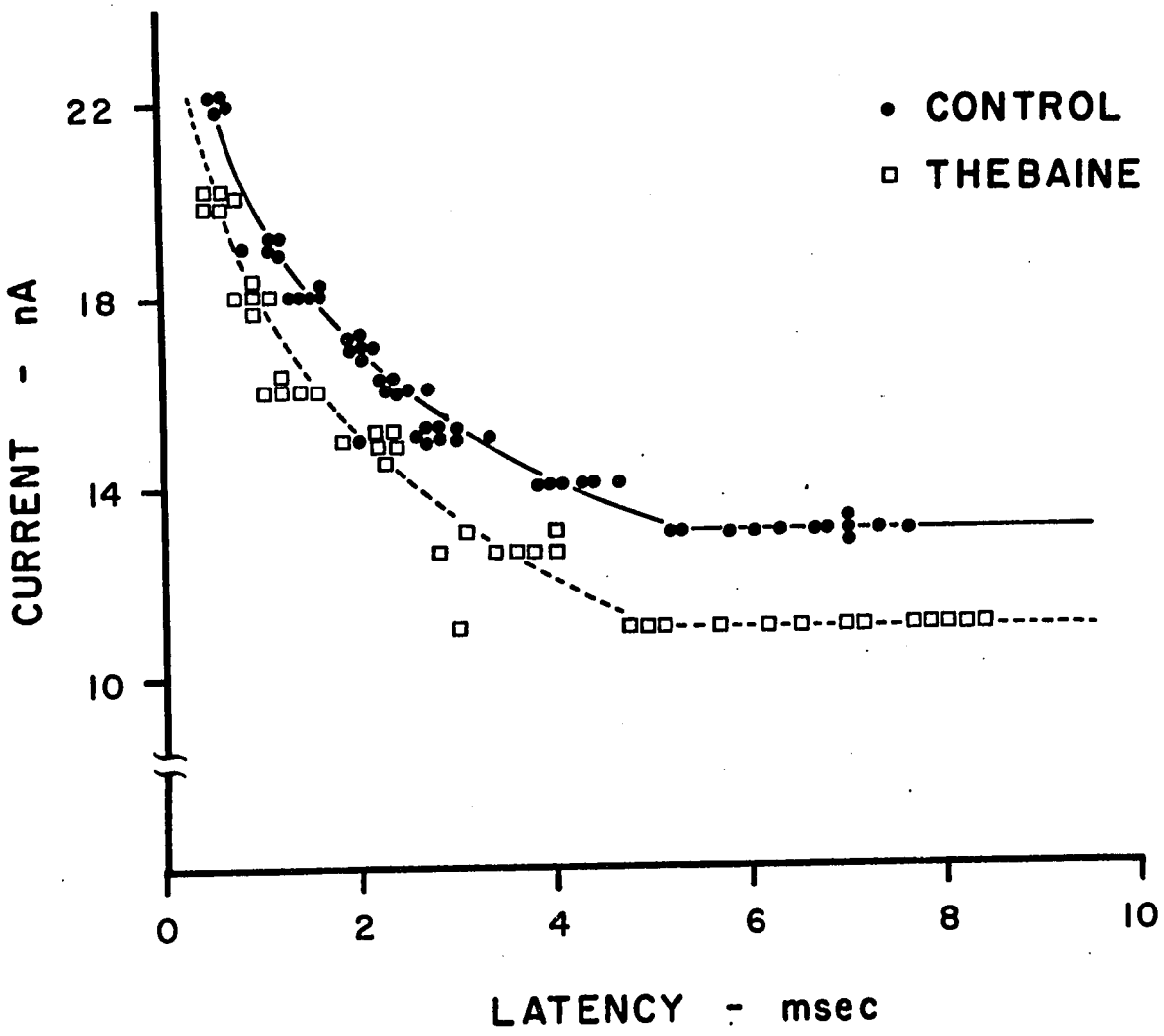

• CONTROL
□ THEBAINE
CURRENT - nA
22
18
14
10
0
2
4
6
8
10
LATENCY - msec

FIGURE 18: Effect of a combination of thebaine and strychnine on direct inhibition of the MSR.

Test responses elicited by stimulation of the hamstring nerve were recorded at the central ends of the cut L_7 and S_1 ventral roots. These responses were conditioned by a stimulus to the quadriceps nerve, which preceded the test stimulus by a variable interval. The inhibitory curve (△) was determined in the presence of a subeffective dose of thebaine (0.44 mg/kg), and redetermined immediately after the intravenous administration of 0.47 mg/kg of strychnine (■).

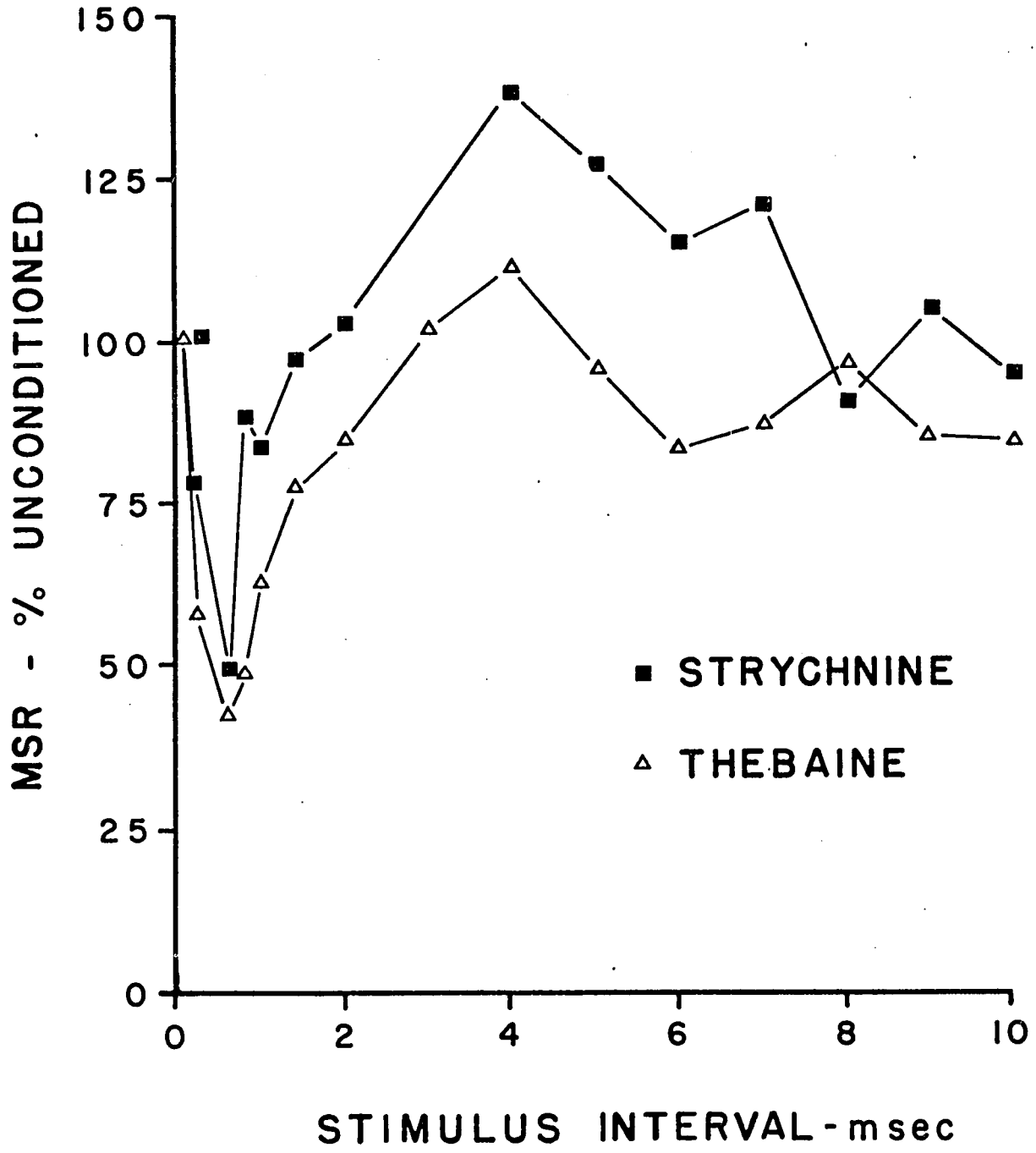

150
125
100
75
50
25
0
MSR - % UNCONDITIONED
0
2
4
6
8
10
STIMULUS INTERVAL - msec
STRYCHNINE
THEBAINE

FIGURE 19: Effect of nalorphine on direct inhibition of the MSR, in the presence of thebaine.

Test responses elicited by stimulation of the S_1 dorsal root were recorded at the S_1 ventral root and conditioned by a stimulus to the L_6 dorsal root. The inhibitory curve (△) was obtained 5 mins after the intravenous administration of 0.87 mg/kg of thebaine. 20 mins later, nalorphine (10 mg/kg) was injected and the inhibitory curve redetermined in the presence of both drugs (▼).

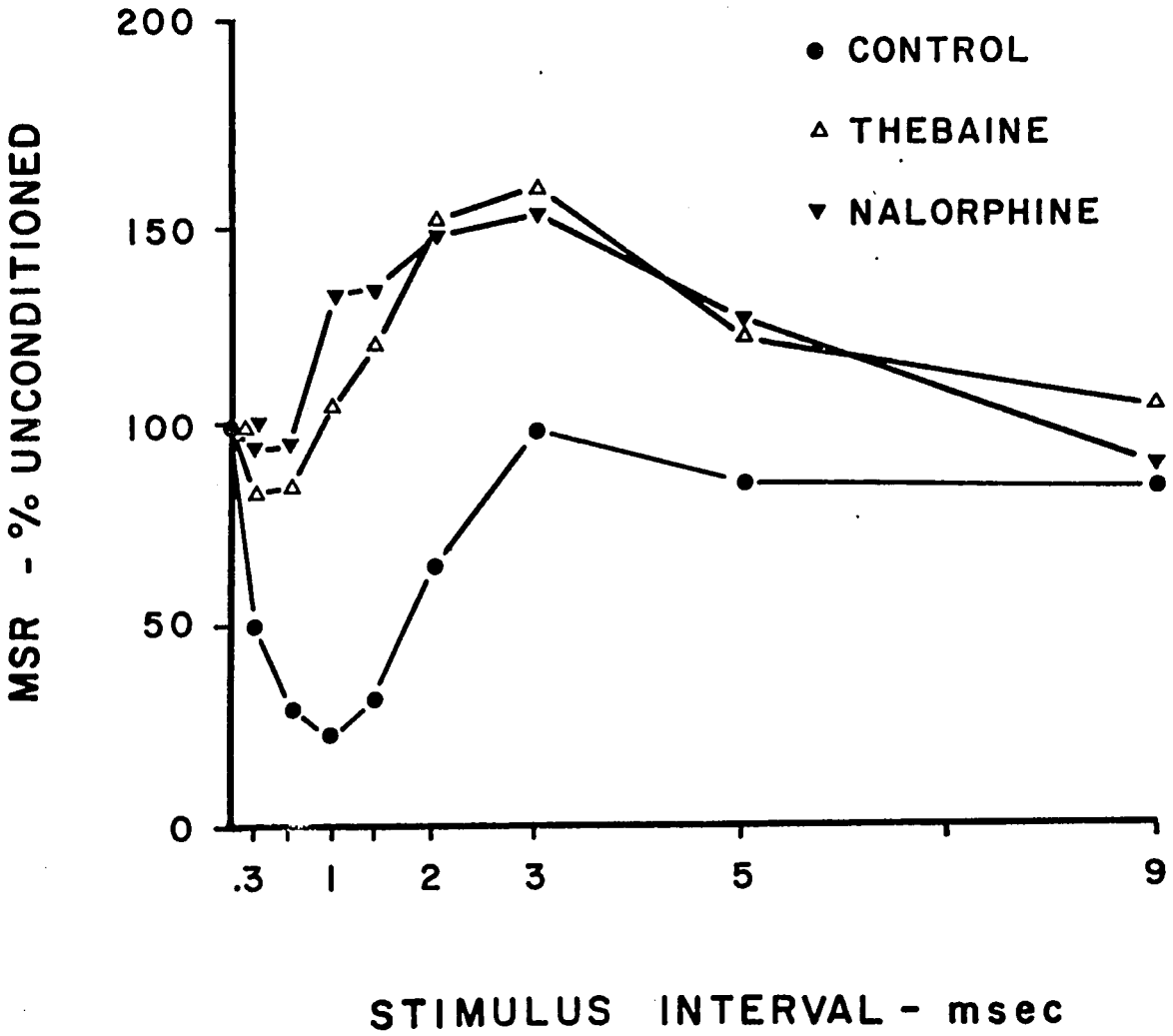

200
150
100
50
0
MSR - % UNCONDITIONED
CONTROL
THEBAINE
NALORPHINE
.3
1
2
3
5
9
STIMULUS INTERVAL - msec

FIGURE 20: Effect of thebaine on spontaneous activity of spinal motoneurons, recorded at a ventral root.

The figure presents photographic records of representative responses obtained in a single experiment. The traces to the left are a continuous recording of the level of spontaneous activity in the L_7 ventral root before drug administration. The traces to the right were recorded during one of the recurrent bursts of intense spontaneous activity which resulted from the intravenous injection of 1.83 mg/kg of thebaine. The calibration pulse represents 0.2 mV and 45 msec.

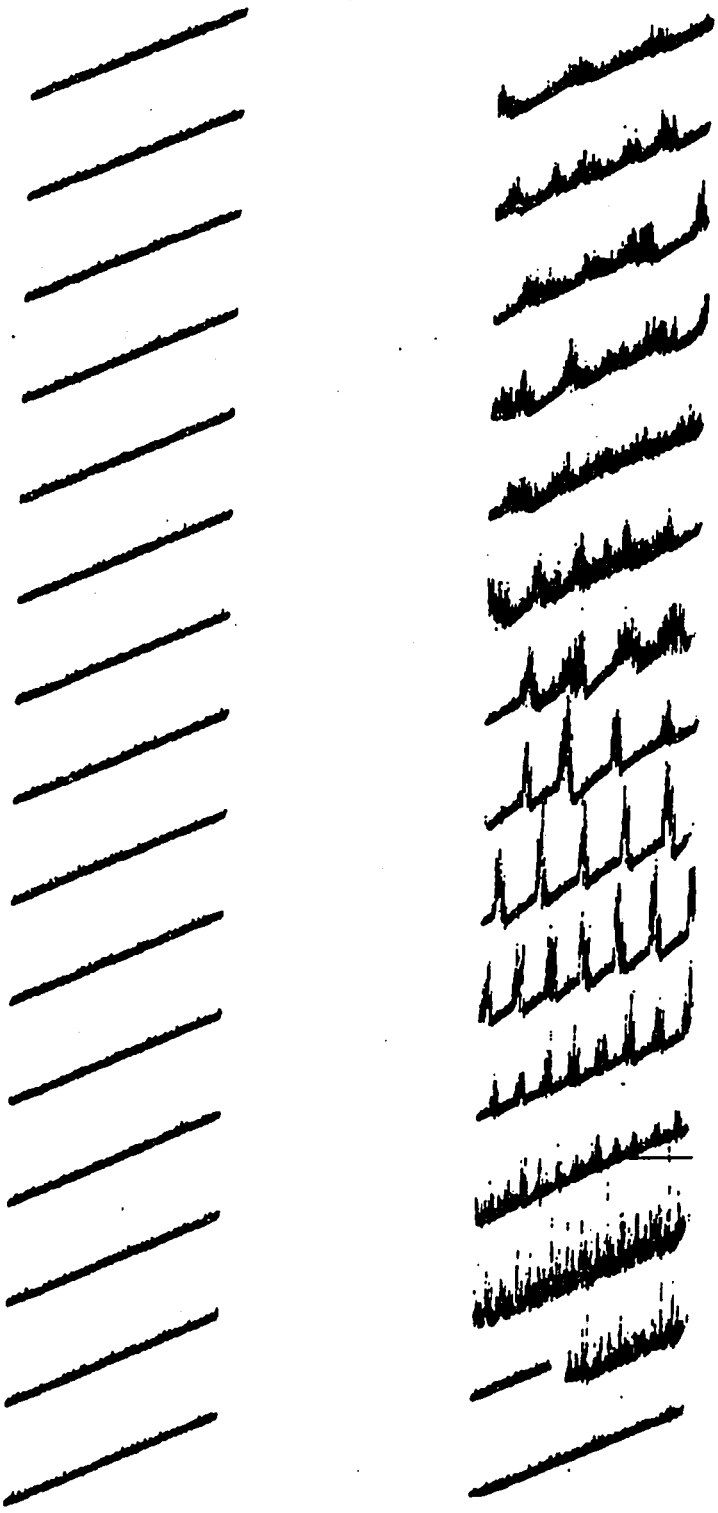
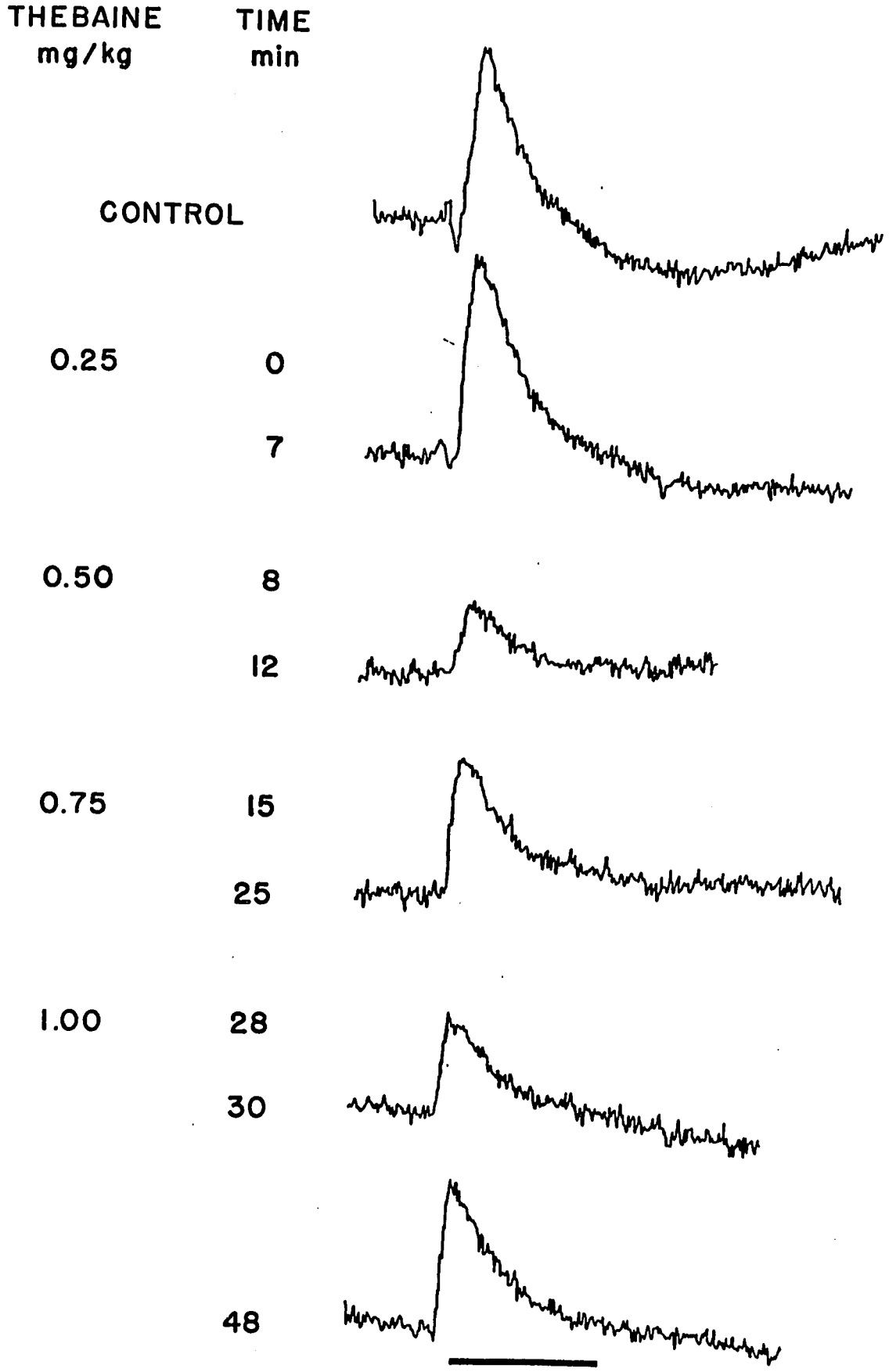

CONTROL
THEBAINE

FIGURE 21: Effect of thebaine on monosynaptic EPSP of spinal motoneurons.

Monosynaptic EPSP were evoked by stimulation of Ia afferents in the hamstring nerve and recorded intracellularly with a potassium citrate microelectrode. The traces represent the sum of 110 consecutive oscilloscope sweeps, recorded by a computer of average transients. The calibration bar represents 5 msec. The membrane resting potential of this cell was unchanged during the experiment.


THEBAINE
mg/kg
TIME
min
CONTROL
0.25
0
7
0.50
8
12
0.75
15
25
1.00
28
30
48