NEUROPHARMACOLOGICAL STUDIES OF THE SYMPATHETIC REFLEX ARC

by

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I. INTRODUCTION

A. Statement of the Problem

The development of techniques for recording neural activity from preganglionic white rami communicantes has furnished an approach for the analysis of the organization of sympathetic efferent activity in the spinal cord. Studies of the spontaneous activity and reflex activation of sympathetic preganglionic neurons isolated from supraspinal control have demonstrated the integrity of sympathetic reflex arcs and have provided insight into the integrative capability of spinal mechanisms controlling the sympathetic outflow. Comparison of the reflex activity of sympathetic preganglionic neurons with that of somatic motoneurons reveals both similarities and differences in the central organization of these distinctly separate efferent systems.

Information concerning the effects of pharmacological agents on sympathetic preganglionic neurons and their central reflex pathways is almost non-existent, in marked contrast to the exponential accumulation of knowledge and the proliferation of research pertaining to the pharmacology of the peripheral sympathetic nervous system. The realization that preganglionic neurons represent the "final common path" of the sympathetic nervous system serves to emphasize the importance of this gap in information. The vulnerability of spinal somatic reflex connections to metabolic modification or pharmacologic intervention is well known. Likewise, preganglionic neurons and their reflex connections should be readily accessible to the influence of drugs; functional alteration at these sites remains an unexplored mechanism whereby pharmacological

agents may alter sympathetic activity.

Therefore, the present investigation represents an initial study of the neuropharmacology of sympathetic preganglionic neurons. This thesis describes the effects of several selected drugs on the spontaneous and reflex activity of sympathetic preganglionic neurons in the unanesthetized spinal cat. The selection of drugs for study was made in consideration of the several aims of the investigation: (i) to expand present understanding of the basic physiology of sympathetic reflex arcs, (ii) to compare the effects of drugs on spinal sympathetic reflexes with their recognized effects on spinal somatic reflexes, (iii) to gain insight into potential transmitter agents involved in the activation or inhibition of preganglionic neurons, (iv) to describe the pharmacological activity of agents that produce sympathetic effects by an action on sympathetic mechanisms in the spinal cord.

In keeping with the proposed goals, the selection of mephenesin, strychnine, and picrotoxin were made for several reasons. The recognized effects of each of these drugs on somatic synaptic transmission in the spinal cord provide a basis of comparison for the present study. In addition, their mechanisms of action are at least partially understood. Their use as pharmacological tools, especially strychnine and picrotoxin, has been instrumental in establishing current concepts of central synaptic transmission, particularly in regard to inhibitory mechanisms. Furthermore, additional insight regarding the mechanism of vasopressor effects of analeptic agents may be gained by correlation of the peripheral sympathetic response with the neural activity of spinal sympathetic centers.

The recent histological demonstrations of monoamine-containing neurons in the central nervous system has aroused new speculation regarding the possible transmitter function of monoamines. In particular, a prominent convergence of descending axons containing monoamines has been found to terminate in the intermediolateral columns of the spinal cord, that is, in the vicinity of sympathetic preganglionic neurons (Carlsson et al., 1964; Dahlström and Fuxe, 1965). The possibility of adrenergic transmission to sympathetic preganglionic neurons prompted the present studies of the effects of the monoamine precursors, dihydroxyphenylalanine and 5-hydroxytryptophan, on preganglionic neural activity.

B. Physiology of Spinal Sympathetic Reflexes

The capability of the isolated spinal cord to mediate sympathetic reflexes has been demonstrated repeatedly. For example, vasomotor changes in acute or chronic spinal animals have been observed following electrical stimulation of peripheral nerves (Sherrington, 1906; Langley, 1924; Brooks, 1933), mechanical stimulation of the viscera (Downman and McSwiney, 1946; Mukherjee, 1957), and thermal or mechanical stimulation of the skin (Sahs and Fulton, 1940; Kuntz, 1945; Richins and Brizzee, 1949). Reflex sudomotor adjustments in spinal animals have also been described (Wang and Brown, 1956; Wang, 1964). Similar vasomotor and sudomotor reflexes have been demonstrated in spinal man (Riddoch, 1917; Leriche and Fontaine, 1927; Guttmann and Whitteridge, 1947; Pollock et al., 1951). Such observations of peripheral sympathetic activity indicate the existence of sympathetic reflexes at the

spinal level and provide some evidence for their segmental arrangement; however, this indirect approach can contribute only limited knowledge of the neurogenic mechanisms involved.

Most electrophysiological approaches to the study of sympathetic efferent mechanisms have emphasized supraspinal control to the neglect of purely spinal contributions. The few, previous studies in which spinal mechanisms have received attention are not very elucidating. Alexander (1945) noted that the spontaneous activity of spinal sympathetic centers of the cat varied inversely with blood flow or oxygen supply in the spinal cord. Postganglionic recordings from the deafferented spinal cord led to the conclusion that the pO_2 in the cord may affect the central excitatory state of preganglionic neurons. Subsequently, Alexander (1946) reported a loss of reflex responses evoked by sciatic nerve stimulation and recorded from the inferior cardiac nerve when the spinal cord was transected, although irregular tonic activity persisted. Likewise, Schaefer (1960) found no evidence of reflex pathways between skin or muscle afferents and preganglionic neurons except by way of medullary centers; segmental connections apparently did not exist. Such evidence is contrary to the numerous examples of peripheral vasomotor and sudomotor adjustments in spinal animals and man.

Recently, definitive neurophysiological studies of sympathetic preganglionic neurons by Perl and associates have resolved much of the controversy surrounding their functional capabilities in the isolated spinal cord (Beacham and Perl, 1964a,b; Fernandez de Molina, Kuno, and Perl, 1965; Fernandez de Molina and Perl, 1965; Franz, Evans, and Perl, 1966).

Conventional electrophysiological recordings from upper thoracic and upper lumbar sympathetic preganglionic rami or extracellular and intracellular microelectrode recordings from preganglionic neurons in the functionally decerebrate cat provided the results of these studies. These results will be discussed in some detail as essential background for the present investigation.

Preganglionic fibers regularly exhibited a background discharge that could often be modified by noxious as well as mild thermal or mechanical stimuli. Such stimuli could increase or decrease the resting discharge and could be either specific in type and localization or relatively nonspecific. Long-term (25-60 sec), cyclic increases in background discharge were often directly and temporally related to periodic fluctuations in the systemic blood pressure; increments in blood pressure lagged the increased neural discharge, and both patterns could be altered by hemodynamic changes. This relationship indicates a basic mode of vasomotor control at the spinal level.

A reflex discharge of preganglionic neurons was commonly evoked by excitation of small myelinated afferent fibers (conduction velocity, 10-35 m/sec) in dorsal roots or spinal, limb, or visceral nerves. Reflexes were localized within several segments of afferent entry to the spinal cord. Responses involved a few or many units and could be graded according to the strength of the afferent volley. The duration of reflex latency and its variability excluded the possibility of monosynaptic connections between primary afferent fibers and preganglionic neurons; the greater latency of viscerosympathetic reflexes indicated that they have a more complex central pathway than do somatosympathetic reflexes. Excitatory convergence of afferent fibers in

different nerves upon a limited pool of excitable neurons was demonstrated by common excitation of single preganglionic units and by mutual occlusion of the evoked discharge.

The frequent failure of spontaneously active units to engage in the reflex discharge or to be affected by it and the intense mass discharge during temporary asphyxia indicated that the number of preganglionic neurons available to spinal reflexes included only a portion of the sympathetic outflow. Furthermore, although localized changes in blood flow and transient increases in systemic arterial pressure could be reflexly induced, reflex changes in heart rate either directly or through adrenal medullary secretion could not be demonstrated; evidence of reflex piloerection is similarly absent. Thus, reflex or background discharges have been related only to vasomotor control; the possibility that a portion of the reflexly-activated sympathetic outflow may be sudomotor appears likely on the basis of electrodermal studies (Wang, 1964).

Maximal reflex responses were usually obtained at stimulus intensities that were 1.5 to 2 times the reflex threshold. However, stimulus intensities sufficient to include nonmyelinated afferent fibers frequently produced an intense, prolonged after-discharge following the initial, low-threshold response in lumbar rami. Splanchnic stimulation was more effective than stimulation of lumbar spinal nerves in producing an after-discharge. Although after-discharge could be evoked in the midthoracic region by splanchnic stimulation, it was not seen in upper thoracic rami or in response to stimulation of intercostal nerves.

The excitability of sympathetic reflexes following a maximal response recovered rapidly, so that a large fraction of the pool could be discharged again after 20-30 msec. The recovery of reflex responsiveness was variable for different units; one single unit studied was re-excitable after 4 msec, but others were depressed for over 40 msec. Usually, the size of population reflex responses declined when frequencies of afferent stimulation exceeded 0.5/sec, but some preganglionic units would follow up to 20/sec before failing. Results of extracellular and intracellular microelectrode recordings from preganglionic neurons during antidromic invasion substantiated their rapid recovery of excitability. The absence of recurrent inhibition by axon collaterals and a small, post-spike hyperpolarization of 20-60 msec duration were consistent with recovery times for spinal reflexes.

Facilitation of sympathetic spinal reflexes was difficult to demonstrate and rarely recruited additional preganglionic neurons into a maximal reflex response. Short trains of tetanic stimulation also failed to facilitate the response, and post-tetanic potentiation was rare and never large. The absence of facilitation indicated a virtual lack of subliminal fringe available for discharge in a reflex response.

Usually, spinal sympathetic reflexes were markedly depressed for several hundred milliseconds by a prior conditioning volley in the same nerve or in a different nerve that also evoked a reflex response upon stimulation. In some experiments, the comparatively small size of the conditioning reflex indicated that the depression was due to inhibition rather than to refractoriness of common units. In one experiment, a single unit was re-excitable in less than 4 msec

by the second of two afferent volleys in a spinal nerve but was inhibited for 200 msec by a conditioning volley in the splanchnic nerve that evoked a discharge of the same, single unit.

Inhibition required 20-30 msec for onset and reached a maximum 10-30 msec later. Complete recovery required up to 400 msec. The rapid recovery of reflex excitability discussed above was frequently obscured by this type of afferent inhibition. In such cases, recovery was often biphasic: an initial steep recovery phase corresponded to determinations of reflex recovery in the absence of inhibition, whereas the second phase was similar to the prolonged inhibition where failure of recovery in common preganglionic units could not have been a significant factor. In other experiments, separation into two phases was less obvious. Division of afferent fibers into two types, excitatory and inhibitory, on the basis of differences in conduction velocity was unsuccessful; the possibility of mixed influences from one afferent nerve has not been eliminated. The mechanism and the central site of inhibition of sympathetic preganglionic neurons remain completely obscure.

C. Spinal Cord Pharmacology

Since the recognized effects of selected drugs (mephenesin, strychnine, picrotoxin) on spinal somatic mechanisms are to serve as a basis of comparison for the present study of the effects of these drugs on spinal sympathetic mechanisms, a succinct review of the pertinent pharmacology of each drug is necessary. In addition, the rationale for their inclusion as pharmacological tools in the present experimental approach may become more apparent. Accordingly, discussions

are limited to effects of the drugs on the spinal cord, except where reference to other neuronal systems may contribute to an understanding of their spinal effects. The interpretation of the effects of dihydroxyphenylalanine and 5-hydroxytryptophan on the spinal cord is less secure than that of the other three drugs. However, a brief summary of relevant observations concerning their effects on somatic transmission and the central distribution of monoamines should explain their use in the present investigation.

1. Mephenesin

Mephenesin is the prototype of a series of drugs that selectively depress certain neuronal systems controlling muscle tone and movement, thereby producing relaxation of skeletal muscle without loss of consciousness by an action on the central nervous system. The problems surrounding interpretations of the mechanism of muscle relaxation by these drugs have recently been reviewed by Smith (1965); in short, the mechanism of their relaxant action has not been elucidated. Nevertheless, efforts to define this mechanism have disclosed prominent effects on synaptic transmission in the central nervous system. A preferential depression of polysynaptic reflexes over monosynaptic reflexes in the spinal cord and in some supraspinal pathways has led to their characterization as "interneuronal blocking agents". However, not all polysynaptic pathways are selectively depressed (King and Unna, 1954), and depression of monosynaptic reflexes can be demonstrated, although reduction of polysynaptic reflexes is clearly greater (Henneman, Kaplan, and Unna, 1949; Kaada, 1950; Taverner, 1952; Latimer, 1956).

Longo (1961) found significant depression of repetitive firing in some interneurons by mephenesin, but a prototype interneuron, the Renshaw cell, was only slightly affected (McIntyre, Mark, and Steiner, 1956; Longo, Martin, and Unna, 1960). The marked depression of synaptic recovery of spinal reflexes exhibited by mephenesin provides a basis for the reduction of repetitive activity and may underlie its mechanism of action (Esplin, 1963). If repetitive activity is necessary for synaptic transmission through interneurons, its depression could account for selective failure of polysynaptic reflexes.

2. Strychnine

No drug affecting the central nervous system has been investigated more intensively than strychnine. Although all portions of the central nervous system are affected, its predominant effects are generated within the spinal cord. Strychnine and related substances have been found to depress all types of postsynaptic inhibition in the spinal cord, and their convulsant properties have been ascribed to this mechanism. Bradley, Easton, and Eccles (1953) initially demonstrated that postsynaptic inhibition of monosynaptic reflexes is markedly depressed by subconvulsive doses of strychnine. Subsequently, intracellular recording from motoneurons revealed that this effect is due to depression of the inhibitory postsynaptic potential (IPSP) (Coombs, Eccles, and Fatt, 1955). These observations have been confirmed for all spinal postsynaptic inhibitory mechanisms on which strychnine has been tested (Curtis, 1959, 1963; Eccles, 1964) with the exception of one type which appears to be resistant to strychnine (Green and Kellerth, 1966). The ability to block postsynaptic inhibition

selectively has provided a valuable research tool for the study of synaptic transmission in the central nervous system. Some large and prolonged IPSPs and postsynaptic inhibition of neurons located in the higher centers of the brain are markedly resistant even to large doses of strychnine. However, topical strychninization has been reported to depress postsynaptic inhibition and IPSPs in some cortical neurons (Pollen and Marsan, 1965).

Although unconditioned monosynaptic reflexes are variably affected by strychnine, depending on the dose and the type preparation used, the amplitude of polysynaptic reflexes is markedly increased in all types of preparation (Kaada, 1950; Bernhard, Taverner, and Widén, 1951; Brooks and Fourtes, 1952). The increase in polysynaptic reflexes has been attributed to strychnine depression of inhibitory synapses along the polyneuronal pathways, thereby recruiting additional neurons from the subliminal fringe (Bradley, et al., 1953). Alteration of monosynaptic reflexes may depend on the balance of background inhibition and facilitation in the particular preparation.

Wall and Horwitz (1951) reported that strychnine did not evoke spontaneous, synchronous firing in sympathetic preganglionic neurons but that stimulation in the presence of strychnine produced repetitive firing. On the other hand, Langley (1924) obtained marked, spontaneous increases in the systemic blood pressure of spinal cats with strychnine. Stimulation of peripheral nerves produced a much greater vasomotor response after strychnine than before, suggesting increased excitability of sympathetic centers in the spinal cord. Significantly, strychnine appeared to increase the excitability of only the vasomotor and somatic mechanisms of the spinal cord; reflex or spontaneous effects on

piloerection, sweating, intestinal motility, or cardioacceleration were notably absent. Likewise, higher centers of autonomic integration appeared to be stimulated only weakly by strychnine. Langley's studies of the effects of strychnine on spinal autonomic centers stand very much alone.

3. Picrotoxin

Picrotoxin, unlike strychnine, produces convulsions more readily in the intact animal than in the spinal preparation. On this basis, the primary site of action of picrotoxin has been ascribed to supraspinal centers, and the marked stimulation of medullary respiratory and vasomotor centers, especially when these are depressed, has contributed to its reputation as a medullary analeptic.

Interest in the effects of picrotoxin on the spinal cord has been aroused by its demonstrated effect on presynaptic inhibition. In presynaptic inhibition the excitatory presynaptic terminals of primary afferent fibers are depolarized, thereby diminishing the afferent spike potential and the amount of transmitter released; consequently, the motoneuron may be insufficiently depolarized to generate an impulse. Eccles, Schmidt, and Willis (1963) found that picrotoxin depresses and shortens both the dorsal root potential (DRP), which is used as a convenient, indirect measure of the presynaptic depolarization of primary afferent fibers (PAD), and the resulting presynaptic inhibition of monosynaptic reflexes in cats. Similar effects on the isolated toad spinal cord were demonstrated by Schmidt (1963). The depression of presynaptic inhibition by picrotoxin is less complete than the blockade of postsynaptic inhibition by strychnine. The convulsive activity of picrotoxin is not attributed solely to its depressant action on presynaptic inhibition (Eccles, 1964). Strychnine does

not depress presynaptic inhibition or the DRP; rather, it may enhance them, possibly by increasing the activity of interneurons in the presynaptic inhibitory pathway. The enhancement of presynaptic inhibition by strychnine and its depression by picrotoxin in subconvulsive doses argue against occlusion of interneurons to account for the depressed PAD.

The specificity of picrotoxin as a depressant of presynaptic inhibition may be suspect, since two other convulsant drugs which do not block postsynaptic inhibition, pentylenetetrazol and beta-methyl-beta-ethylglutarimide, were also found to depress presynaptic inhibition (Eccles et al., 1963). On the basis of only a few experiments these observations were not considered significant, because the doses required were considered to be excessive and some of the results were inconsistent. These discrediting observations have been ignored in subsequent discussions of presynaptic inhibition (Curtis, 1963; Eccles, 1964, 1965; Schmidt, 1964, 1965). Furthermore, the powerful presynaptic inhibition of polysynaptic pathways such as the flexor reflexes (Eccles, Kostyuk, and Schmidt, 1962) has not been examined pharmacologically. The failure to examine the effects of picrotoxin on the presynaptic inhibition of polysynaptic reflexes is surprising in view of the thorough study of its depression of presynaptic inhibition of monosynaptic reflexes (Eccles et al., 1963). Thus far, the demonstration of selective depression of presynaptic inhibition by picrotoxin is less convincing than that of selective blockade of postsynaptic inhibition by strychnine.

4. Dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan (5-HTP)

The uneven distribution in the central nervous system of choline acetylase, the enzyme responsible for the synthesis of acetylcholine, led to the proposal by Feldberg and Vogt (1948) that in some central pathways cholinergic neurons alternated with non-cholinergic neurons. Familiarity with peripheral synaptic junctions between cholinergic preganglionic neurons and adrenergic postganglionic neurons in sympathetic ganglia provided impetus for investigations designed to elucidate possible adrenergic systems in the central nervous system. Formerly, measurements of the concentrations of norepinephrine and other catecholamines in various parts of the central nervous system could not define their possible functional significance because discrete neuronal localization was impossible with the available techniques.

More recently, a specific and sensitive histochemical fluorescence method for the cellular localization of catecholamines and 5-hydroxytryptamine has been developed (Carlsson et al., 1962; Falck et al., 1962) and applied to studies of their discrete localization in the central nervous system (Fuxe, 1965; Hillarp, Fuxe, and Dahlström, 1966). A large number of neuronal systems in the central nervous system have been demonstrated to store and form dopamine, norepinephrine (NE), or 5-hydroxytryptamine (5-HT). The highest concentration of such amines are found in the varicosities of the synaptic terminals, but low concentrations also exist in the cell bodies and non-terminal axons. Pharmacological methods have been used to increase or decrease the concentrations of amines in all parts of these central neurons and have aided in tracing their location and pathways. The central monoamine-

containing neurons display the same basic properties and pharmacological reactions as the peripheral adrenergic neurons, which provides strong evidence for the existence of neurotransmission by monoamines in the central nervous system. This evidence has recently been reviewed by Hillarp et al. (1966).

Particularly relevant to the present investigation is the discovery that sympathetic preganglionic neurons receive a direct and very dense, terminal innervation from NE- and 5-HT-containing fibers that descend from medullary neurons by relatively discrete bulbospinal tracts (Carlsson et al., 1964; Dahlström and Fuxe, 1965). Quantitatively, the majority of such descending fibers terminate in the dorsal and, especially, in the ventral horns of the spinal cord, but their distribution is much more diffuse than those that converge on the intermediolateral columns. Retrograde cell changes following spinal root transections have assisted demonstrations that terminals containing NE or 5-HT make intimate contacts with cell bodies of sympathetic preganglionic neurons and alpha-motoneurons, and possibly, with interneurons. The location of cell bodies and fiber tracts of neurons that contain NE or 5-HT and terminate in the sympathetic columns has led to suggestions that they serve inhibitory, vasodepressor, or vasodilator functions; however, the evidence for any of these roles is scanty.

Although NE and 5-HT do not readily cross the blood-brain barrier, their central actions may be studied after intravenous injection of precursors that cross the barrier. Fuxe (1965) demonstrated that DOPA and 5-HTP are decarboxylated and converted to NE and 5-HT, which accumulate with a high degree of specificity in their respective neurons; pretreatment with

monoamine oxidase inhibitors greatly facilitates this accumulation.

The effects of DOPA or 5-HTP on spinal reflexes have been attributed to increased synthesis and overflow of NE or 5-HT from their particular neurons, which suggest possible transmitter functions. Carlsson, Magnusson, and Rosengren (1963) reported facilitation of flexor reflexes by the administration of DOPA or 5-HTP to spinal rabbits. Subsequent studies in spinal cats by Lundberg (1965) add support to the view that these precursors modify spinal reflexes by the formation and release of NE and 5-HT from descending pathways. Both DOPA and 5-HTP depress short latency transmission from the flexor reflex afferents to primary afferents, motoneurons, and to ascending pathways; a secondary, long-latency discharge of flexor motoneurons evoked by tetanic stimulation of flexor reflex afferents is then revealed. In addition, 5-HTP increases excitability of flexor and extensor motoneurons so that monosynaptic reflexes are increased and spontaneous discharges appear in ventral roots. Inhibition of decarboxylase, an enzyme necessary for the formation of NE and 5-HT, prevents the effects of DOPA and 5-HTP, which indicates that the precursors act only after conversion to NE and 5-HT and not directly. Inhibition of monoamine oxidase potentiates the effects of both DOPA and 5-HTP on spinal reflexes. Phenoxybenzamine blocks the effects of DOPA but not those of 5-HTP which, however, are partially blocked by an antagonist of 5-HT; evidently, the NE and 5-HT act on different receptors.

Although the effects of DOPA and 5-HTP on spinal reflexes are poorly understood, the evidence supports the view that they are mediated by NE and 5-HT, respectively. Additional support has been obtained by Weight

and Salmoiraghi (1965, 1966) who demonstrated facilitation or depression of activity in a variety of spinal cord units by electrophoretically applied NE or 5-HT; in some motoneurons, NE depressed injury discharge and blocked antidromic spike invasion. Only depression of spinal neurons by NE and, to a lesser extent, by 5-HT was found in similar experiments by Engberg and Ryall (1965).

In view of the dense convergence of monoamine-containing terminals that make intimate contact with sympathetic preganglionic neurons and the demonstrated activity of monoamine precursors on spinal somatic reflexes, it seemed particularly important to examine the effects of DOPA and 5-HTP on spinal sympathetic reflexes in the present investigation.

II. METHODS

A. Surgical Preparation

Experiments were conducted on unanesthetized, adult cats, between 1.7 and 4 kg body weight. Under ether anesthesia, the trachea was cannulated for subsequent connection to a positive-pressure respirator. Both carotid arteries were ligated in the neck, and one was cannulated and connected to an aneroid manometer for continuous visual monitoring of blood pressure. Under deep ether anesthesia the spinal cord was transected at the atlanto-occipital junction by blunt dissection. Ether administration was then discontinued, and respiratory rate and depth were regulated to maintain end-tidal CO_2 at 4.0 to 5.5% (monitored continuously by an infrared Beckman CO_2 detector, Spinco, LB-1) by an Ensco positive-pressure respirator. The vertebral blood supply to the head was occluded, producing complete pupillary dilatation and loss of reflex movements of the head. An indwelling polyethylene cannula was tied into a radial vein for the intravenous administration of drugs. Deep body temperature was maintained between 36 and 38° C by an external heating plate regulated by a Thermistemp Temperature Controller that monitored rectal temperature through a termistor probe. Gallamine triethiodide was injected intravenously as needed to produce skeletal muscle paralysis throughout the dissection and experimental procedures.

A dorsal midline incision in the upper thoracic or upper lumbar region provided entrance to the dissecting field. Gross dissection and removal of muscle covering the dorsal aspect of the vertebrae and the adjacent, lateral areas of the left side were accomplished with the aid of cautery. Preparations were routinely

placed on their right side and held by metal clamps grasping the dorsal thoracic or transverse lumbar spinal processes. The clamps were rigidly attached to the slotted, metal, animal board (Figure 1A). Upper thoracic sympathetic rami entering the stellate ganglion were partially exposed retropleurally after removal of sufficient intercostal muscle and the heads of ribs 2, 3, and 4. Although care was taken to keep the pleura intact, occasional tears occurred; these were repaired by fine suture, methylcellulose sponge, and fat tissue. Lumbar rami were partially exposed retroperitoneally after removal of muscle and the first three transverse lumbar spinal processes. Adjacent intercostal or lumbar spinal nerves were dissected free and wrapped in Ringer-soaked cotton for subsequent stimulation or recording. Major blood vessels in the operative field were ligated before cutting. Exposed tissues were covered with mineral oil in a pool formed by tying skin flaps to a rigidly supported metal ring.

Final dissection of sympathetic white rami communicantes was performed under mineral oil with the aid of a binocular dissecting microscope (3.5 to 15 X magnification). Preganglionic white rami could be identified by their appearance and location and by their entry into the appropriate sympathetic ganglia and juncture with the appropriate spinal nerve (Figure 1B). Rami were sectioned at the ganglia and were carefully dissected free and cleaned of connective tissue with the aid of knives made from razor blade chips. The presence of persistent background activity and reflexly-evoked discharge provided final identification of preganglionic elements.

In some of the experiments conducted in the lumbar region, a segment of the ipsilateral splanchnic nerve crossing the dorsal crus of the diaphragm was dissected

free and sectioned distally for subsequent stimulation of visceral afferent fibers.

B. Recording Techniques

A block diaphragm of the stimulating and recording equipment is shown in Figure 2. Afferent volleys in spinal and splanchnic nerves were delivered by pairs of fine, platinum-iridium, wire electrodes. Rectangular pulses, 0.25 msec in duration, were applied through a stimulus isolation transformer from a dual stimulator (Ramey-Bosler, Model 58) which also supplied triggering pulses for a cathode-ray oscilloscope and a digital averaging computer.

Efferent electrical activity in preganglionic rami and spinal nerves was led from the central end with pairs of fine platinum-iridium leads and amplified by Tektronix Type 122 low-level preamplifiers. The band pass of the preamplifiers was adjusted within the range of 8 cycle/sec to 1 kc/sec to obtain optimum recording conditions for each experiment. Amplified signals were displayed on a Tektronix 502 Dual-beam Oscilloscope and were photographed by a Grass Model C-4 Kymograph Camera that was triggered manually. Neural activity was continuously monitored by an audio unit. Respiratory movement artifact in the thoracic region was minimized by partially occluding the respiratory hoses and by restricting the movement of disconnected ribs with a blunt retainer that was rigidly attached to the animal board. In addition, rotation of the vertebral clamps allowed partial suspension of the animal, thereby eliminating significant contact with the animal board. Ipsilateral pneumothorax was performed in several experiments where necessary.

The reflexly-evoked, preganglionic discharge was small in amplitude, seldom

greater than 60 μV, and often only slightly greater than background noise. Furthermore, consecutive reflex responses underwent fluctuations in size indicative of phasic changes in neuronal excitability that did not correlate with respiratory movements or heart rate. In order to obtain meaningful quantitative data in many of the experiments, it was necessary to average 25 to 100 evoked responses by a signal-averaging digital computer (Enhancetron 1024, Model ND-800, Nuclear Data, Inc.). The use of this instrument greatly improved the signal-to-noise ratio and facilitated quantitative comparisons of series of evoked reflex responses. The multiscaling mode of the computer was used for two types of measurements: (i) at single, slow sweep speeds (minutes), to detect changes in spontaneous activity; and (ii) at repetitive, fast sweep speeds (milliseconds), to measure changes in the relative firing frequency of units comprising an evoked reflex response. The multiscaling mode of operation was used only when time-frequency data were of interest. Response amplitude was always measured by the averaging mode. Input for the Enhancetron was led from the vertical amplifier output of either beam of the oscilloscope. Enhancetron output was displayed on the other beam of the oscilloscope and photographed. Enlarged (6.5X) film-reader projections of photographic records were measured for quantitative evaluation of the data.

The reproducibility of computer measurements in the present experiments was usually within $\pm$ 5% and never exceeded $\pm$ 10%. Deviations beyond these limits were attributable to the effect of drugs or to noteworthy changes in the condition of the preparation.

C. Experimental Procedures

Recording of preganglionic activity prior to five or six hours following spinal cord transection was prevented by the time required for the extensive operative procedures. An additional period of one to two hours was routinely allowed to insure stable recording conditions. Consequently, at least seven or eight hours elapsed between spinal transection and the start of experimental procedures.

Depending upon the stability of reflex responses and the condition of the preparation, experimental procedures were frequently continued for up to 18 hours after spinal transection. Continuous, visual monitors of blood pressure and end-tidal CO_2 provided reliable indicators of the stability of a preparation. Drug-induced changes in blood pressure were also monitored visually and recorded in the protocol. Blood pressure usually became stabilized near 70 mm Hg, which appeared to maintain the animals in satisfactory condition for prolonged periods. When necessary, a small volume (5-10 ml) of 6% dextran in saline was injected intravenously to maintain this homeostatic condition. The use of cautery, ligation of cut blood vessels, and care to avoid infusion of heparin solution from the blood pressure recording apparatus prevented significant blood loss.

In general, drug-induced blood pressure changes were allowed to subside before reflex testing procedures were continued. When experimental data were collected during marked blood pressure alterations, e.g., pressor effects during intense, drug-induced activity, such changes were recorded in the protocol at frequent intervals.

When stability of the preparation and brevity of drug effect permitted, the effect of a drug on an experimental parameter was tested periodically until partial

reversal of the effect. This procedure confirmed impressions that observed changes were drug-induced.

During reflex testing procedures, responses were evoked at rates of 0.2/sec or 0.5/sec. During long-term testing above 0.5/sec, responses became progressively smaller with increasing rates. The averaging technique required at least 25 evoked responses to provide averages of sufficient amplitude for accurate measurement. Consequently, several minutes were required to obtain a single measurement. In interaction studies, measurements were routinely taken at 6 or 7 intervals between 20 and 200 msec. Measurements at shorter intervals were often impractical because of the duration of the conditioning reflex and its positive after-potential. Such series with appropriate control measurements required 20-30 minutes to complete. It was felt that the accuracy of results obtained during more prolonged series of measurements would be adversely affected by changing drug levels, since the duration of action of most of the drugs studied was relatively short.

Stable solutions of mephenesin, strychnine sulfate, or picrotoxin in isotonic saline were injected intravenously and followed by a 1-ml saline wash. The concentrations of strychnine and picrotoxin solutions was such that no more than 1 ml/kg of the solution was injected during a single administration. The low solubility of mephenesin required a larger injection volume (up to 5 ml/kg) to obtain effective drug levels. d,l-DOPA and d,l-5-HTP were dissolved in warm saline at pH 4, and up to 2 ml/kg of freshly prepared solution was required for injection at higher dose levels.

III. RESULTS

A. Mephenesin

1. Spontaneous activity

The spontaneous activity recorded from sympathetic preganglionic rami in different preparations ranged from its complete absence to vigorous firing that involved many preganglionic units. Vigorous, spontaneous activity was routinely depressed within several minutes by 25 mg/kg of mephenesin. Larger doses produced proportionately greater depression.

Figure 3A illustrates the depression of spontaneous activity in a T3 preganglionic ramus by two, consecutive 25 mg/kg doses of mephenesin. The depression of a high level of spontaneous activity in an L1 ramus by 50 mg/kg of mephenesin is illustrated by the single traces in Figure 3B.

Depression of spontaneous sympathetic activity by mephenesin was not accompanied by a decrease in blood pressure as might be anticipated from the demonstrated vasomotor influences of the sympathetic outflow. Since the volume of mephenesin solutions injected was necessarily large (7-15 ml), a small vasomotor depression, if present, may have been offset by the increased circulating blood volume from the injection.

Immediately following the administration of mephenesin, a transient vasodepressor response occurred; this was then supplanted by a mild vasopressor effect for several minutes. The fall in blood pressure was probably due to a direct effect of mephenesin on vascular smooth muscle, and the subsequent rise was probably due to blood volume expansion by the injection, since it was

duplicated by injection of equivalent amounts of saline. In two of three experiments in which spontaneous sympathetic activity was brisk, the depressor effects produced a sharp increase in neural activity, and the subsequent pressor effects produced an equivalent decrease. However, changes in blood pressure induced by mephenesin did not significantly alter low levels of spontaneous sympathetic activity. The reciprocal influence of blood pressure on spontaneous activity has been ascribed (Alexander, 1945) to changes in blood flow and oxygen supply to the spinal cord.

2. Evoked reflex responses

Mephenesin markedly depressed the amplitude of sympathetic reflex responses evoked by stimulation of afferent fibers in visceral or somatic nerves. Evoked responses were reduced by doses of mephenesin (25-50 mg/kg) that have little effect on monosynaptic reflex pathways. Submaximal responses were almost completely blocked, and maximal responses were usually reduced to less than 50%. Changes in size were clearly defined by measuring the average of 25 to 50 evoked reflex responses. Examples of response averages of depressed reflexes are shown in Figure 4A. Large decrements were also evident in single traces (Figure 4B).

Depression of evoked responses by successive doses of mephenesin was additive, and the degree of depression was dose-dependent. In keeping with the short duration of action of mephenesin, its effect was reversible within one to two hours. The graded depression and the partial recovery of two responses from two, successive doses of mephenesin (25 mg/kg,each) are illustrated graphically with sample reproductions of response averages in Figure 4A.

In several preparations, an apparent prolongation of reflex latency by mephenesin was found to stem from the loss of small, early components of the reflex response. The latency of remaining, large-amplitude components did not change. However, the loss of small, early and late components from depressed responses tended to shorten their duration. Otherwise, the latency and duration were not significantly altered despite an appreciable depression of amplitude.

When latency of two reflex responses in the same preparation differed significantly, the one having the longer latency was more severely depressed by mephenesin. Consequently, the viscerosympathetic reflex pathway was more susceptible to mephenesin than the shorter-latency, somatosympathetic reflex pathway.

3. After-discharge

The after-discharge recorded from lumbar preganglionic neurons in response to activation of nonmyelinated afferent fibers was moderately depressed by 50 mg/kg of mephenesin. The depression was detected on the audio monitor and was recorded by the computer as a decrease in total frequency of discharge during successively evoked responses, as shown in Figure 3C. After-discharges were less sensitive than the initial, low-threshold responses to the depressant effect of mephenesin.

4. Interaction

The effect of mephenesin on the inhibition of sympathetic test reflex responses by a conditioning volley in the same or in a different nerve was determined in six experiments at intervals from 25 to 200 msec. Figure 5 illustrates two such experiments. In A, the reflex response evoked in a T3 ramus by stimulation of

the T4 spinal nerve was inhibited by prior stimulation of the T3 spinal nerve. In B, both conditioning and test stimuli were applied to the T4 spinal nerve. Despite significant depression of evoked responses, mephenesin did not alter the degree or the recovery of inhibition. Small, random deviations from control curves were within the limits of accuracy of measurement. Therefore, it appears that the inhibitory pathways do not differ from the excitatory pathways in their susceptibility to the depressant effect of mephenesin. In this series of experiments, no examples of rapid reflex recovery in the absence of inhibition were encountered.

B. Strychnine

1. Spontaneous activity

Doses of strychnine sufficient to induce spontaneous discharge of motoneurons always induced spontaneous discharge of sympathetic preganglionic neurons as well. Initiation of intense preganglionic discharges by rapid injection of strychnine required a definite threshold dose which differed among preparations. This dose ranged from 0.06 to 0.1 mg/kg. The onset of paroxysmal activity was characterized by an abrupt, intense discharge that was initially continuous. The continuous discharge gradually diminished in intensity for several minutes until it was replaced by periodic bursts which decreased in intensity, frequency, and regularity during the next 15-30 minutes. Occasional, intense discharges were followed by variable periods of relative inactivity. Suprathreshold doses of strychnine produced a similar but more prolonged pattern of paroxysmal activity. The onset was more gradual when strychnine was injected slowly during 10 to 15

minutes. The time-frequency records shown in Figure 6A and B illustrate typical discharge patterns.

Simultaneous recordings of activity in both preganglionic rami and adjacent segmental nerves showed a close correlation of their respective discharges. Prolonged bursts of activity in motoneurons were accompanied by prolonged bursts of preganglionic activity; sympathetic activity was proportional to somatic activity in intensity and duration. Brief, rhythmic "strychnine spikes" of motoneurons were sometimes joined by brief "strychnine spikes" of preganglionic neurons; however, small, transient bursts were often obscured by the general increase in activity of the sympathetic fibers. Intense discharges of both efferent systems were easily evoked by mild stimuli such as limb flexion, touching, or light pinching of the preparation. These discharges were similar in form to those occurring spontaneously. Examples of simultaneously recorded somatic and preganglionic paroxysms after strychnine are shown in Figure 7. The possibility of collateral innervation by recurrent, cholinergic axons between motoneurons and preganglionic neurons to account for the coincidence of discharges was considered; however, the cholinergic blocking agent, dihydro-beta-erythroidine (DHE, 4 mg/kg) failed to alter the synchrony of their respective discharges.

Strychnine-induced discharges of preganglionic neurons were always followed by sharp increases in blood pressure. The amplitude and duration of vasopressor responses were proportional to the severity and duration of these discharges. The onset of pressor effects followed bursts of activity in preganglionic rami by at least 5 seconds, and maximum elevation of blood pressure was reached within an additional 5 to 20 seconds. Following brief, isolated preganglionic discharges,

the blood pressure often rose 50-80 mm Hg and returned to the pre-existing level within 30 seconds. In most instances the neural event ceased before the brief pressor cycle began. Where the initial discharges were intense, as in Figure 6A, bursts recurred too continuously to permit fluctuations in blood pressure between isolated discharges. Nevertheless, Figure 6A illustrates the close correlation between the preganglionic discharge rate the peripheral vasomotor consequences. The pressor effect of short bursts of activity is shown in Figure 6B. The brevity of such pressor effects indicated their mediation by the vasomotor outflow rather than by sympathoadrenal discharge or by the ensuing cardioacceleration.

Following prolonged, severe paroxysmal activity, blood pressure often remained lower than before strychnine injection, which suggested some postictal depression of central vasomotor tone.

2. Evoked reflex responses

The effects of strychnine on evoked sympathetic reflex responses were complex and variable. These effects can best be described under three categories: (a) Occlusion, (b) Enhancement or no change, and (c) Depression.

a) Occlusion. During intense, preganglionic discharges induced by strychnine, evoked sympathetic reflex responses were always depressed. Attempts to evoke responses during bursts of activity invariably failed, and responses that could be evoked between bursts were usually much smaller than control responses. As the interval between consecutive, spontaneous discharges expanded during their declining stage, interposed reflex responses that were equal to or greater than (see below) the size of pre-drug responses could be evoked several seconds after the preceding burst. Although no accurate measurements were made, the

duration and degree of depression following a paroxysm were directly proportional to the intensity of the burst.

The depression of evoked reflex responses during paroxysmal activity was attributed to occlusion or post-activation depression of preganglionic neurons. The complete occlusion or marked depression of units comprising an evoked response indicated their inclusion in the population of preganglionic neurons activated by strychnine.

The average of successive reflex responses evoked during paroxysmal activity was always smaller than control averages due to the occlusion or depression of many of the individual responses. Figure 8A illustrates the graded depression of two reflex responses during intense activity induced by three successive doses of strychnine. As shown in Figure 8B, somatic reflex responses were also partially occluded by intense, spontaneous activity of motoneurons, but this occlusion was less complete than that of sympathetic responses evoked at the same time. During the occlusion period, bursts of sympathetic activity were more readily precipitated by the second of two afferent volleys in close succession than by a single afferent volley.

b) Enhancement or no change. Following termination of occlusive activity, 8 of the 23 evoked preganglionic reflex responses studied were enhanced by strychnine; 8 other responses remained unchanged. Submaximal responses were more regularly and more markedly enhanced by strychnine than were responses evoked by maximal or supramaximal stimuli. Figure 9A shows the averages of submaximal and maximal responses in the same sympathetic reflex pathway and their alteration by two consecutive doses of strychnine. The enhancement of the

submaximal response was appreciably greater than that of the maximal response. Subtle changes were revealed only by the averaging technique, but marked enhancement of reflex responses could also be detected in single traces (Figure 9B and C). When compared with the increase of polysynaptic somatic reflex responses by strychnine, the augmentation of sympathetic reflex responses was clearly less (Figure 9C).

The frequent failure of strychnine to recruit additional preganglionic units into a maximal reflex response provides further evidence that the reflexly excitable pool of preganglionic neurons is limited by the absence or insignificance of a subliminal fringe. In 2 of 3 experiments in which it was possible to record from single units, the units occasionally discharged twice to a single afferent volley. Although the effect of strychnine on single unit discharge was not examined, an increased tendency for multiple firing would account for increases in the size of even maximal reflexes. Evidence for the induction of repetitive activity by strychnine is discussed in the next section which deals with after-discharge.

c) Decrease. The remaining 7 (of 23) sympathetic reflex responses studied were slightly decreased in size after strychnine paroxysms terminated. The response decrement could not be attributed to deterioration of the preparation or to changes in blood pressure or ventilation. Depression of sympathetic reflexes after paroxysmal activity may reflect a postictal reduction in excitability of preganglionic neurons. Activation of previously dormant interneuronal inhibitory mechanisms by strychnine could also account for diminished reflex responses, but interaction studies did not support this possibility.

3. After-discharge

The large after-discharges regularly evoked in lumbar rami by activation of nonmyelinated afferent fibers were prolonged by strychnine, but the intensity of these discharges was not detectably altered.

Although after-discharge of preganglionic neurons in the upper thoracic region is normally absent, brief, secondary discharges were sometimes evoked during mild, spontaneous activity induced by strychnine (Figure 10A and B). This type of after-discharge revealed by strychnine was evoked at stimulus intensities that were presumably too weak to activate nonmyelinated fibers. Some of the more prominent units comprising such low-threshold discharges appeared to fire repetitively (Figure 10A), which suggests that strychnine may induce or enhance repetitive discharges or reflexly-activated preganglionic units, thereby prolonging the duration of the evoked response. Alternatively, strychnine may reveal a small, late component that is normally depressed by a mechanism that is not readily apparent, or the after-discharge may be a small, strychnine paroxysm evoked by the afferent volley.

4. Interaction

Studies of the effects of strychnine on recovery of reflex excitability and inhibition were complicated by several factors. First, as described in the introduction, it was virtually impossible in most experiments to make a clear distinction between reflex recovery and inhibition. Previous studies (Beacham and Perl, 1964b; Fernandez de Molina et al., 1965) have indicated that reflex excitability recovered rapidly in the absence of inhibition. However, rapid recovery is usually obscured by prolonged intrasegmental or intersegmental inhibition.

The two processes become interwoven because of mixed excitatory and inhibitory influences from one afferent trunk and extensive convergence of afferent fibers from different nerve trunks. In some experiments, convergence was minimized by the comparatively small size of the conditioning reflex. Nevertheless, at brief intervals between conditioning and test stimuli, the respective contributions of post-activation depression and frank inhibition could not be estimated with any certainty.

Second, the changes induced in the size of the conditioning or the test response by strychnine were occasionally not parallel, which indicated a greater change in excitability of one central pathway than the other. For example, if the conditioning reflex response was increased by strychnine and the test reflex response was not, inhibition was generally increased. If the converse occurred, inhibition was decreased. Such changes are not unexpected if it is assumed that both inhibitory and excitatory central pathways are similarly affected by drugs.

Third, reliable interaction data could not be obtained during intense spontaneous discharges because of pronounced and irregular occlusion of the reflex responses. However, two responses evoked at short intervals (12 to 100 msec) by stimulation of the same afferent trunk were often affected differently by occlusion. Normally, the second response was much smaller than the first; if the first response was partially occluded, the second response was often equal to or larger than the first, but never greater than the singly-evoked control response before strychnine. The first afferent volley may have inhibited spontaneous activity sufficiently to allow the second volley to evoke a greater reflex.

Since postsynaptic inhibition is depressed for several hours by subconvulsive

doses of strychnine (Curtis, 1959), studies of its effect upon inhibition of sympathetic reflexes in the absence of paroxysms appeared valid. Doses of strychnine used in these studies were equal to or greater than those reported to block postsynaptic inhibition of motoneurons. Routinely, sufficient strychnine was given to elicit a period of spontaneous paroxysms and consequent pressor effects, which were allowed to subside before interaction experiments were begun. The results of such trials were always compared with control trials obtained just prior to drug administration. Following small doses of strychnine, no more than 15 to 30 min separated control and drug trials. Measurements of 25 to 50 averaged responses provided quantitative data for comparisons.

The effect of strychnine on the intersegmental inhibition of sympathetic reflexes is illustrated in Figure 11. Part A shows the marked inhibition of a reflex response in a T3 ramus evoked by the T4 spinal nerve when it was preceded by a conditioning volley in the T2 spinal nerve. The small size of the conditioning reflex response relative to the unconditioned test reflex response is illustrated in the inset at the right of the figure. Such experiments indicated that the prolonged depression of sympathetic reflexes is due to inhibition rather than to refractoriness of common preganglionic units. Strychnine (0.07 mg/kg) failed to alter the degree or the temporal recovery of the inhibition.

The inhibition of a reflex evoked from a somatic nerve (L2) by a conditioning volley in a visceral nerve (splanchnic) is shown in Figure 11B. Although the conditioning reflex is much larger than the unconditioned test reflex in this experiment, the recovery curves do not differ significantly from those in A except that their biphasic nature is more apparent. The slightly decreased

inhibition after strychnine (0.08 mg/kg) cannot be considered significant, since it may have resulted indirectly from the slight depression of both reflexes.

Figure 12 depicts the effect of strychnine on the inhibition of a reflex response evoked by the second of two afferent volleys in the same spinal nerve. In A, the recovery of excitability of a reflex response in the T3 preganglionic ramus resembles the responses in Figure 11, which indicates that the depression is primarily due to inhibition. In order to test the possibility of collateral inhibition of preganglionic neurons by recurrent cholinergic axons, this preparation was treated with dihydro-beta-erythroidine (5 mg/kg) which blocks central cholinergic transmission from recurrent axons of motoneurons to Renshaw cells. As shown, the drug did not affect inhibition. Strychnine (0.1 mg/kg) was similarly without effect on early inhibition but appeared to intensify its later stages; this unexplained effect was not seen in any other experiment and no significance is attached to it.

The rapid recovery of excitability of the lumbar sympathetic reflex response illustrated in Figure 12B indicates that an unusually small amount of intrasegmental inhibition was present. Recovery was about 50% in 10 msec and was almost complete in an additional 100 msec. As in the other experiments, strychnine did not induce any significant change in recovery.

From the foregoing examples, which are typical of this series of experiments, it must be concluded that strychnine has no effect on the central segmental mechanisms by which sympathetic preganglionic neurons are inhibited. This provides strong evidence that the classical postsynaptic inhibition in the spinal cord is not involved in spinal sympathetic reflexes.

C. Picrotoxin

1. Spontaneous activity

The onset of action of systemically administered picrotoxin is quite slow, in contrast to that of strychnine and mephenesin. Within 15 to 30 min after injection of picrotoxin, spontaneous activity in preganglionic neurons began to increase markedly. The intensity of this spontaneous activity then gradually increased to a peak of intense paroxysmal discharges about one hour after injection. The discharges were intermittent, and their frequency and duration declined slowly during the next 2 to 3 hours. Although the spinal preparations varied considerably in their sensitivity to the drug, doses of 1 to 1.5 mg/kg were usually sufficient to produce paroxysmal activity of preganglionic neurons.

The general level of spontaneous preganglionic activity was also increased by picrotoxin. Spontaneous discharges occurred irregularly and varied in intensity and duration (Figure 13A); recurrent bursts of activity were less rhythmic than those induced by strychnine. Intense discharges often could be evoked by touching or pinching the extremities.

Increased spontaneous activity of motoneurons usually appeared earlier and lasted longer than that of preganglionic neurons. However, intense, spontaneous discharges of both efferent systems usually coincided, as shown in Figure 13B. Shortly after prolonged or severe, coincident bursts of activity, a second burst of motoneuronal activity often occurred without a corresponding burst of preganglionic activity; preganglionic neurons appeared to be more susceptible than motoneurons to short-term, post-activation depression.

During the general increase in spontaneous preganglionic activity induced by picrotoxin, blood pressure was increased by 5 to 10 mm Hg. Following spontaneous bursts of activity, blood pressure always rose rapidly, the increment being proportional to the intensity and duration of the burst. The short latency and brief duration of such pressor effects indicated that they were directly induced by a prominent vasomotor component of the discharge. The brevity of the pressor effect would rule against significant sympathoadrenal involvement; piloerection was never seen. As the excitatory effect of picrotoxin on preganglionic neurons declined, systemic blood pressure usually stabilized at a level below that existing before picrotoxin. The extent of this vasodepression appeared to be causally related to the intensity and duration of paroxysmal activity, which indicated a decrement of vasomotor tone due, probably, to postictal depression of sympathetic preganglionic neurons. Some recovery of blood pressure was noted several hours after intense, spontaneous activity subsided.

2. Evoked reflex response

Picrotoxin, in doses sufficient to increase spontaneous activity of preganglionic neurons, regularly increased the size of evoked sympathetic reflex responses. Figure 14 illustrates the time courses and representative response averages of two evoked reflexes that were enhanced by picrotoxin. The series depicted in A shows the effect of two successive doses of picrotoxin. In this experiment, the response remained above control size for more than one hour after the first dose and was further increased by the second dose, which enhanced it for almost three hours.

In Figure 14B the response was increased for more than two hours by a single dose of picrotoxin, after which it became smaller than the initial control. Such

late depression was observed in several experiments and may reflect a degree of postictal depression of preganglionic neurons. Since postictal depression of reflex responses in two preparations occurred in the absence of postictal depression of blood pressure, the depression of responses could not be attributed to hemodynamic deterioration. A significant increase in the size of evoked responses was evident in records of single responses as well as in their response averages (Figure 15A).

The enhancement of submaximal responses was usually greater than that of maximal responses, and threshold stimuli frequently evoked an appreciable response where none could be detected before picrotoxin. Significantly, all reflex responses studied were increased by picrotoxin. Although the increase of some maximal responses was small and relatively brief, an equal number were markedly enhanced.

Responses were variably occluded during and shortly after intense preganglionic paroxysms, which made experimental procedures impractical. However, many of the reported measurements were made during brisk background activity, at which time responses were not occluded but were generally enhanced.

The latency and duration of reflex discharges were not altered by picrotoxin, except as discussed in the following section on after-discharge.

3. After-discharge

The effect of picrotoxin on the after-discharge evoked in lumbar rami by activation of nonmyelinated afferent fibers was not studied. However, a brief after-discharge of upper thoracic reflex responses was often induced by picrotoxin at stimulus intensities that were too low to activate nonmyelinated fibers (Figure 15B). Such secondary responses appeared during the declining phase of paroxysmal activity and were more prominent when a reflex response was evoked infrequently.

This effect of picrotoxin may be attributable to a brief paroxysm evoked by the afferent volley, an increased tendency of preganglionic neurons to fire repetitively, or to the depression of an inhibitory influence that is normally operative.

4. Interaction

Studies of the effects of picrotoxin on interactions between reflex responses were subject to the same complications that were described in connection with strychnine (II. C. 4.): (a) uncertain separation of reflex recovery and inhibition, (b) unequal changes in the size of conditioning and test reflexes, and (c) occlusion of reflexes by intense spontaneous activity. In addition, the slow onset of action by picrotoxin made dose titration more difficult, since dosages that produced some paroxysmal activity were required to produce an effect on interactions. Consequently, 1 to 2 hr separated control and drug trials to ensure adequate drug activity and to allow the period of occlusion to subside. Averages of 25 to 50 consecutive, evoked responses were used for analyses of the effect of picrotoxin on interactions between sympathetic reflexes.

In Figure 16, the reflex response in the T3 ramus evoked by stimulation of the T4 spinal nerve was conditioned by one (A) or four (B) volleys in the T2 spinal nerve. The greater degree of inhibition produced by four conditioning volleys is apparent. Stimuli were maximal for the respective responses. Changes in the reflex ratio (conditioning response average:test response average) appeared to be an important determinant of the effect of picrotoxin on inhibition, as will become evident in connection with Figure 17B.

In the experiments illustrated in Figure 16, inhibition was not altered by a dose of 1.6 mg/kg of picrotoxin. However, an additional 1 mg/kg did produce

a significant reduction of inhibition. Unfortunately, the response at the 200-msec test interval in A was occluded by intense paroxysms; the broken line is an estimate based on the other intervals at which reliable data were obtained. In B, where four conditioning pulses preceded the test reflex, failure to alter inhibition at short intervals was probably due to the predominance of depressed reflex recovery of common preganglionic units. The fourth conditioning pulse occurred 12 msec after the first, and the depression of recovery after four volleys was probably greater and more prolonged than after one volley. However, at the longer intervals where depression is due to inhibitory mechanisms, the inhibition was notably reduced. It will be noted that the reflex ratio did not change appreciably in these experiments.

The reflex ratio remained almost constant throughout the experiment depicted in Figure 17A, and inhibition was depressed at 2 and 4 hr after 1.5 mg/kg of picrotoxin. Unusually severe and prolonged, spontaneous activity prevented earlier trials in this preparation. Inhibition had returned to control levels by 5 hr, despite postictal depression of both reflex responses. Conditioning and test stimuli were both maximal for their respective reflex responses in this experiment. The failure of picrotoxin to alter depression at the shortest interval (25 msec) indicates a predominance of post-activation depression of preganglionic neurons common to both reflexes. Inhibition is generally weak at short intervals but becomes maximal at 40 to 60 msec, whereas depression of reflex recovery is maximal at short intervals and weak by 40 to 60 msec. The failure to alter reflex recovery in this experiment is consistent with such observations and is comparable to results of the experiment illustrated in Figure 16B.

In 8 of 9 experiments, inhibition of test reflexes by a volley in the same or in a different nerve was decidedly depressed by picrotoxin. The single exception is instructive and requires description. The importance of considering the reflex ratio becomes apparent in the experiment illustrated in Figure 17B. The conditioning reflex response was about one-fourth maximal, and the test reflex response was maximal. Picrotoxin enhanced the conditioning response much more than the test response, as reflected in the 50% increase in reflex ratio. As a consequence, instead of partially blocking the inhibition, picrotoxin in doses of 1.2 and 2.2 mg/kg actually produced a graded increase of inhibition. Two and one-half hours after the larger dose, the increased inhibition as well as the reflex ratio had partially declined toward control levels. If it can be assumed that the inhibitory mechanisms of the conditioning reflex response were increased in proportion to the increase of its excitatory mechanisms, then an increase in inhibition of the test response could also be expected. It follows that any blockade of inhibition produced by picrotoxin might be obscured by concurrent recruitment of additional inhibitory mechanisms by the conditioning volley. The configuration of the recovery curves indicates almost pure inhibition, which is accountable to the comparatively small pool of preganglionic neurons discharged by the conditioning reflex and minimal convergence by the two afferent pathways.

Picrotoxin appeared to depress the inhibition of sympathetic reflexes in a consistent manner provided that simultaneous changes in the conditioning and test reflex responses were equivalent, i.e., the reflex ratio did not change. On the other hand recovery of reflex excitability was unaffected by picrotoxin.

D. Dihydroxyphenylalanine (DOPA) and 5-Hydroxytryptophan (5-HTP)

Only the effects of DOPA and 5-HTP on the spontaneous activity and evoked reflex responses of sympathetic preganglionic neurons and somatic motoneurons were examined in this series of experiments. Basic understanding of the effects of these agents on interactions between somatic reflex responses was considered insufficient to provide insight into potential effects on interactions between sympathetic reflexes.

1. Spontaneous activity

a. DOPA. - The spontaneous activity of sympathetic preganglionic neurons was regularly increased by DOPA. In four experiments, doses between 50 and 100 mg/kg increased the frequency of discharge within 10 to 20 min. The activity increased gradually for up to 1 hr, after which it slowly declined to near control levels by 2 hr. DOPA did not induce spontaneous activity in the normally quiescent motoneurons. Figure 18 illustrates the increase of sympathetic but not of somatic activity by DOPA; a second dose given two hours after the first shows the reproducibility of this effect. Enhancement of spontaneous activity by DOPA was less than that by strychnine or picrotoxin and was not characterized by intense bursts of activity.

Immediately following injection of DOPA, blood pressure was markedly increased and remained so for 15 min to 2 hr depending on the dose and on unknown variables of the preparation. The increment ranged from 35 mm Hg after a dose of 50 mg/kg to 145 mm Hg after 200 mg/kg. The dose response relationship was not constant among four preparations in which DOPA was tested. A ganglionic blocking agent, hexamethonium (8 mg/kg), did not alter the pressor effect of

DOPA, which supported the impression gained from preganglionic recordings that the pressor effect was exerted on peripheral receptors. The subsequent mild increase in spontaneous preganglionic activity did not significantly influence blood pressure.

b. 5-HTP. - The spontaneous activity of preganglionic neurons was not appreciably affected by 5-HTP (50 mg/kg). However, bursts of spontaneous activity in motoneurons were induced after a latency of about 20 min and were still maintained after 3 hr. Occasionally, the larger and more prolonged bursts of activity in motoneurons were accompanied by brief bursts of activity in preganglionic neurons as illustrated in the records of Figure 19.

Like DOPA, 5-HTP produced a prolonged vasopressor effect. In one preparation, 50 mg/kg doubled the normal blood pressure of 70 mm Hg, which was not re-established for more than 1 hr. The pressor effect of 5-HTP is probably a direct one on peripheral structures.

2. Evoked reflex response

a. DOPA. - Administration of DOPA in doses of 50-100 mg/kg routinely enhanced the size of evoked, sympathetic reflex responses. During the initially marked, peripheral vasopressor effect of DOPA, both sympathetic and somatic reflex responses were severely depressed. It is not known whether the initial depression is a direct effect of DOPA or an indirect effect of the increased blood pressure. However, in several experiments, pressor doses of norepinephrine enhanced sympathetic reflex responses for a short time after its administration, which suggests the former possibility. When the pressor effect of DOPA had diminished, the enhancement of sympathetic reflex responses became apparent. Such responses

were progressively enhanced for up to 3 hr after administration of DOPA. The complete time course of enhancement has not been determined, but there appeared to be a slight decline after 2.5 to 3 hr in three preparations in which the effect was followed for these periods. The progressive enhancement of response averages for two, evoked sympathetic responses is shown in Figure 20; A shows the effect of a single dose of DOPA, and C shows the effect of two doses spaced one hour apart.

The enhancement of preganglionic reflex responses by DOPA is apparently not due to a general increase in neuronal excitability, because somatic responses evoked at the same time were notably depressed. Somatic reflex responses evoked by stimulation of intercostal nerves are also mediated by polysynaptic reflex pathways (Downman, 1955) which are, however, less complex than those mediating sympathetic reflexes. Concurrent sympathetic and somatic reflex responses evoked by stimulation of the same spinal nerve before and at several intervals after the administration of DOPA are shown in Figure 20B and D. Depression of somatic responses during enhancement of sympathetic responses by DOPA was a consistent observation.

b. 5-HTP. - Doses of 50 mg/kg of 5-HTP produced severe depression of sympathetic reflex responses. Although responses were not tested during the early, acute vasopressor phase, trials between 35 min and 3.5 hr indicated that depression of sympathetic reflex responses by 5-HTP was progressive with time, at least up to 3.5 hr. The effect of the drug was not followed for longer periods. Figure 21 illustrates the progressive depression of a submaximal response (A) and of a maximal response (B) by 5-HTP.

Short-latency somatic reflex responses were also severely depressed by 5-HTP, but some recovery was noted within 3 hr. The depression of simultaneously-evoked sympathetic and somatic responses by 5-HTP and the partial recovery of the somatic response is shown in Figure 21C; the onset of sympathetic depression was more gradual than that of somatic depression. The effect of 5-HTP on reflex responsiveness could not be attributed to changes in cardiovascular or respiratory conditions which remained optimal throughout these experiments.

IV. DISCUSSION

The use of the functionally decerebrate preparation in the present study confined the anatomical site of action of centrally acting drugs to the spinal cord and eliminated the effect of drugs on supraspinal structures that regulate spinal neurons and parasympathetically innervated effector organs. Regulation of end-tidal pCO_2 and of body temperature within approximate physiological limits, and continuous, visual monitoring of blood pressure provided reasonable assurance of the stability of reflex responses and the state of preparations during experiments. The few preparations in obviously poor hemodynamic conditions were not included in the results.

The signal-averaging technique greatly facilitated analyses of the experimental data, which significantly expanded the scope and enhanced the quality of this investigation. The complexity and the irregularity of evoked signals were simplified by averaging a sufficient number of responses to overcome most of their variability. In addition, improvement in the signal-to-noise ratio enabled analyses of small responses that would otherwise have been obliterated by amplifier noise. The stability of control response averages apparent in Figure 14 demonstrates the reproducibility of the computer during periods of less than one hour; repeated testing of control responses for periods of several hours has verified the reliability of this technique for detecting changes in the average size or pattern of evoked responses.

The activity of several agents that produce sympathetic effects by an action on the spinal cord was described with the RESULTS. The most significant

observation in this regard dealt with the spontaneous paroxysms that were elicited in preganglionic neurons by both strychnine and picrotoxin and that were invariably followed by sudden and prominent elevations of blood pressure. The close correlation between the intensity and duration of the vasopressor effect and that of the neural discharge demonstrates the interdependence of the central and the peripheral events. The short latency and brief duration of elevations in blood pressure following isolated bursts of preganglionic activity indicate negligible contributions from sympathoadrenal discharge; the pressor effect from sympathoadrenal discharge is slower in onset and much more prolonged, especially in the absence of compensatory reflexes, than was seen in the present study. Furthermore, efferent fibers in upper thoracic rami from which preganglionic activity was usually sampled are not distributed to the adrenal medulla. Cardioacceleration, which often followed intense neural discharges, occurred only secondarily to the vasopressor response and is attributable to the effect of circulating pressor amines. Consequently, the vasopressor effects of strychnine and picrotoxin in the spinal animal are undoubtedly mediated by discharges of preganglionic neurons with predominant vasomotor distribution and function. A similar conclusion was reached by Langley (1924) on the basis of purely peripheral observations of the effects of strychnine on spinal autonomic centers. The major influence of the vasomotor outflow in the maintenance of blood pressure during hypoxia in rabbits has also been recently demonstrated by Korner and White (1966).

In contrast with the prominent vasomotor responses and in agreement with Langley's studies with strychnine, intense spontaneous paroxysms elicited by

strychnine or picrotoxin never induced detectable piloerection. Even the prolonged, intense spontaneous preganglionic activity accompanying temporary or terminal asphyxia (Iggo and Vogt, 1960; Franz et al., 1966) produces only mild piloerection (Langley, 1924; Franz, unpublished observations). Pilomotor neurons may require longer than vasomotor neurons to recover from the effects of spinal transection or they may depend upon supraspinal structures for maximal activation.

Incidental observation of the foot pads provided no evidence of sudomotor responses to strychnine or picrotoxin. Ladpli (1962) found that electrodermal reflexes began to return only after a period of days following spinal transection, which suggests unresponsiveness of sudomotor neurons during the present acute experiments. Nevertheless, the possibility of sudomotor components in the intense spontaneous discharges cannot be eliminated; electrodermal recording in acute and chronic spinal preparations would provide an additional approach to the pharmacological study of spinal sympathetic centers, since the distribution of sudomotor neurons in cats appears to be more restricted than that of vasomotor neurons (Wang, 1964).

The depression of blood pressure following cessation of prolonged or severe, drug-induced sympathetic activity suggests postictal depression of vasomotor neurons similar to that seen after intense stimulation of motoneurons by an analeptic drug. Postictal depression of sympathetic reflexes provides additional support for such a conclusion. Postictal depression of blood pressure or reflexes after strong stimulation by drugs and its absence after mild stimulation serve to emphasize the advisability of a conservative approach to analeptic therapy. In

addition, the failure to elicit spinal vasomotor and somatic discharges independently indicates a lack of selectivity for strychnine and picrotoxin at the spinal level.

Despite significant depression of spontaneous sympathetic activity by mephenesin, vasodepression could not be demonstrated. Although expansion of plasma volume by the volume of fluid necessary to solubilize the drug may have offset a temporary vasodepression, several other possibilities could account for the lack of effect. First, a large portion of the spontaneous activity that was depressed may have served other than vasomotor functions. Second, the low resting blood pressure (about 70 mm Hg) may have been adequately maintained by relatively few discharging neurons, especially in combination with the expansion of blood volume. Regardless of the cause, the fact that spontaneous activity could be depressed at all by small doses of mephenesin suggests that it is maintained by afferent impulses that drive preganglionic neurons through reflex pathways that are vulnerable to the depressant effects of the drug. If the spontaneous activity originates in the neuron itself, mephenesin should exert little effect, if any.

The present understanding of the physiology of spinal sympathetic reflexes has been significantly expanded by the use of pharmacological agents with recognized effects on spinal somatic reflexes. Comparison of the effects of these drugs on each reflex system has facilitated interpretation of the experimental observations.

The spontaneous activation of preganglionic neurons in unison with motoneurons by both strychnine and picrotoxin is difficult to resolve, considering the dissimilar mechanisms by which they are purported to act (Curtis, 1963;

Eccles, 1964, 1965). Activation of motoneurons by strychnine is attributed solely to blockade of postsynaptic inhibition. However, no evidence of postsynaptic inhibition of preganglionic neurons and their reflex pathways or depression of this inhibition by strychnine was found in the present study. Yet, preganglionic neurons were as vulnerable to the spontaneous hyperexcitability induced by strychnine as were motoneurons. The similar activation of both efferent systems by picrotoxin is more easily resolved, because it seems to produce similar effects on presynaptic inhibition of both systems (see below), and because its effect on somatic presynaptic inhibition does not entirely account for the convulsions it produces. Nevertheless, the parallel discharge of both systems, either spontaneously or in response to mild stimuli, suggests the activation of a common pathway by a similar mechanism of action for both drugs. Common pathways appear to connect the same group of visceral afferent fibers with both preganglionic neurons and motoneurons (Franz et al., 1966); interpolated interneurons common to both pathways provide a possible site of drug action that would account for the synchronous nature of spontaneous discharges. The possibility of excitatory cholinergic axon collaterals between motoneurons and preganglionic neurons seems unlikely due to the failure of DHE, a central cholinergic blocking agent, to alter their discharge patterns.

The depression of evoked reflex responses by mephenesin supports previous conclusions (Beacham and Perl, 1964a; Franz et al., 1966) that the reflex pathways for spinal sympathetic reflexes are polysynaptic. The doses of mephenesin required to demonstrate significant depression of sympathetic responses were too small to have affected somatic monosynaptic reflexes but were

sufficient to produce a prominent reduction of somatic polysynaptic reflexes (Henneman et al., 1949; Taverner, 1952).

Frequent failure of strychnine to enhance the size of evoked sympathetic reflex responses may be related to the inability of previous workers to facilitate the response (Beacham and Perl, 1964b; Franz et al., 1966). The lack of a significant subliminal fringe available for discharge was suggested by these workers to account for failure to recruit additional preganglionic neurons into a maximal reflex response. Likewise, the absence of a significant subliminal fringe may account for the small number of reflex responses that were significantly enhanced by strychnine. The facilitation or marked enhancement by strychnine of somatic polysynaptic reflexes is attributable to a large subliminal fringe which can be recruited into a reflex response. The greater enhancement of submaximal than of maximal sympathetic responses by strychnine may also be due to the recruitment of normally undischarged preganglionic neurons into the submaximal response. The ability of strychnine to produce significant enhancement of some maximal responses indicates that a subliminal fringe in the preganglionic reflex pool is not always absent.

In contrast to strychnine, picrotoxin consistently enhanced the size of even maximal sympathetic reflex responses. Although enhancement was small for about half of the responses, the larger increments were considerably greater than those produced by strychnine. In view of the depressant effect of picrotoxin but not of strychnine on the inhibition of sympathetic reflexes (see below), reduction of a possible inhibitory influence in the preganglionic pool would enable more neurons to respond to the afferent volley. However, the inhibition

that is depressed by picrotoxin reaches a maximum at least 20 to 30 msec after the height of the reflex discharge; it has not been possible to determine its earliest onset, but some inhibition may occur early enough to interfere with the response. The possibility of concurrently active inhibitory and excitatory effects of the afferent volley has been suggested to account for the sudden cessation of preganglionic reflex discharges (Beacham and Perl, 1964b).

Hongo and Ryall (1966) have recorded repetitive discharges of preganglionic neurons to single afferent volleys. Responses recorded from preganglionic rami often suggest multiple discharges of some units. In the present experiments, repetitive firing of single units after strychnine or picrotoxin appeared to contribute to the after-discharges in thoracic rami and to the spontaneous paroxysms (Figures 7 and 10A). Enhancement of repetitive activity during reflex discharge provides another means whereby strychnine or picrotoxin may increase the size of reflex responses.

The unsuccessful attempt to depress differentially the inhibitory and excitatory afferent pathways to preganglionic neurons with mephenesin indicates that the two pathways are similar in complexity. If the inhibitory pathway was the more complex of the two, then its depression by mephenesin should have been greater in proportion to the depression of the excitatory pathway, and inhibition would have lessened. On the contrary, inhibition of sympathetic responses was unchanged, despite significant depression of both conditioning and test responses.

Strychnine was also without effect on inhibition of sympathetic reflexes provided that changes in the size of the conditioning and test reflexes were

proportionate. Since strychnine blocks postsynaptic inhibition of motoneurons in the spinal cord, its failure to affect sympathetic inhibition in any way provides strong evidence that the classical postsynaptic inhibition plays no part in the inhibition of spinal sympathetic reflexes. Furthermore, the absence of recurrent inhibition by preganglionic axon collaterals (Fernandez de Molina et al., 1965) has now been demonstrated pharmacologically by the inefficacy of both strychnine and DHE on reflex recovery and inhibition.

Several observations, both physiological and pharmacological, suggest that presynaptic inhibition may be largely responsible for the inhibition of spinal sympathetic reflexes. First, the temporal course of sympathetic inhibition is very similar to that of presynaptic inhibition (Frank and Fourtes, 1957; Eccles et al., 1962; Eccles et al., 1963; Eccles, 1964). Both are slow in onset (maximal at 40-60 msec) and long in duration (up to 1 sec). Second, in both systems, inhibition is increased by several conditioning volleys, but its time course is not altered significantly (Figure 16; Eccles, 1964). Third, presynaptic inhibition of monosynaptic somatic reflexes is markedly depressed by picrotoxin but not by strychnine (Eccles et al., 1963). Likewise, inhibition of sympathetic reflexes in the present study was significantly and consistently depressed by picrotoxin, except where it was obscured by disporportionate changes in the conditioning and test reflexes (Figure 17B). The strong similarity of sympathetic inhibition to presynaptic inhibition with respect to time course, repetitive conditioning, and the effect of picrotoxin indicates that inhibition of spinal sympathetic reflexes is presynaptic. Unfortunately, the effect of picrotoxin on the presynaptic inhibition of polysynaptic somatic reflexes

(Eccles et al., 1962) has not been reported, so that comparison with that more appropriate system cannot be made.

The effects of DOPA and 5-HTP on the spontaneous and reflex activity of preganglionic neurons furnish additional evidence regarding the possible role of monoamines in synaptic transmission within the central nervous system. Relevant background material dealing with monoamine-containing neurons in the central nervous system has been outlined in the INTRODUCTION and need not be repeated. However, some additional consideration of the dense convergence of monoamine-containing axons upon preganglionic neurons is requisite to an interpretation of the observed effects of monoamine precursors.

The details of the localization and pharmacology of monoamine-containing neurons that terminate in the spinal cord have been described by Carlsson et al. (1964), Dahlström and Fuxe (1965), and Hillarp et al. (1966). Two types of terminals, one containing NE and the other containing 5-HT, separate and converge upon the intermediolateral columns where they form a dense plexus of both types of terminals in close proximity to preganglionic neurons. The terminals containing NE intimately enclose the cell bodies and may form axo-somatic synapses, but the 5-HT terminals lie between the cell bodies where they may contact cell processes. The cell bodies of neurons giving rise to these NE- and 5-HT-containing terminals are localized in relatively discrete areas of the medulla oblongata, and their axons descend in the lateral funiculi and terminate in the intermediolateral columns; about half the fibers cross at the spinal level. No cell bodies of such neurons are found in the spinal cord.

There is evidence that these neurons function by releasing their amines from the synaptic terminals. Andén et al. (1964, 1965) found release of NE and 5-HT from the isolated spinal cords of mice upon in vitro stimulation, and electrical stimulation of the medulla oblongata combined with synthesis inhibition in vivo caused a marked depletion of NE and 5-HT in the spinal cord (Dahlström et al., 1965). Lundberg's neurophysiological studies (1965) also support the view that NE and 5-HT serve transmitter functions in the spinal cord. His pharmacological evidence which was summarized in the INTRODUCTION and the enhancement of central stores of NE or 5-HT by DOPA or by 5-HTP, respectively (Fuxe, 1965), provide strong evidence that intraneuronal amines are formed from their precursors which readily penetrate the central nervous system.

No previous descriptions of the effects of DOPA and 5-HTP on reflexes of sympathetic preganglionic neurons are available. However, the effect of these agents on somatic reflexes as described by Lundberg (1965) provide a basis of comparison for that portion of the present study. In agreement with his observations (depression of short-latency transmission from flexor reflex afferents to motoneurons by both DOPA and 5-HTP and enhancement of motoneuron excitability to the point of spontaneous discharge), short-latency somatic reflexes were depressed by the administration of DOPA (Figure 20) and 5-HTP (Figure 21) and spontaneous discharge of motoneurons followed administration of 5-HTP (Figure 19) but not of DOPA (Figure 18). These effects were accomplished with smaller doses than were used by Lundberg. The reflex connections between afferent fibers of body-wall nerves and motoneurons appear to be of the flexor type (Downman, 1955), and are, therefore, similar to those examined by Lundberg.

It should be emphasized that the alterations of somatic and preganglionic reflex responses by DOPA and 5-HTP in the present experiments persisted long after the pressor effects had subsided and cannot be attributed to cardiovascular changes.

The enhancement of sympathetic reflex responses and spontaneous activity after DOPA and the depression of reflex responses after 5-HTP strongly indicate mechanisms that are separate from those affecting somatic reflexes. Furthermore, the progressive nature of the enhancement or depression indicates that DOPA and 5-HTP do not act directly but only after sufficient time to allow for metabolic transformation. Their effects on spinal reflexes have been attributed to the synthesis and release of the respective monoamines, NE and 5-HT, by the appropriate type neurons (Dahlström and Fuxe, 1965; Lundberg, 1965). The slow onset of effect on the spontaneous and reflex activity of preganglionic neurons by DOPA and 5-HTP is consistent with such a scheme, as is the relatively long duration of their action, which far outlasts their peripheral pressor effects.

This neurophysiological evidence, combined with the histochemical localization of axon terminals and cell bodies and the pharmacological alteration of intraneuronal monoamines, suggests the following proposal: No cell bodies containing monoamines are present in the spinal cord; therefore, such neurons are not interposed between afferent fibers and preganglionic neurons. However, if the terminals of axons that descend from the medulla and converge upon preganglionic neurons take up circulating DOPA or 5-HTP and convert them to NE or 5-HT, these may be released spontaneously to act upon the preganglionic neurons. An increase in their excitability by the release of NE or a decrease

in excitability by 5-HT would account for the observed enhancement or depression of sympathetic reflexes. Going one step further, it may be proposed that NE may act as an excitatory transmitter and 5-HT may act as an inhibitory transmitter for the supraspinal control of sympathetic preganglionic neurons. According to this proposal, the excitatory pathway for sympathetic impulses from the medulla to the effector organs would be composed of three alternating neurons in series: bulbospinal adrenergic, preganglionic cholinergic, and postganglionic adrenergic. Such an alternating chain of adrenergic and cholinergic neurons is reminiscent of the alternating cholinergic-non-cholinergic central pathways originally proposed by Feldberg and Vogt (1948).

Studies designed to explore the effect of DOPA and 5-HTP on preganglionic activation by these direct bulbospinal pathways would largely resolve the credibility of this hypothesis. With the spinal preparation, additional pharmacological studies would also provide worthwhile substantiation of the present results.

V. SUMMARY

The effects of several classes of pharmacological agents on the spontaneous and reflex activity of sympathetic preganglionic neurons in spinal cats have been investigated by electrophysiological techniques in combination with computer analysis.

Spontaneous and reflex activity were significantly depressed by mephenesin in doses known to depress polysynaptic reflex pathways. Its failure to alter the intersegmental inhibition of sympathetic reflexes indicated a similarity in the complexity of central inhibitory and excitatory pathways.

Strychnine and picrotoxin produced intense, spontaneous bursts of coincident activity in preganglionic neurons and motoneurons. The temporal characteristics of the ensuing vasopressor effects indicated their mediation by vasomotor preganglionic neurons. No evidence for similar activation of pilomotor, sudomotor, cardioaccelerator, or sympathoadrenal preganglionic neurons was found. Although sympathetic reflex responses were consistently enhanced by picrotoxin, the frequent failure of strychnine to do so indicates that the preganglionic pool available for reflex discharge is spatially limited.

The inability of strychnine to depress the inhibition of sympathetic reflexes provides strong evidence that classical postsynaptic inhibition is not involved in spinal sympathetic reflexes. Inhibition of sympathetic reflexes was significantly depressed by picrotoxin and was similar to presynaptic inhibition in time course and in response to repetitive conditioning. Therefore, it was concluded that sympathetic inhibition is mediated by a presynaptic mechanism.

Dihydroxyphenylalanine (DOPA) enhanced the spontaneous and reflex activity of sympathetic preganglionic neurons. Reflex activity was depressed by 5-hydroxytryptophan (5-HTP). The progressive nature of these responses suggested that they did not act directly but through the formation of their metabolites, norepinephrine (NE) and 5-hydroxytryptamine (5-HT). The presence of NE or 5-HT in neurons that descend from the medulla and converge upon preganglionic neurons and the effects of their precursors on sympathetic activity has led to the proposal that NE serves an excitatory transmitter function and 5-HT serves an inhibitory transmitter function for the supraspinal control of preganglionic neurons.

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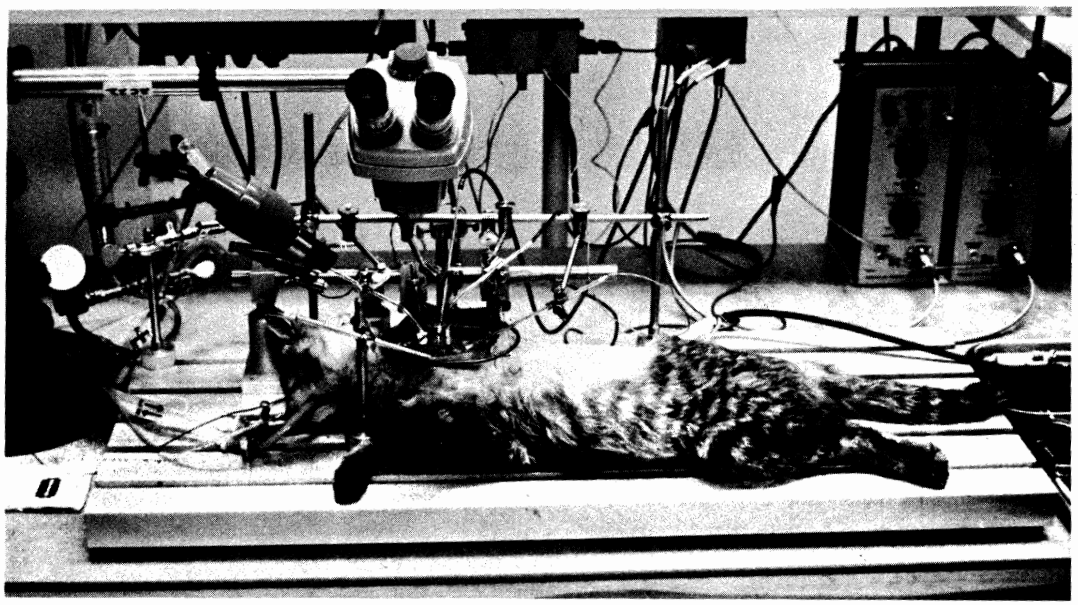
Fig. 1. Experimental set-up.

A. Thoracic preparation. The lower bar behind the cat supported the vertebral clamps, and the stimulating and recording electrodes were suspended from the upper bar. Each bar was secured to the slotted animal board. The dissecting microscope and auxiliary microscope light remained in place for periodic examination of the field during experiments. Electrode selector boxes (top, center) were mounted under a shelf at the rear of the table. Pre-amplifiers (right) were rigidly supported between the shelf and table.

B. Operative field of preparation in A. Spinal nerves (sn) T2, T3, or T4 were stimulated to evoke thoracic sympathetic reflexes; T2 and T4 are shown mounted on stimulating electrodes. The T2 and the T3 white rami communicantes (r) from which sympathetic preganglionic activity was recorded are mounted on recording electrodes. The prominent sympathetic trunk (ST) enters the stellate ganglion (SG) at the far left. The T1 ramus also enters the ganglion from the left just above the ganglion. T3 ramus was unusually short in this preparation because it entered an aberrant T3 ganglion (below T3 ramus) instead of the stellate ganglion as it normally does. Magnification, 4 X actual size.

A



B

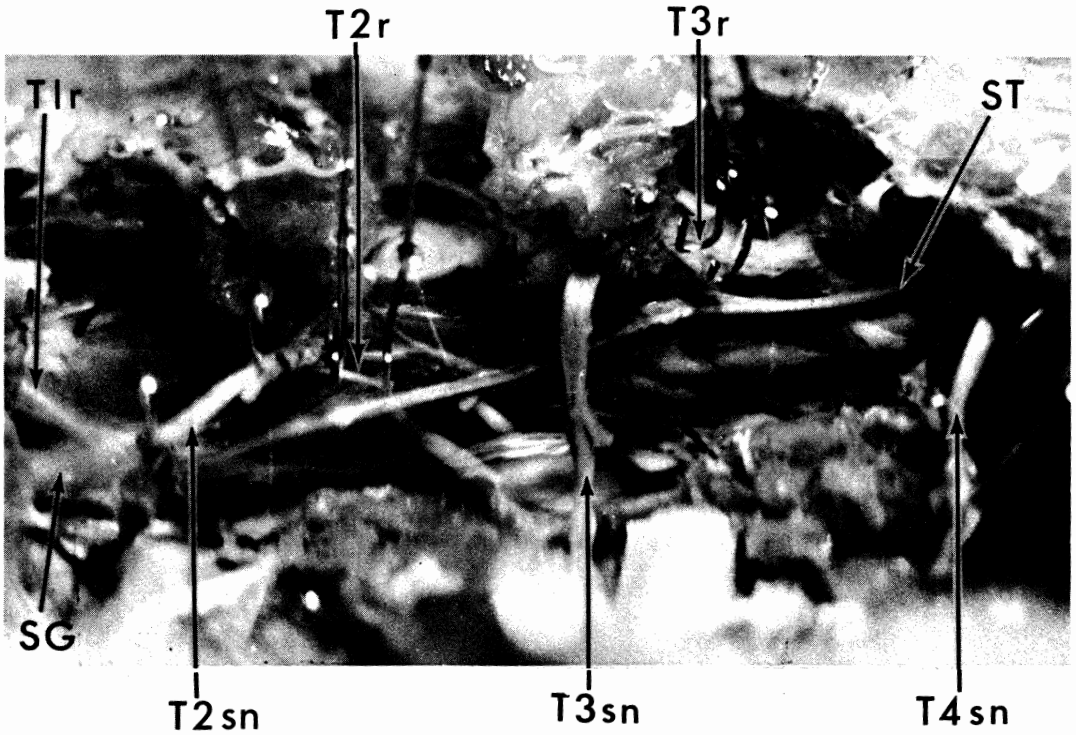
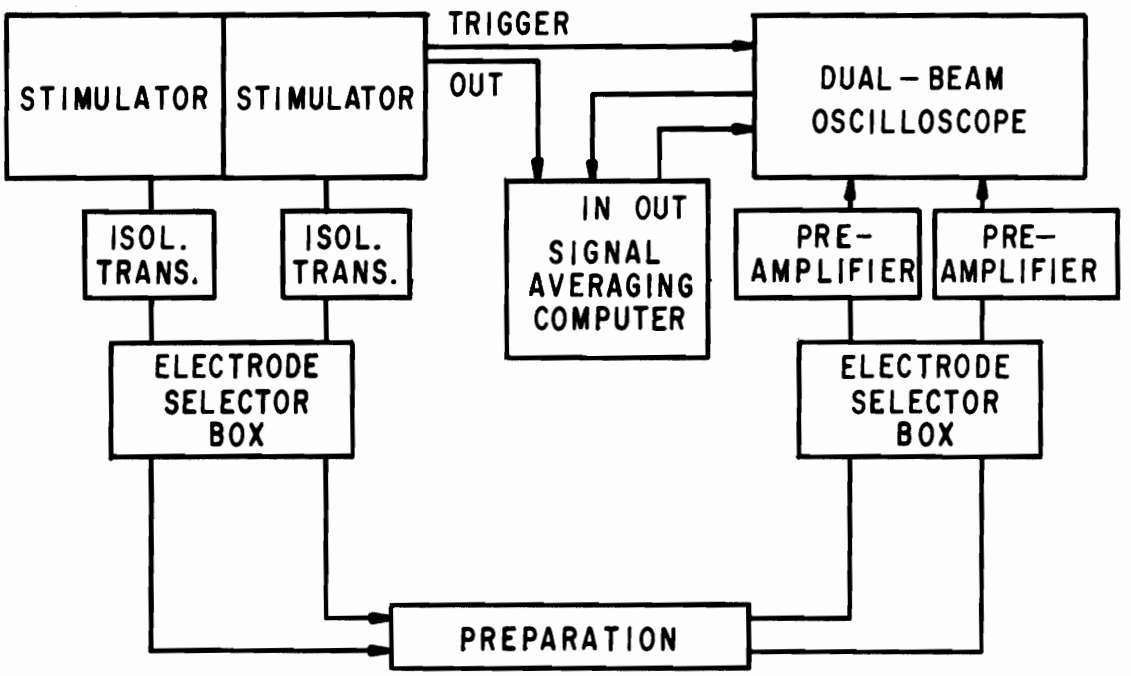


Fig. 2. Block diagram of stimulating and recording equipment.

The function and type of equipment illustrated are explained in the text.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ISOL.
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ELECTRODE
SELECTOR
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PREPARATION

Fig. 3. Depression of spontaneous preganglionic activity and after-discharge by mephenesin.

A. Relative frequency of spontaneous activity in T3 preganglionic ramus was recorded during two, single, 1024-sec (17 min) sweeps by the multiscaling mode of the Enhancetron. At 5 min (arrows) after the start of each sweep, 25 mg/kg of mephenesin was administered. Approximately 5 minutes separated the two sweeps. The base line indicates 0 frequency.

B. Spontaneous activity during single sweeps was recorded from an L1 preganglionic ramus before and 15 min after 50 mg/kg of mephenesin. Time marker: 100 msec.

C. Depression of after-discharge (arrows) in L1 preganglionic ramus 10 min after 50 mg/kg of mephenesin. Relative frequency of evoked discharge was added by the Enhancetron during 25, 1-sec sweeps. Responses were evoked by stimulation of the splanchnic nerve at an intensity presumed to be sufficient to activate nonmyelinated fibers. The initial, large peak is the sum of the low-threshold response frequency.

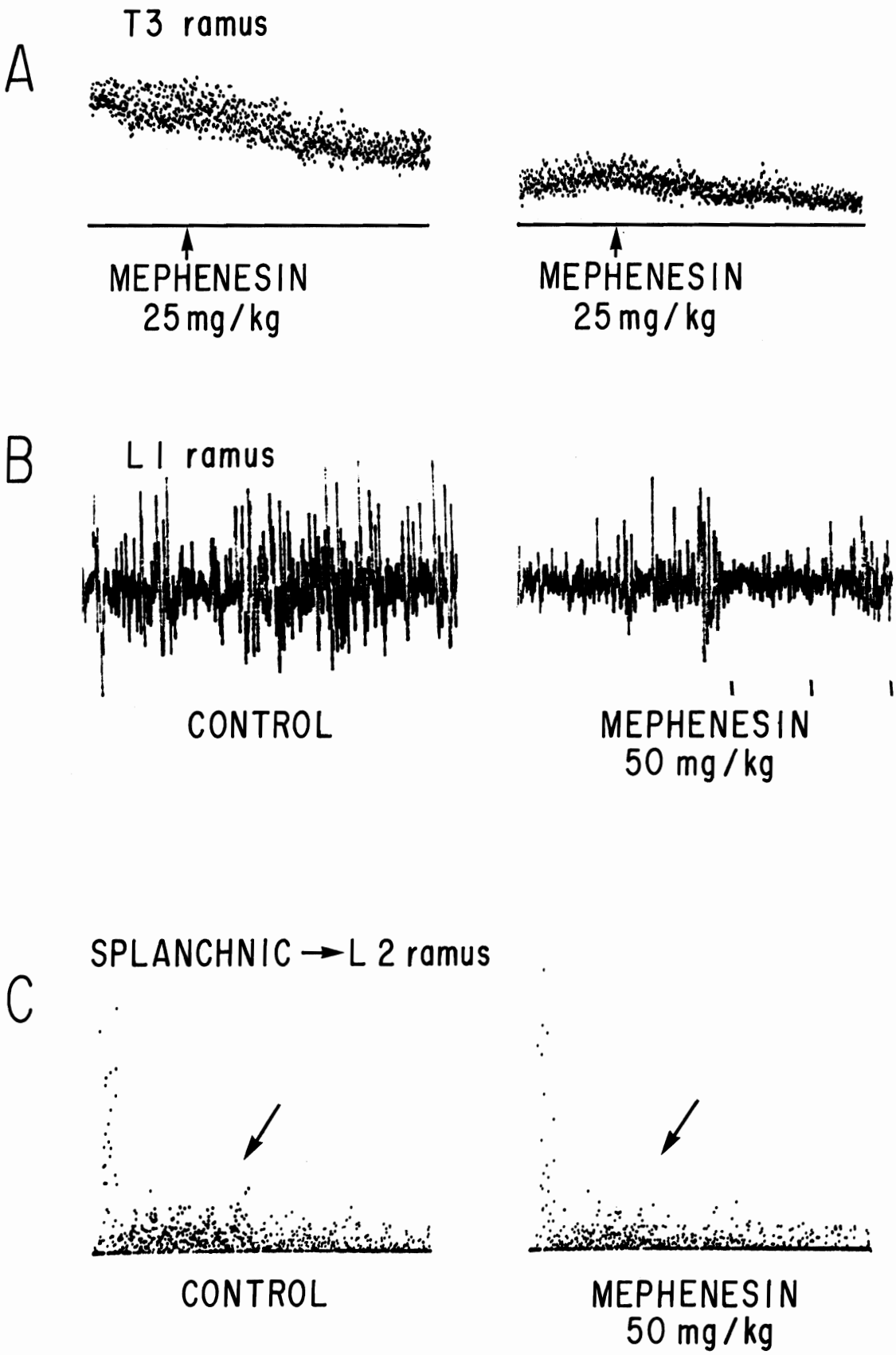
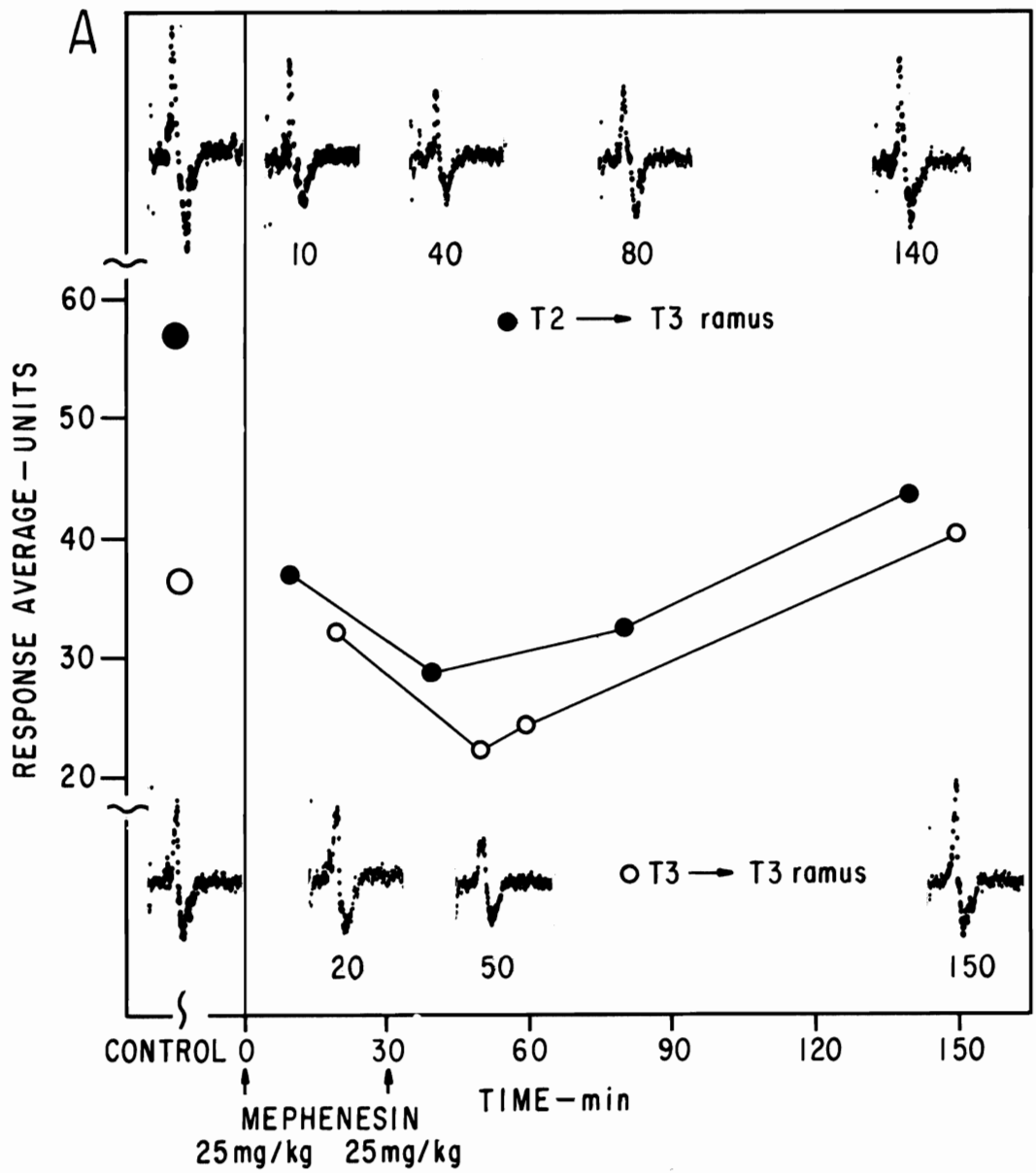

A
T3 ramus
MEPHENESIN
25 mg/kg
MEPHENESIN
25 mg/kg
B
L 1 ramus
CONTROL
MEPHENESIN
50 mg/kg
C
SPLANCHNIC → L 2 ramus
CONTROL
MEPHENESIN
50 mg/kg

Fig. 4. Depression of sympathetic reflex responses by mephenesin.

A. Ordinate, response average in arbitrary units; abscissa, time after first dose of mephenesin. Stimulation of the T2 or the T3 spinal nerve evoked maximal reflex responses in the T3 preganglionic ramus. Depression of responses was graded by two successive doses of mephenesin (arrows). Each point represents the average of 25 evoked responses. Large symbols prior to the 0 time represent control averages several minutes before administration of mephenesin. Response averages from which the data were obtained are reproduced above or below the respective points; numbers below each record correspond to the times indicated on the abscissa.

B. Same experiment as in A. Depression of single, maximal reflex responses (T2 to T3r) by 50 mg/kg of mephenesin (cumulative). Numbers below each record indicate times corresponding to the abscissa in A. Arrows below records denote stimulus artifact. Time marker: 10 msec.


A
10
40
80
140
60
50
40
30
20
T2 → T3 ramus
RESPONSE AVERAGE – UNITS
T3 → T3 ramus
20
50
150
CONTROL 0
30
60
90
120
150
TIME – min
MEPHENESIN
25 mg/kg 25 mg/kg

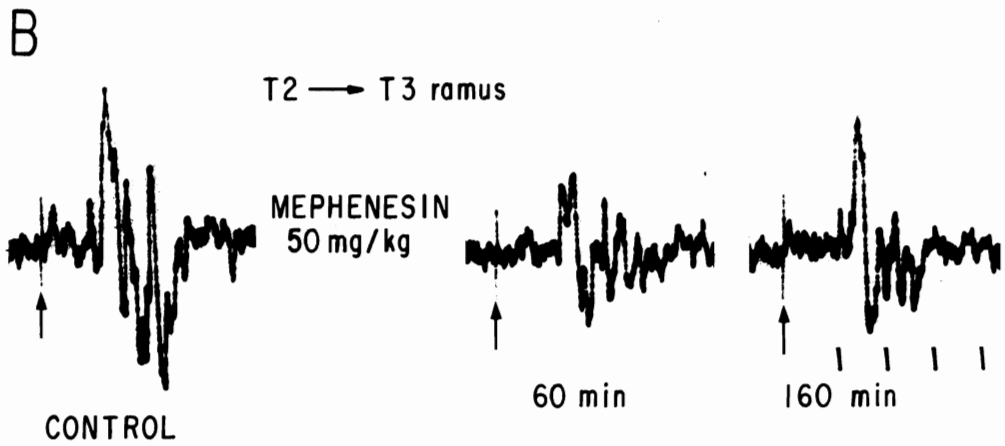

B
T2 → T3 ramus
MEPHENESIN
50 mg/kg
CONTROL
60 min
160 min

Fig. 5. Effect of mephenesin on interactions between sympathetic reflex responses.

These and all subsequent interaction graphs are constructed as follows: Left ordinate, conditioned reflex response average as percentage of the test reflex response average; abscissa, interval between conditioning and test stimuli; in the inset at the right, the filled circle (control symbol) indicates the amplitude of the control test response average which is assigned a value of 1 (right ordinate); the relative amplitude of the test response average after drug treatment is indicated by the appropriate symbol (open symbol in these graphs); cross bars indicate the relative amplitudes of the respective conditioning responses where different than the test response.

A. Maximal reflex response in T3 preganglionic ramus was evoked by stimulation of T4 spinal nerve and conditioned by a maximal volley in T3 spinal nerve. Each point represents the average of 25 evoked responses.

B. Same preparation as in A. Double pulses applied to T4 spinal nerve were maximal for the first reflex response in T3 preganglionic ramus. The response to the second stimulus is plotted as the percentage of the response to the first stimulus. Each point represents the average of 25 evoked responses.

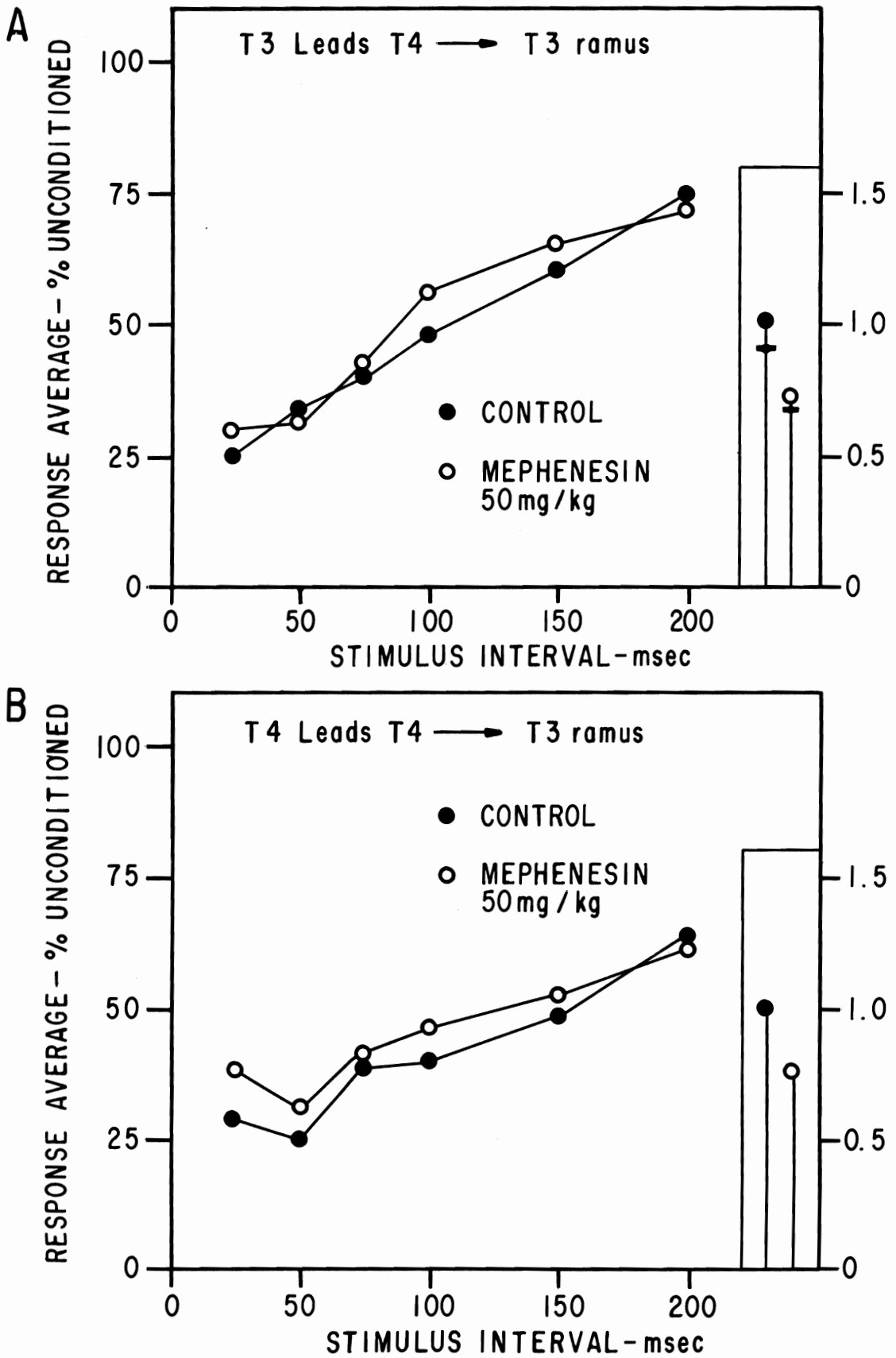

A
T3 Leads T4 → T3 ramus
RESPONSE AVERAGE - % UNCONDITIONED
100
75
50
25
0
1.5
1.0
0.5
0
CONTROL
MEPHENESIN
50 mg/kg
0
50
100
150
200
STIMULUS INTERVAL - msec
B
T4 Leads T4 → T3 ramus
RESPONSE AVERAGE - % UNCONDITIONED
CONTROL
MEPHENESIN
50 mg/kg
STIMULUS INTERVAL - msec

Fig. 6. Enhancement of spontaneous preganglionic activity by strychnine, with accompanying blood pressure changes.

A. Ordinate, mean carotid blood pressure; abscissa, time (min) after start of sweep. At 1 min (arrow), 0.07 mg/kg of strychnine was injected. Blood pressure at 0.5 min intervals is indicated by filled triangles. Relative frequency of spontaneous activity in T4 preganglionic ramus during one 512-sec (8.5 min) sweep was recorded on the multiscaling mode of the Enhancetron. Each dot of the frequency record represents the recorded frequency for 0.5 sec (1024 points). Frequency is relative to ZERO at the abscissa. An ordinate for absolute, preganglionic firing frequency is not included because the number of contributing units could not be determined.

B. Different preparation than in A. Relative frequency of spontaneous activity in T3 preganglionic ramus during one, 512-sec (8.5 min) sweep at 0.4 the gain in A. Ordinate and abscissa are as in A, except the ordinate scale is proportionately smaller. At 1 min (arrow), 0.075 mg/kg of strychnine was injected. Blood pressure measurements at 1 min intervals and at maximum and minimum values are indicated by filled triangles. The broken line connecting the points is estimated.

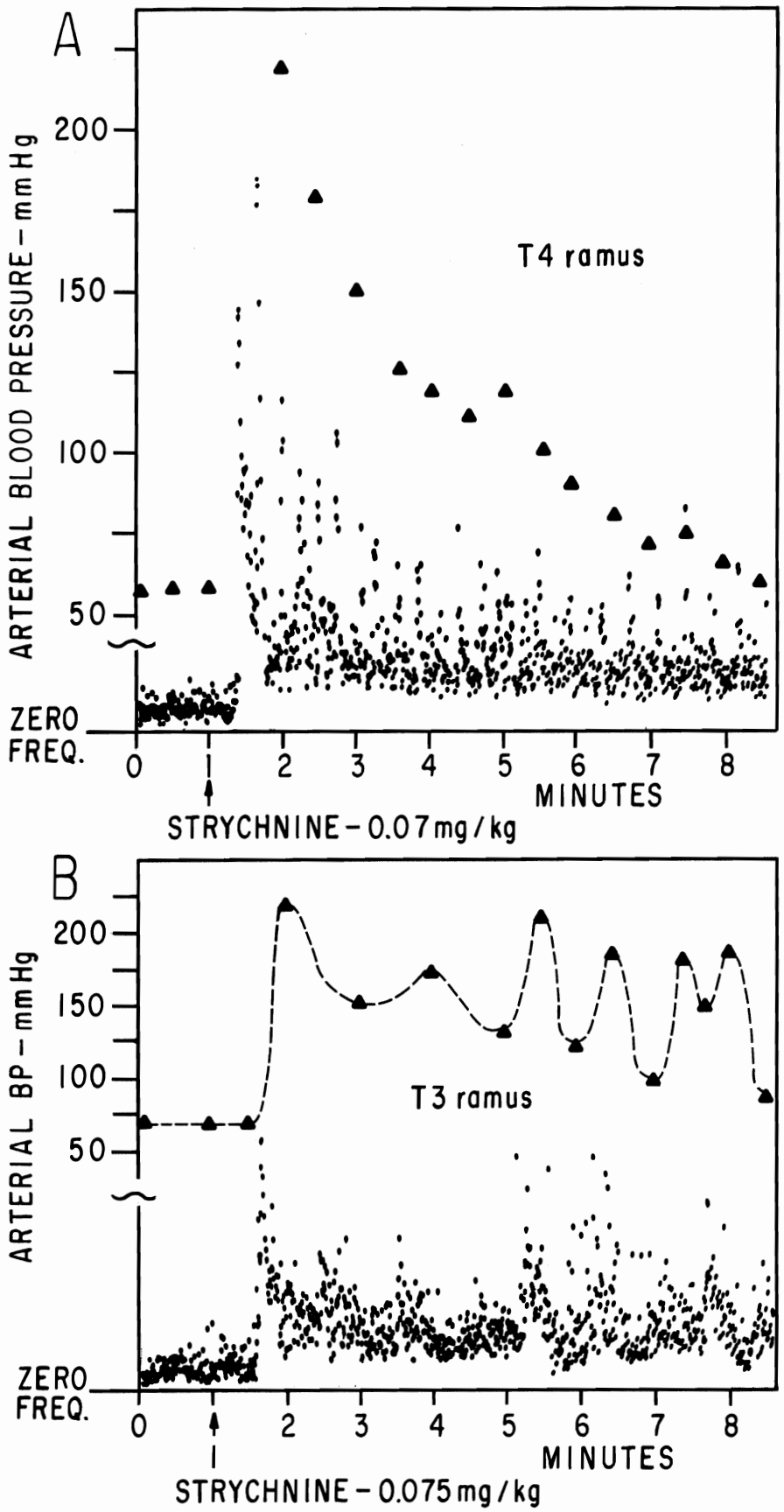

A
ARTERIAL BLOOD PRESSURE – mm Hg
200
150
100
50
ZERO FREQ.
T4 ramus
0
1
2
3
4
5
6
7
8
MINUTES
STRYCHNINE – 0.07 mg / kg
B
ARTERIAL BP – mm Hg
200
150
100
50
ZERO FREQ.
T3 ramus
0
1
2
3
4
5
6
7
8
MINUTES
STRYCHNINE – 0.075 mg / kg

Fig. 7. Spontaneous preganglionic and somatic paroxysms elicited by strychnine.

Spontaneous activity in T3 preganglionic ramus and T3 spinal nerve was recorded simultaneously on moving film. The second and third records are continuous and show the onset and decay of a spontaneous paroxysm, about 1 min after 0.1 mg/kg strychnine. The fourth record shows a brief paroxysm, about 5 min after strychnine. Gain of preganglionic records was twice that for somatic records.

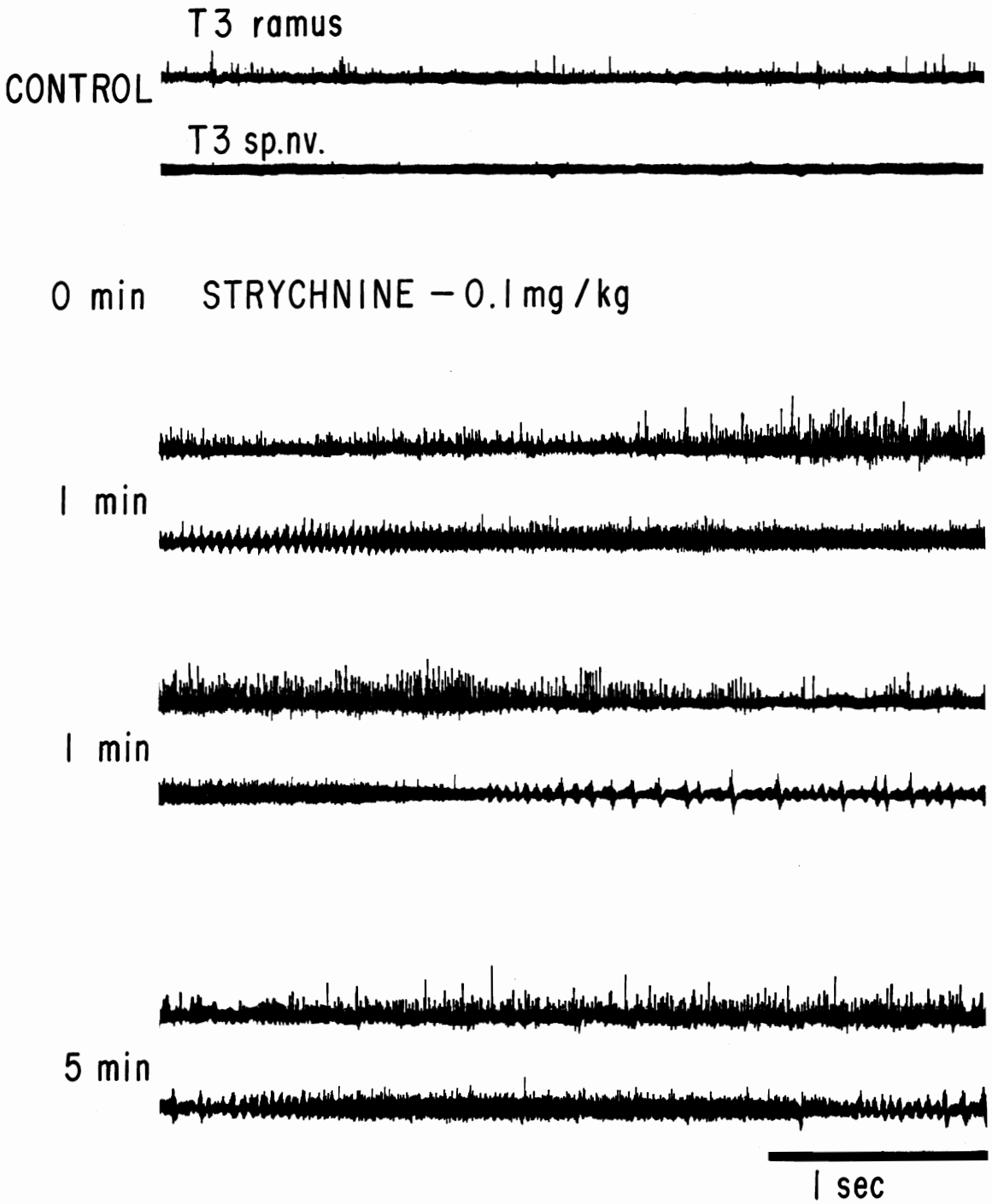
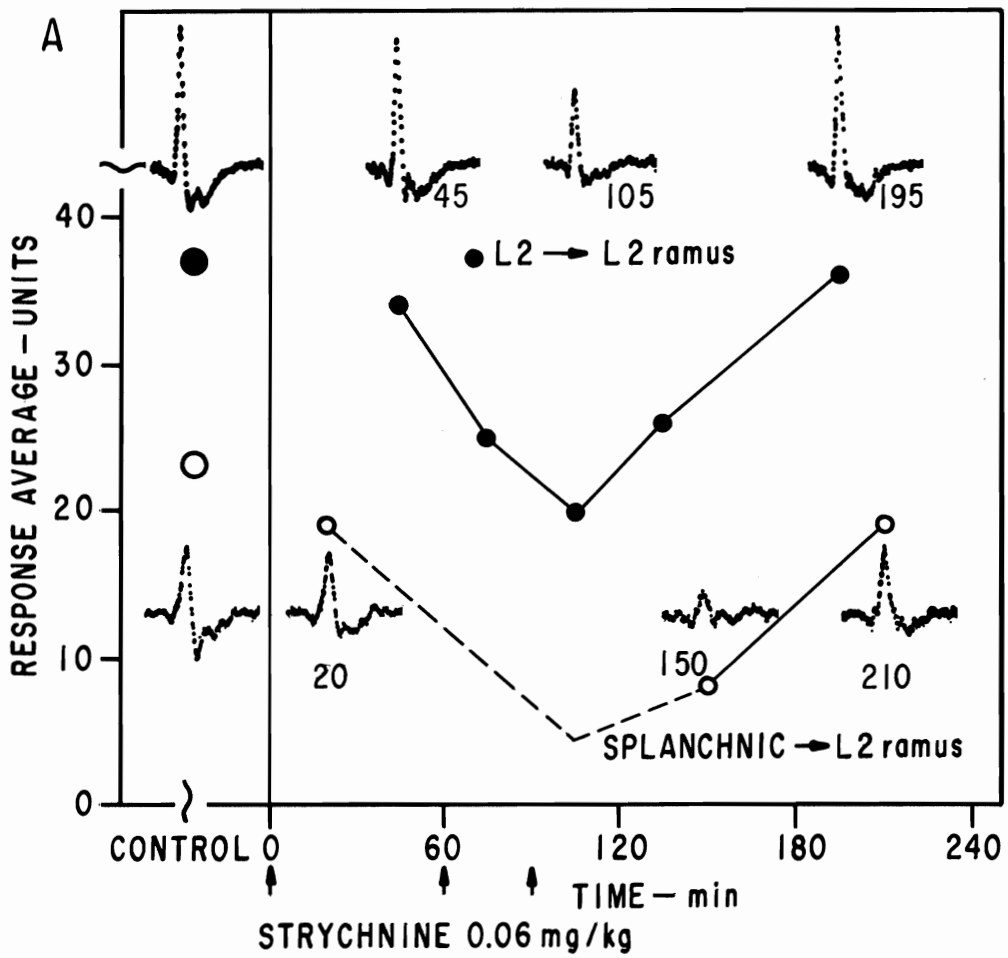

T3 ramus
CONTROL
T3 sp.nv.
0 min
STRYCHNINE – 0.1 mg / kg
1 min
1 min
5 min
1 sec

Fig. 8. Occlusion of sympathetic reflex responses by paroxysmal activity elicited by strychnine.

A. Graph constructed as in Figure 4. Stimulation of the L2 spinal or the splanchnic nerve evoked maximal reflex responses in the L2 preganglionic ramus. Occlusion of responses was graded by increasing paroxysms elicited by three successive doses (0.06 mg/kg, each) of strychnine (arrows). Each point represents the average of 50 evoked responses. The broken line indicates an estimate where no measurements were obtained.

B. Different experiment than in A. Occlusion of single, maximal reflex responses in T3 preganglionic ramus and T3 spinal nerve 10 min after 0.1 mg/kg of strychnine. Responses were evoked simultaneously by stimulation of T4 spinal nerve. Gain of preganglionic records was twice that for somatic records. Arrows below each pair of records denote stimulus artifact. Time marker: 10 msec.


A
RESPONSE AVERAGE - UNITS
40
30
20
10
0
45
105
195
L2 → L2 ramus
20
150
210
SPLANCHNIC → L2 ramus
CONTROL 0
60
120
180
240
TIME — min
STRYCHNINE 0.06 mg/kg

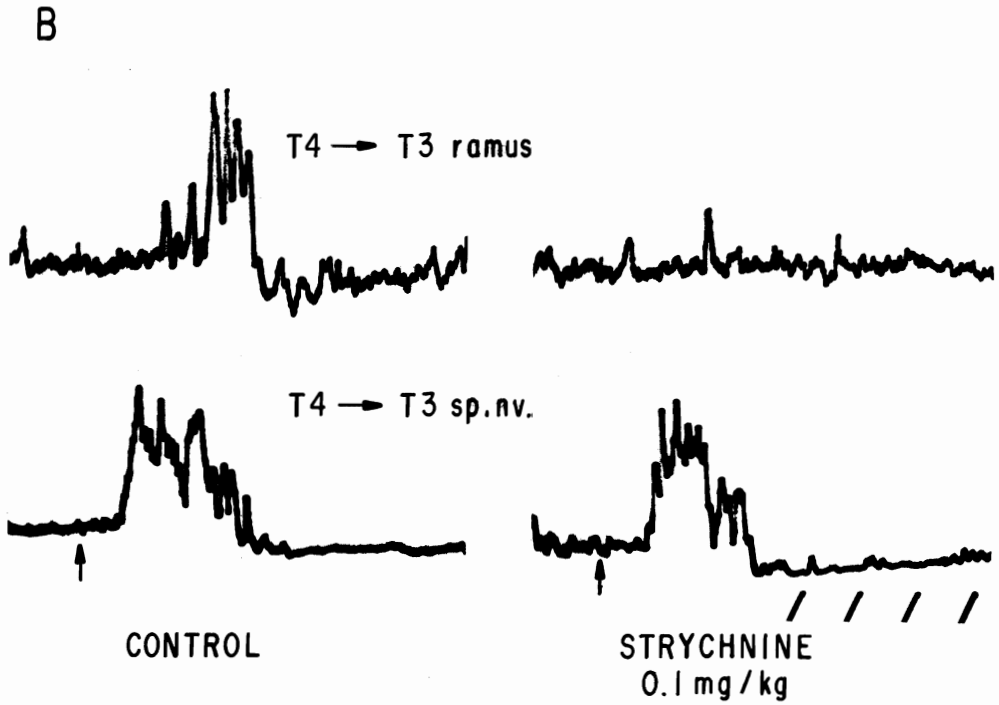
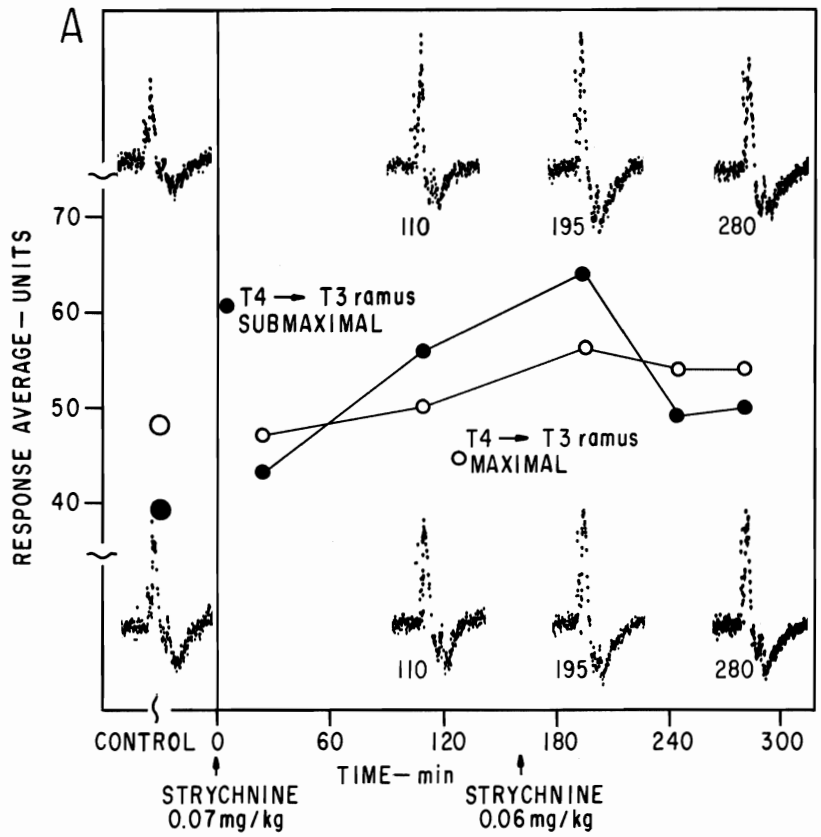

B
T4 → T3 ramus
T4 → T3 sp.nv.
CONTROL
STRYCHNINE
0.1 mg/kg

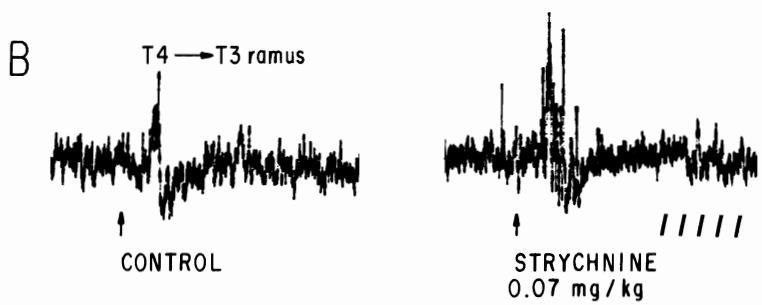
Fig. 9. Enhancement of sympathetic reflex responses by strychnine.

A. Graph constructed as in Figure 4. Submaximal or maximal reflex responses in T3 preganglionic ramus were evoked by stimulation of T4 spinal nerve. Enhancement of responses was graded by two successive doses of strychnine (arrows). Each point represents the average of 32 evoked responses.

B. Enhancement of a single, submaximal response in A, 90 min after 0.07 mg/kg of strychnine. Arrows below records denote stimulus artifact. Time marker: 10 msec.

C. Different preparation than in A and B. Enhancement of single, maximal responses in T3 preganglionic ramus and T3 spinal nerve 20 min after 0.1 mg/kg of strychnine. Responses were evoked by stimulation of T4 spinal nerve. Gain of preganglionic records was twice that for somatic records. Time marker: 10 msec.


A
RESPONSE AVERAGE – UNITS
70
60
50
40
110
195
280
T4 → T3 ramus
SUBMAXIMAL
T4 → T3 ramus
MAXIMAL
110
195
280
CONTROL
0
60
120
180
240
300
TIME – min
STRYCHNINE
0.07 mg/kg
STRYCHNINE
0.06 mg/kg


B
T4 → T3 ramus
CONTROL
STRYCHNINE
0.07 mg/kg

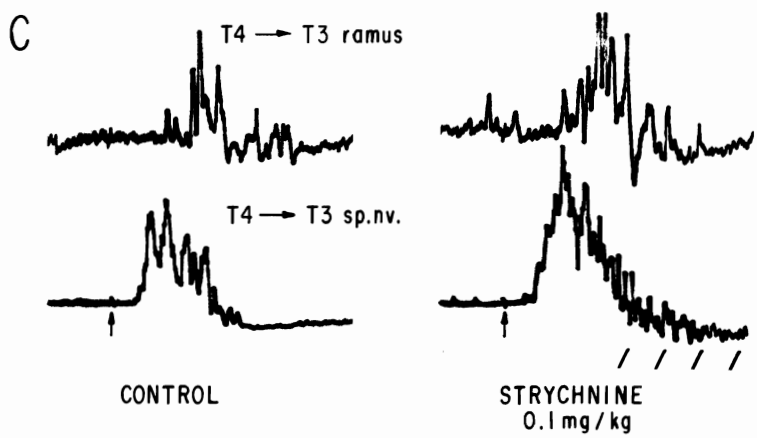

C
T4 → T3 ramus
T4 → T3 sp.nv.
CONTROL
STRYCHNINE
0.1 mg/kg

Fig. 10. Initiation of after-discharge by strychnine.

A. After-discharge (arrow) of single, maximal reflex responses in T3 preganglionic ramus 30 min after 0.2 mg/kg strychnine. Responses were evoked by stimulation of T2 spinal nerve. Time marker: 10 msec.

B. After-discharge (arrow) of maximal reflex responses in T3 preganglionic ramus 30 min after 0.06 mg/kg of strychnine. Relative frequency of evoked discharge was added during 32, 250-msec sweeps. Responses were evoked by stimulation of the T4 spinal nerve.

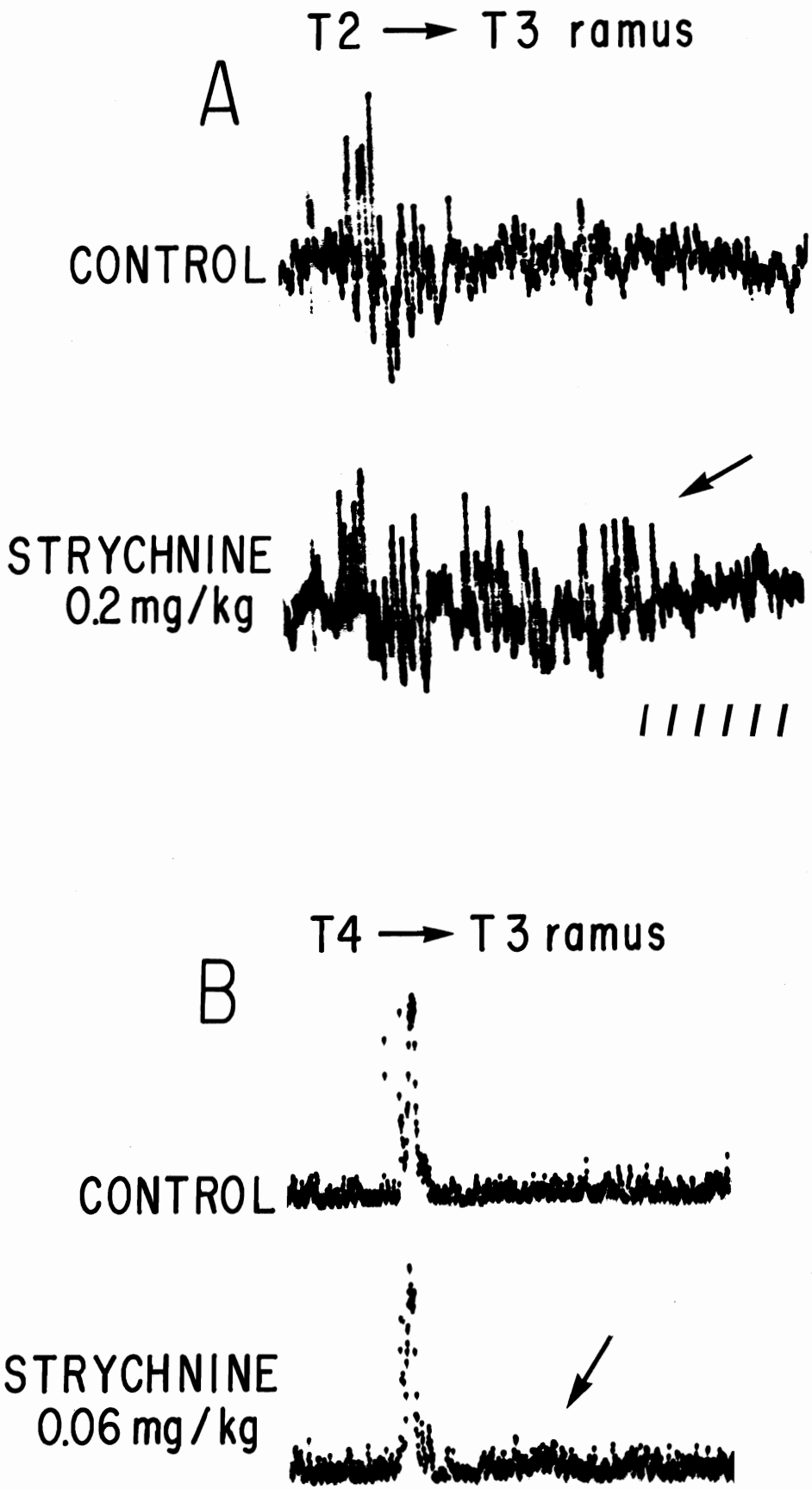

T2 → T3 ramus
A
CONTROL
STRYCHNINE
0.2 mg/kg
T4 → T3 ramus
B
CONTROL
STRYCHNINE
0.06 mg/kg

Fig. 11. Effect of strychnine on interactions between two sympathetic reflex responses in different pathways.

Graphs constructed as in Figure 5.

A. Maximal reflex response in T3 preganglionic ramus was evoked by stimulation of T4 spinal nerve and conditioned by a submaximal volley in T2 spinal nerve. Each point represents the average of 32 evoked responses.

B. Maximal reflex response in L1 preganglionic ramus was evoked by stimulation of L2 spinal nerve and conditioned by a maximal volley in splanchnic nerve. Each point represents the average of 25 evoked responses.

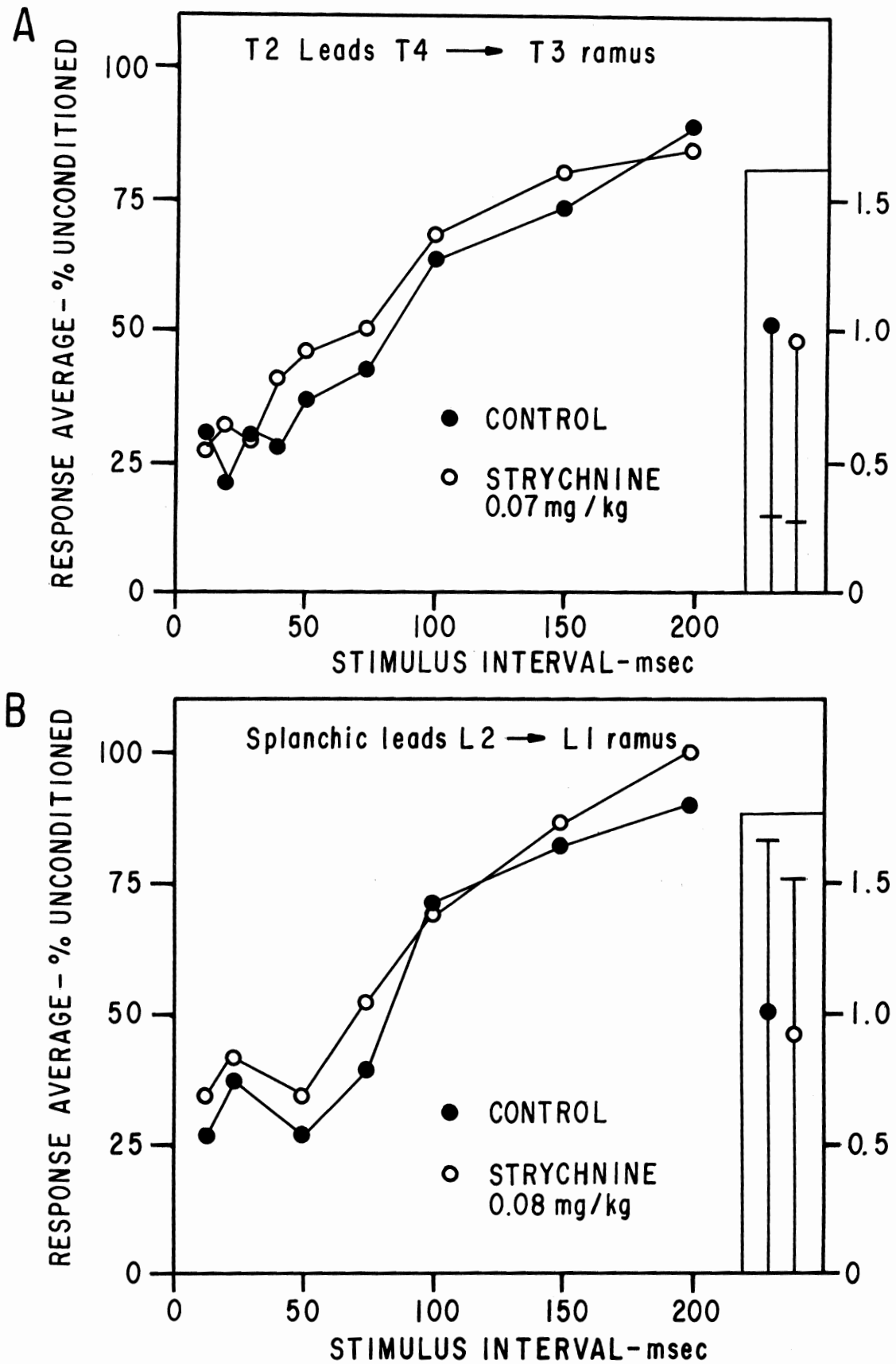

A
T2 Leads T4 → T3 ramus
RESPONSE AVERAGE - % UNCONDITIONED
100
75
50
25
0
0
50
100
150
200
STIMULUS INTERVAL - msec
1.5
1.0
0.5
0
CONTROL
STRYCHNINE
0.07 mg / kg
B
Splanchic leads L2 → L1 ramus
RESPONSE AVERAGE - % UNCONDITIONED
100
75
50
25
0
0
50
100
150
200
STIMULUS INTERVAL - msec
1.5
1.0
0.5
0
CONTROL
STRYCHNINE
0.08 mg/kg

Fig. 12. Effect of strychnine on interactions between sympathetic reflex responses in the same pathway.

Graphs constructed as in Figure 5.

A. Double pulses applied to the T2 spinal nerve were maximal for the first reflex response in T3 preganglionic ramus. The response to the second stimulus is plotted as the percentage of the response to the first stimulus. DHE: dihydro-beta-erythroidine. Each point represents the average of 40 evoked responses.

B. Same type of experiment as in A, but in a different preparation. L2 spinal nerve to L2 preganglionic ramus. Each point represents the average of 50 evoked responses.

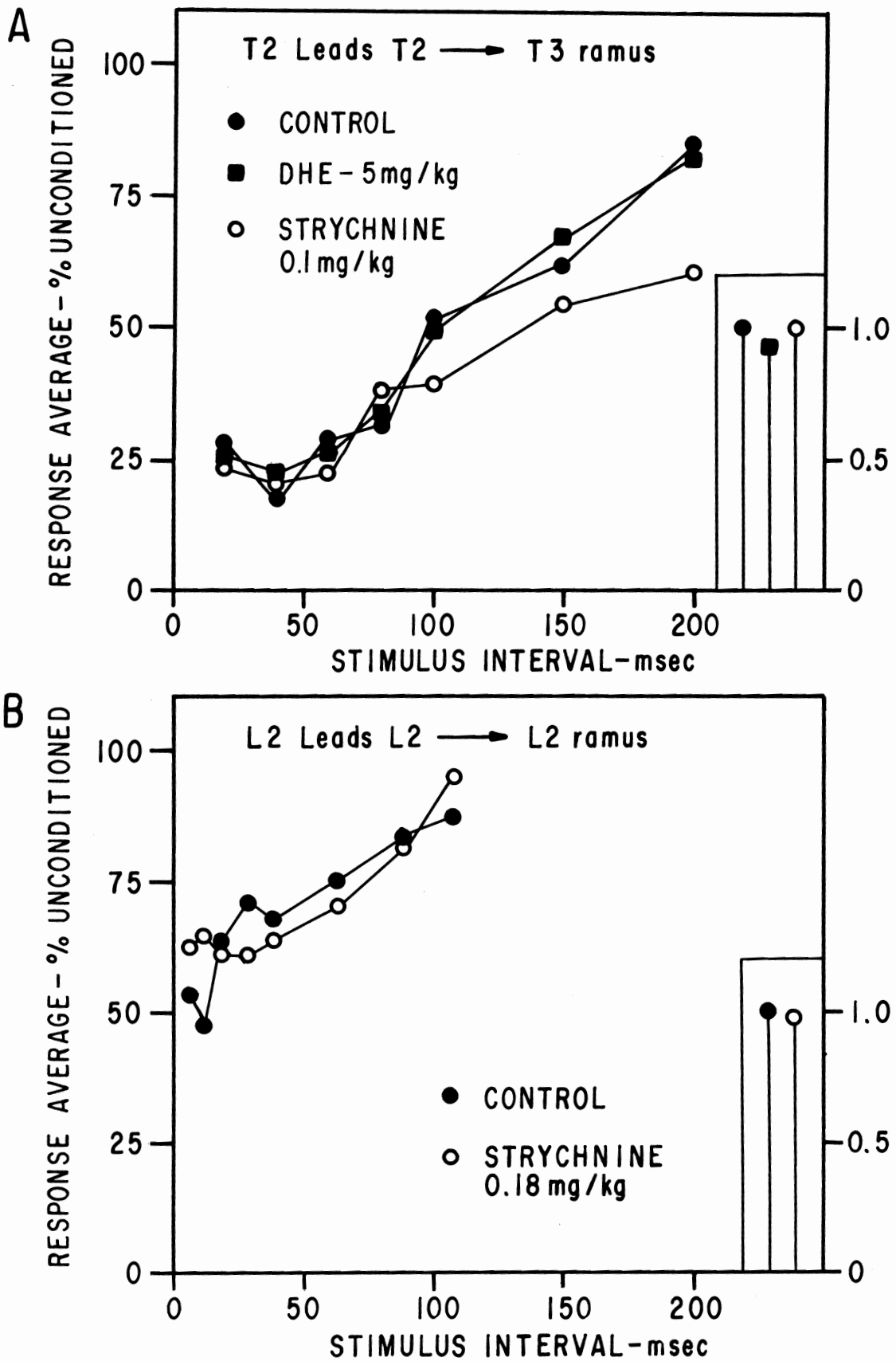

A
T2 Leads T2 → T3 ramus
CONTROL
DHE - 5mg/kg
STRYCHNINE
0.1 mg/kg
RESPONSE AVERAGE - % UNCONDITIONED
100
75
50
25
0
0
50
100
150
200
STIMULUS INTERVAL - msec
1.0
0.5
0
B
L2 Leads L2 → L2 ramus
CONTROL
STRYCHNINE
0.18 mg/kg
RESPONSE AVERAGE - % UNCONDITIONED
100
75
50
25
0
0
50
100
150
200
STIMULUS INTERVAL - msec
1.0
0.5
0

Fig. 13. Enhancement of spontaneous preganglionic and somatic activity by picrotoxin.

A. Relative frequency of spontaneous activity in T3 ramus during one, 512-sec (8.5 min) sweep. Sweep begins about 20 min after 1.5 mg/kg picrotoxin. The base line (Zero frequency) was established during the final 0.5 min of the sweep.

B. Spontaneous activity in T3 preganglionic ramus and T3 spinal nerve was recorded simultaneously on moving film 20 and 40 min after 1.2 mg/kg of picrotoxin. Gains of preganglionic and somatic records were equal.

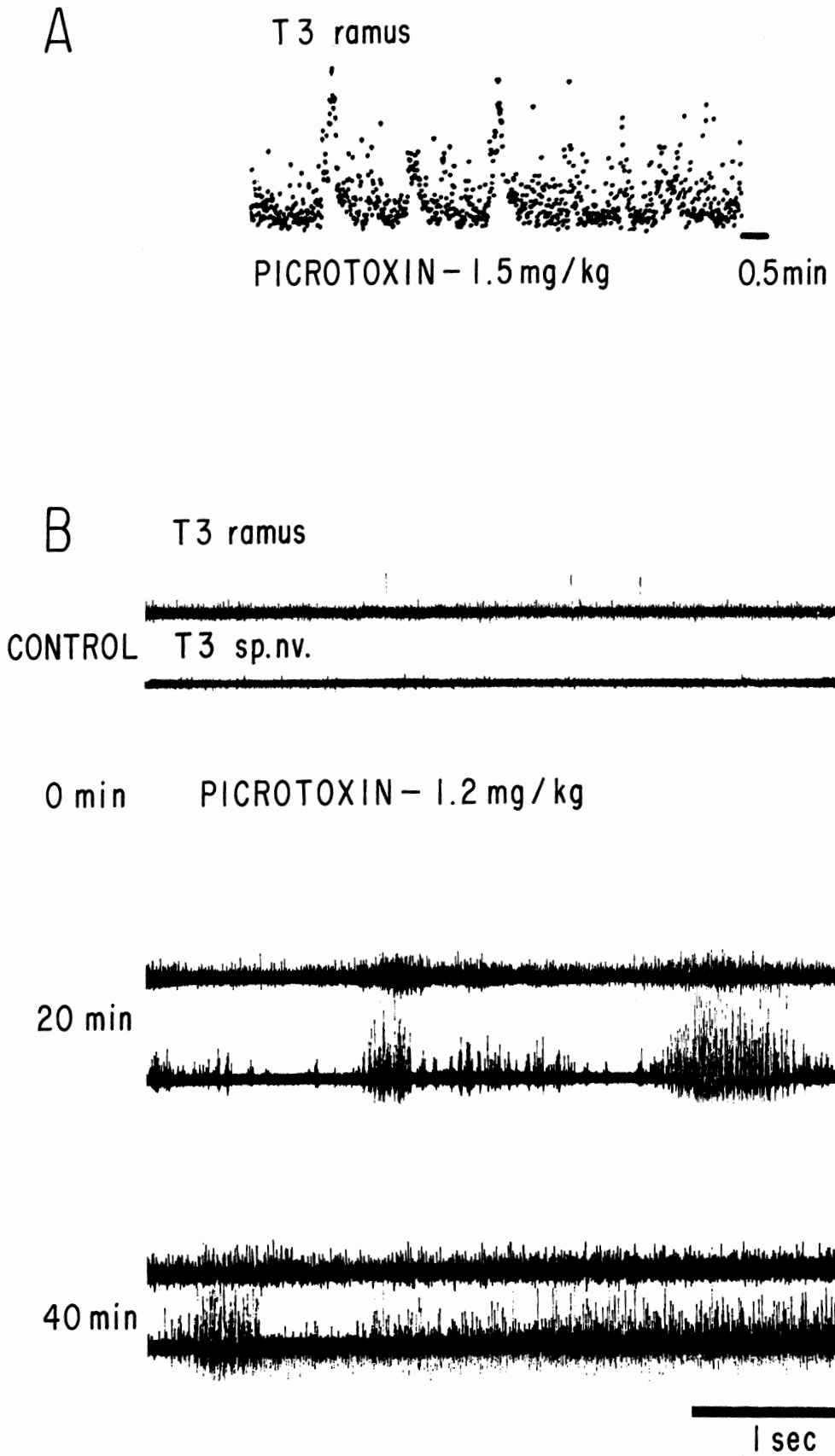

A
T 3 ramus
PICROTOXIN – 1.5 mg/kg
0.5 min
B
T3 ramus
CONTROL
T3 sp.nv.
0 min
PICROTOXIN – 1.2 mg/kg
20 min
40 min
1 sec

Fig. 14. Enhancement of sympathetic reflex responses by picrotoxin.

Graphs constructed as in Figure 4.

A. Stimulation of T4 spinal nerve evoked a maximal reflex response in T3 preganglionic ramus. Enhancement of responses was graded by two successive doses of picrotoxin (arrows). The large symbols represent four control averages obtained intermittently during 50 min prior to the 0 time and illustrate the stability of the control reflex response and the reproducibility of the averaging technique. Each point represents the average of 32 evoked responses.

B. Different preparation than in A. Stimulation of T2 spinal nerve evoked a maximal reflex response in T4 preganglionic ramus. The three control averages were obtained intermittently during 30 min prior to the 0 time. The response was enhanced by a single, 1.5 mg/kg dose of picrotoxin. Some postictal depression is evident at the final two points. Each point represents the average of 25 evoked responses.

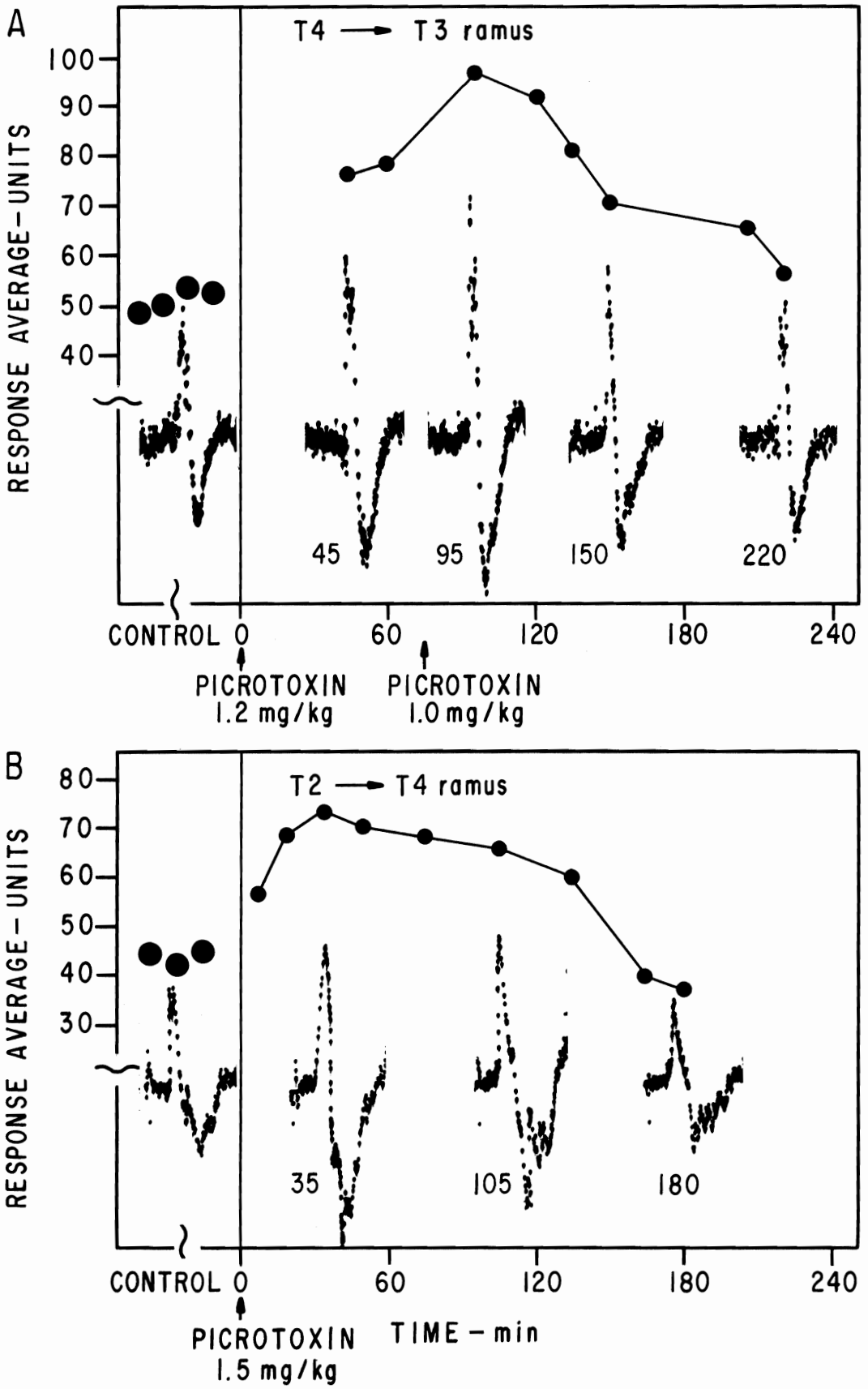
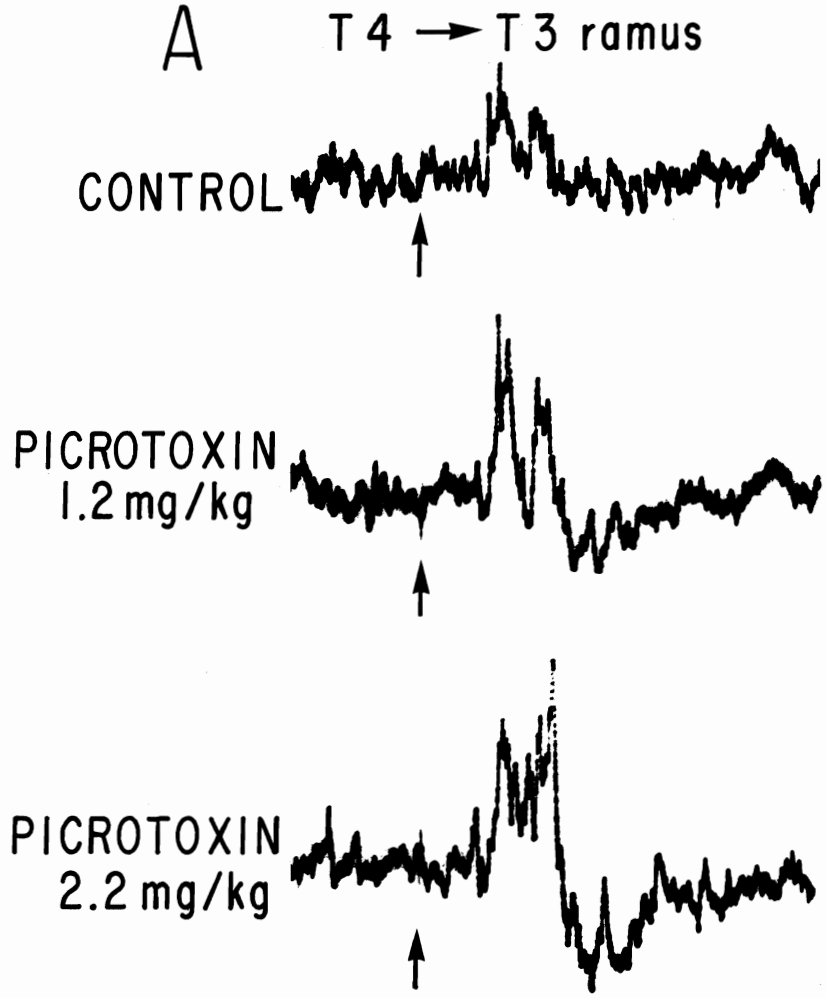

A
T4 → T3 ramus
RESPONSE AVERAGE-UNITS
100
90
80
70
60
50
40
45
95
150
220
CONTROL
0
60
120
180
240
PICROTOXIN
1.2 mg/kg
PICROTOXIN
1.0 mg/kg
B
T2 → T4 ramus
RESPONSE AVERAGE-UNITS
80
70
60
50
40
30
35
105
180
CONTROL
0
60
120
180
240
PICROTOXIN
1.5 mg/kg
TIME - min

Fig. 15. Enhancement of sympathetic reflex responses and initiation of after-discharge by picrotoxin.

A. Same experiment as in Figure 14A. Enhancement of single, maximal sympathetic reflex responses in T3 preganglionic ramus 50 min after 1.2 mg/kg of picrotoxin and 30 min after an additional 1.0 mg/kg of picrotoxin. Responses were evoked by stimulation of T4 spinal nerve. Arrows below each record denote stimulus artifact. Time marker: 10 msec.

B. After-discharge (arrow) of maximal reflex response in T3 preganglionic ramus 60 min after 1.5 mg/kg of picrotoxin. Relative frequency of evoked discharge during 25, 500-msec sweeps. Responses were evoked by stimulation of T2 spinal nerve.


A
T 4 → T 3 ramus
CONTROL
PICROTOXIN
1.2 mg/kg
PICROTOXIN
2.2 mg/kg

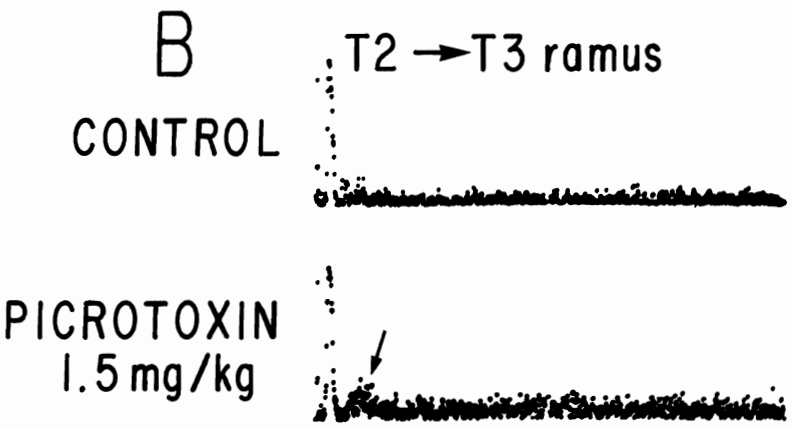

B
T2 → T3 ramus
CONTROL
PICROTOXIN
1.5 mg/kg

Fig. 16. Effect of picrotoxin on interaction between two sympathetic reflex responses in different pathways.

Graphs constructed as in Figure 5. The number above each symbol in the inset at the right is the respective reflex ratio: conditioning response average (cross bar) to test response average (symbol).

A. Maximal reflex response in T3 preganglionic ramus was evoked by stimulation of T4 spinal nerve and conditioned by one maximal volley in T2 spinal nerve. Two hours after the initial 1.6 mg/kg dose of picrotoxin, an additional 1 mg/kg was administered. Interactions were tested 1 hr after the first dose and 0.5 hr after the second dose. The broken line indicates an estimate at the 200 msec interval where responses were occluded. Each point represents the average of 50 evoked responses.

B. Same preparation and type of experiment as in A, except that the test stimulus was preceded by four conditioning stimuli (200/sec) instead of one. Interactions were tested 1.5 hr after the first dose and 0.75 hr after the second dose of picrotoxin. Shaded area on abscissa indicates duration of the conditioning train (12 msec). Each point represents the average of 50 evoked responses.

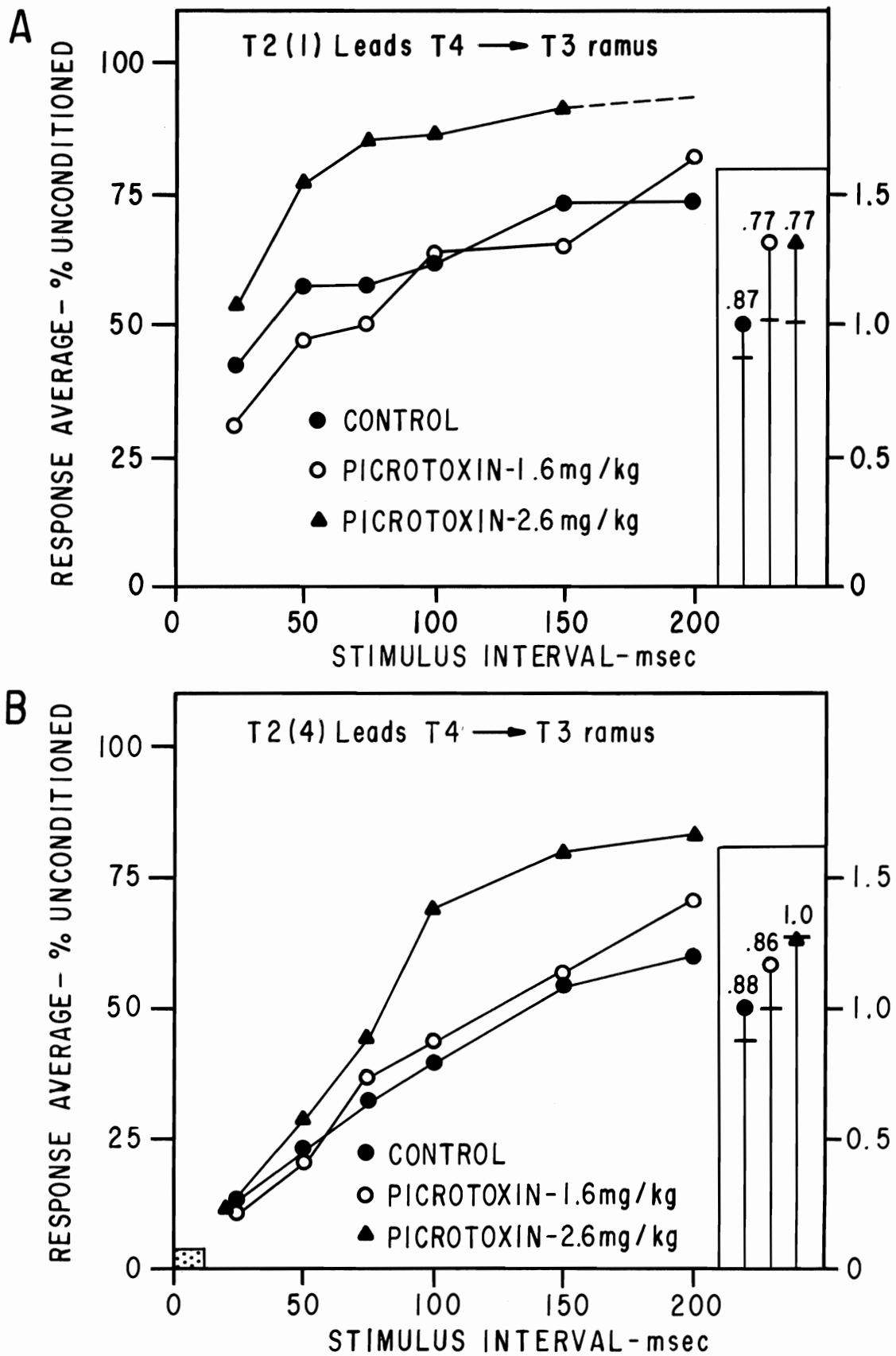

A
T2(1) Leads T4 → T3 ramus
RESPONSE AVERAGE- % UNCONDITIONED
100
75
50
25
0
1.5
1.0
0.5
0
.87
.77
.77
CONTROL
PICROTOXIN-1.6mg/kg
PICROTOXIN-2.6mg/kg
0
50
100
150
200
STIMULUS INTERVAL-msec
B
T2(4) Leads T4 → T3 ramus
RESPONSE AVERAGE- % UNCONDITIONED
100
75
50
25
0
1.5
1.0
0.5
0
.88
.86
1.0
CONTROL
PICROTOXIN-1.6mg/kg
PICROTOXIN-2.6mg/kg
0
50
100
150
200
STIMULUS INTERVAL-msec

Fig. 17. Differential effect of picrotoxin on interactions between two sympathetic reflex responses.

Graphs constructed as in Figures 5 and 16.

A. Depression of inhibition by picrotoxin. Maximal reflex response in T3 preganglionic ramus was evoked by stimulation of T4 spinal nerve and conditioned by one maximal volley in T2 spinal nerve. Interactions were tested at 2, 4, and 5 hr after a single, 1.5 mg/kg dose of picrotoxin. Each point represents the average of 25 evoked responses.

B. Enhancement of inhibition by picrotoxin. Different experiment than in A. Maximal reflex response in T3 preganglionic ramus was evoked by stimulation of T4 spinal nerve and conditioned by one submaximal volley in T2 spinal nerve. An additional 1 mg/kg of picrotoxin was administered 1.25 hr after the initial, 1.2 mg/kg dose. Interactions were tested 1 hr after the first dose and 0.5 and 2.5 hr after the second dose. Each point represents the average of 32 evoked responses.

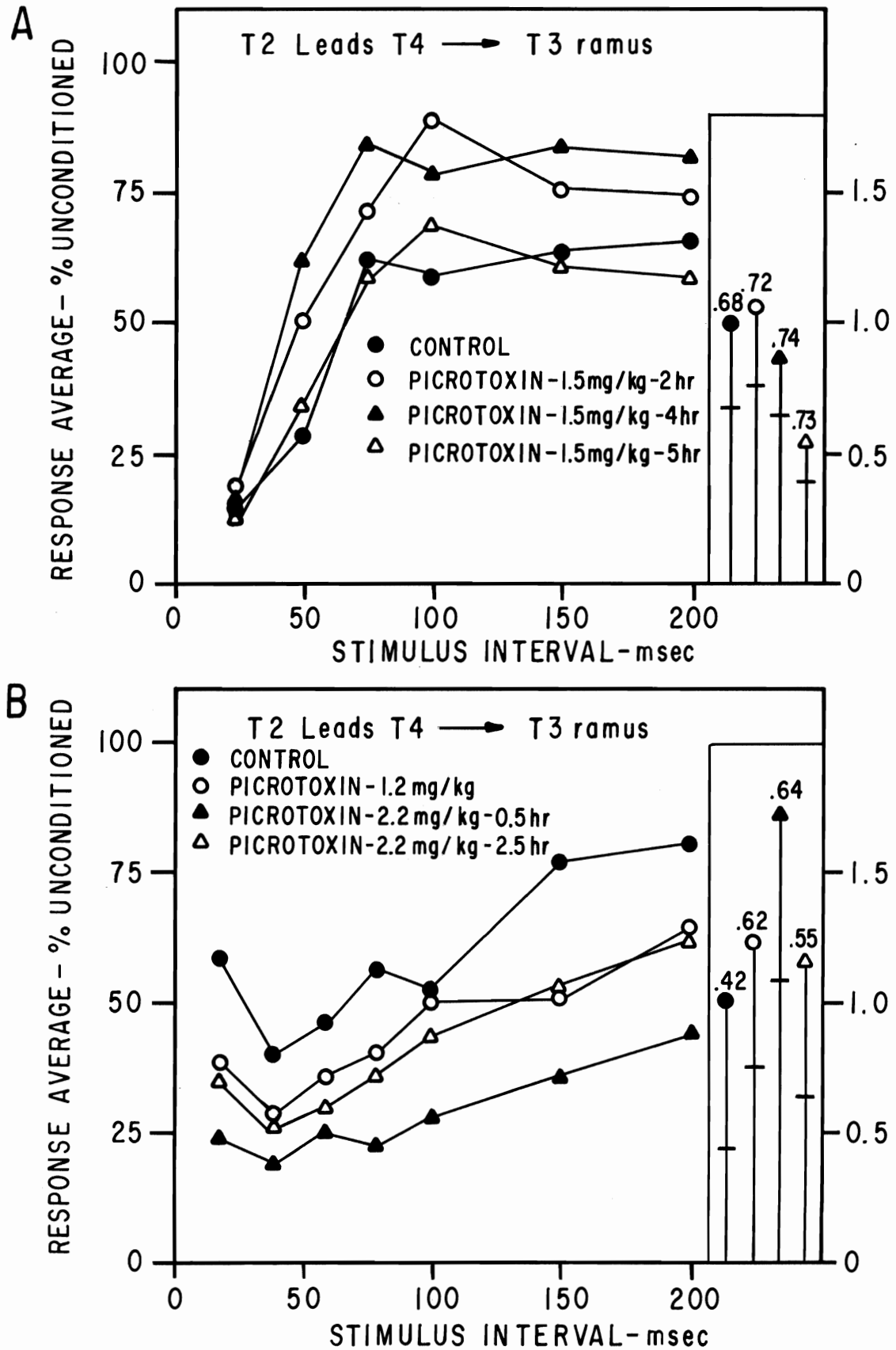

A
T2 Leads T4 → T3 ramus
RESPONSE AVERAGE - % UNCONDITIONED
100
75
50
25
0
CONTROL
PICROTOXIN - 1.5mg/kg - 2hr
PICROTOXIN - 1.5mg/kg - 4hr
PICROTOXIN - 1.5mg/kg - 5hr
.68
.72
.74
.73
1.5
1.0
0.5
0
0
50
100
150
200
STIMULUS INTERVAL - msec
B
T2 Leads T4 → T3 ramus
RESPONSE AVERAGE - % UNCONDITIONED
CONTROL
PICROTOXIN - 1.2 mg/kg
PICROTOXIN - 2.2 mg/kg - 0.5 hr
PICROTOXIN - 2.2 mg/kg - 2.5 hr
.42
.62
.64
.55
STIMULUS INTERVAL - msec

Fig. 18. Enhancement of spontaneous preganglionic activity by DOPA.

Spontaneous activity in T2 preganglionic ramus and T3 spinal nerve was recorded simultaneously on moving film. Separate doses of DOPA were administered 120 min apart. Time designations are relative to administration of the first dose. Gains of preganglionic and somatic records were equal.

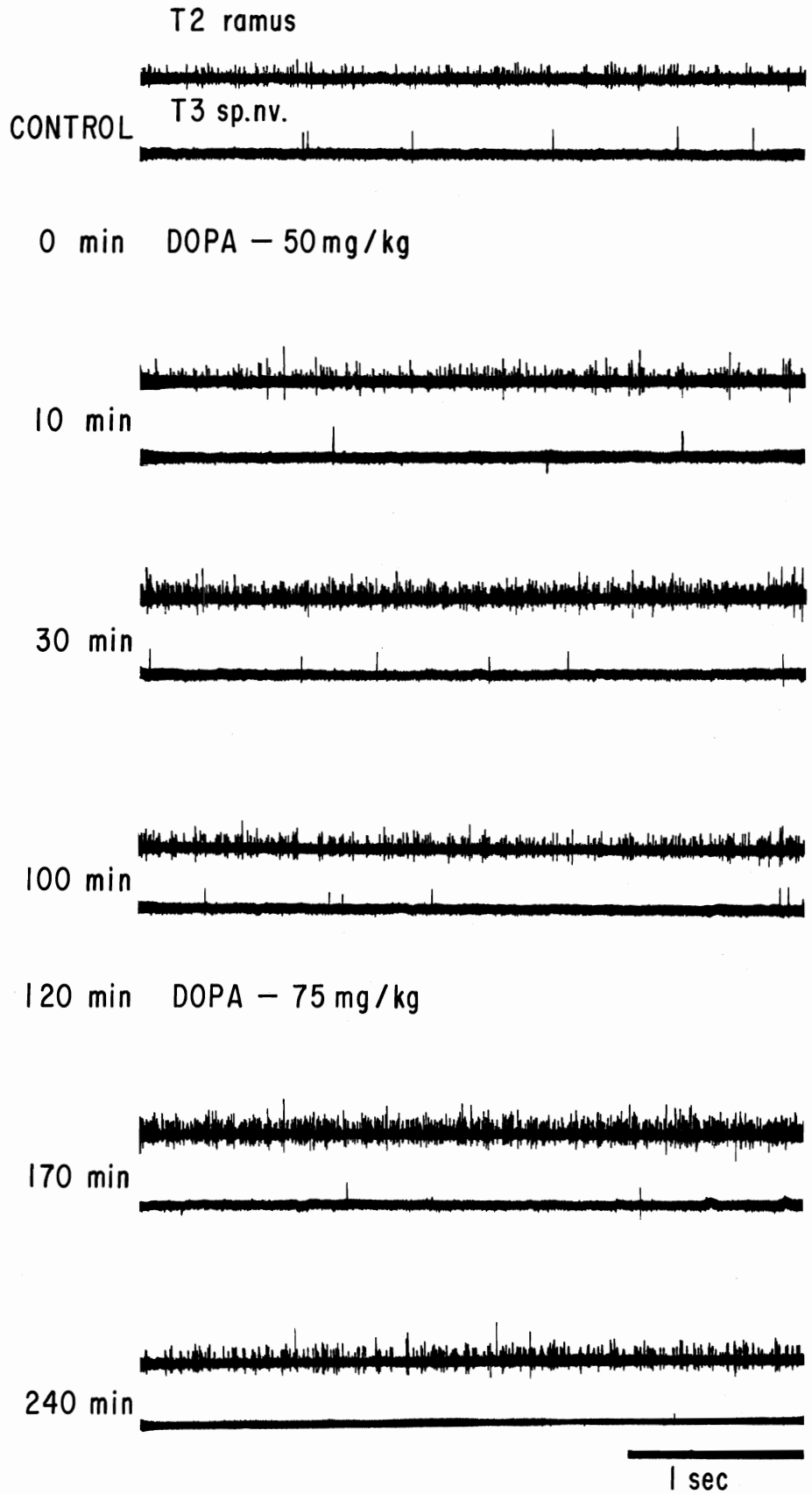

T2 ramus
T3 sp.nv.
CONTROL
0 min
DOPA — 50 mg/kg
10 min
30 min
100 min
120 min
DOPA — 75 mg/kg
170 min
240 min
1 sec

Fig. 19. Effects of 5-HTP on spontaneous preganglionic and somatic activity.

Spontaneous activity in T3 preganglionic ramus and T2 spinal nerve was recorded simultaneously on moving film. Gain of preganglionic records was ten times that of somatic records.

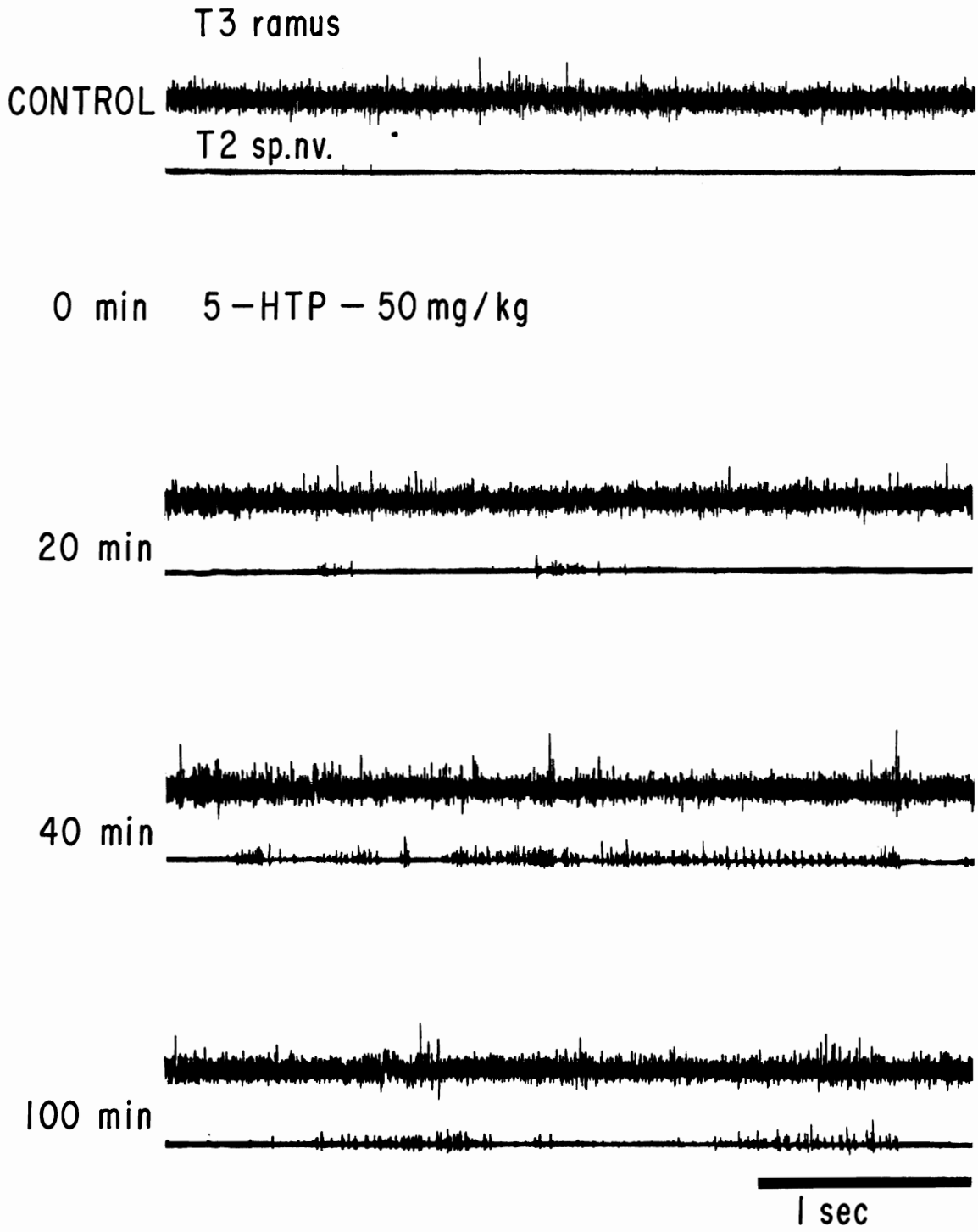

T3 ramus
CONTROL
T2 sp.nv.
0 min
5 – HTP – 50 mg / kg
20 min
40 min
100 min
1 sec

Fig. 20. Enhancement of sympathetic reflex responses and depression of somatic reflex responses by DOPA.

A. Progressive enhancement of maximal responses in T2 preganglionic ramus at the indicated times after administration of 75 mg/kg of DOPA. Responses were evoked by stimulation of T4 spinal nerve. Each record is the average of 50 evoked responses.

B. Same experiment as in A. Enhancement of single, maximal reflex responses in T2 preganglionic ramus by 75 mg/kg of DOPA was accompanied by depression of single, maximal somatic responses in T3 spinal nerve. Responses were evoked simultaneously by stimulation of the T4 spinal nerve. Time designations correspond to those in A. Gain of preganglionic records was five times that of somatic records. Arrows below each record denote stimulus artifact. Time marker: 10 msec.

C. Different preparation than in A. Enhancement of maximal responses in T3 preganglionic ramus by 60 mg/kg and by 40 mg/kg of DOPA administered 60 minutes apart. Time designations are relative to administration of the first dose. Responses were evoked by stimulation of the T4 spinal nerve. Each record is the average of 50 evoked reflex responses.

D. Enhancement of single, maximal reflex responses in L1 preganglionic ramus by 100 mg/kg of DOPA was accompanied by depression of single, maximal somatic responses in L1 spinal nerve. Responses were evoked by stimulation of the L2 spinal nerve. Gain of preganglionic records was twenty times that of somatic records. Time marker: 10 msec.

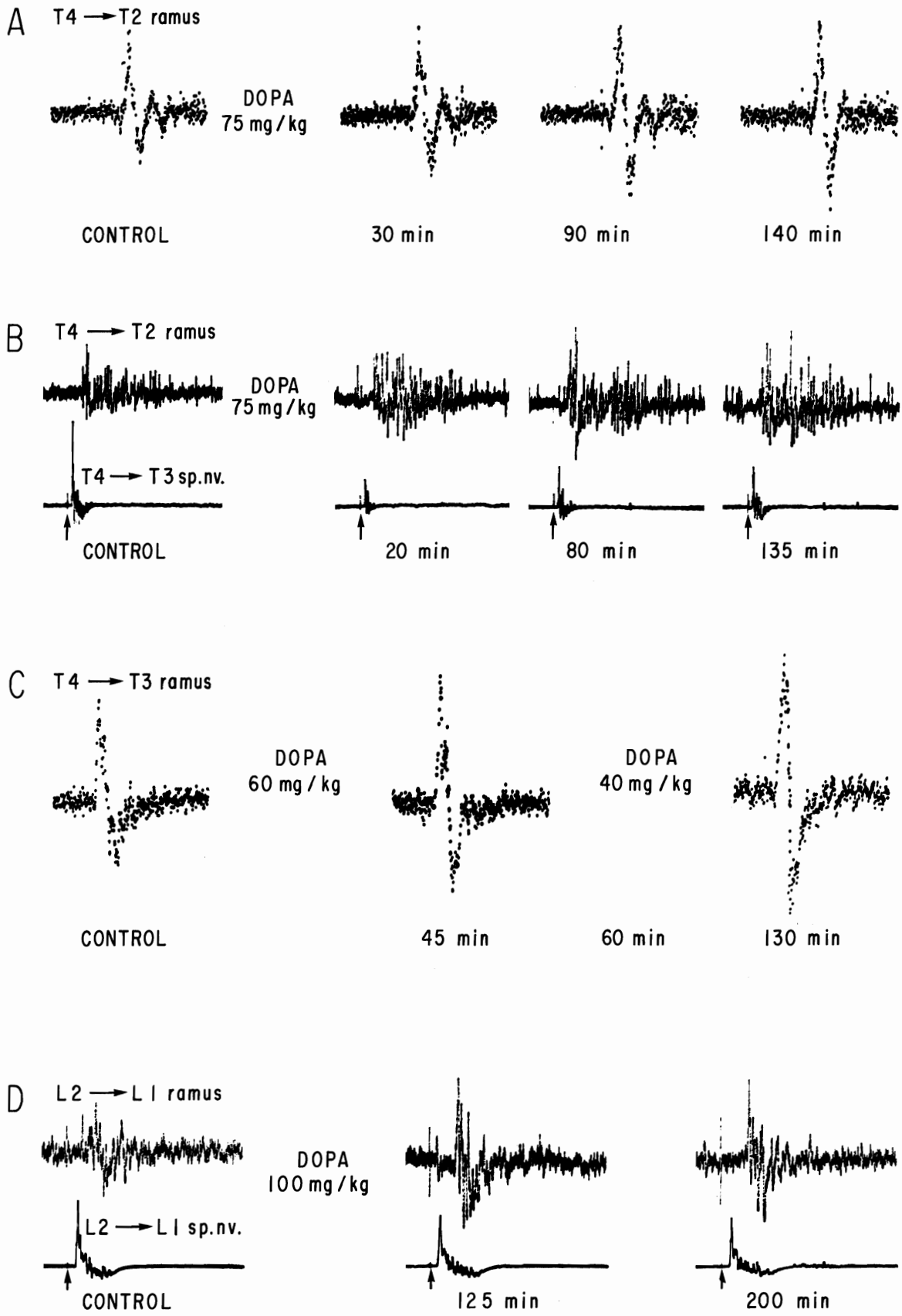
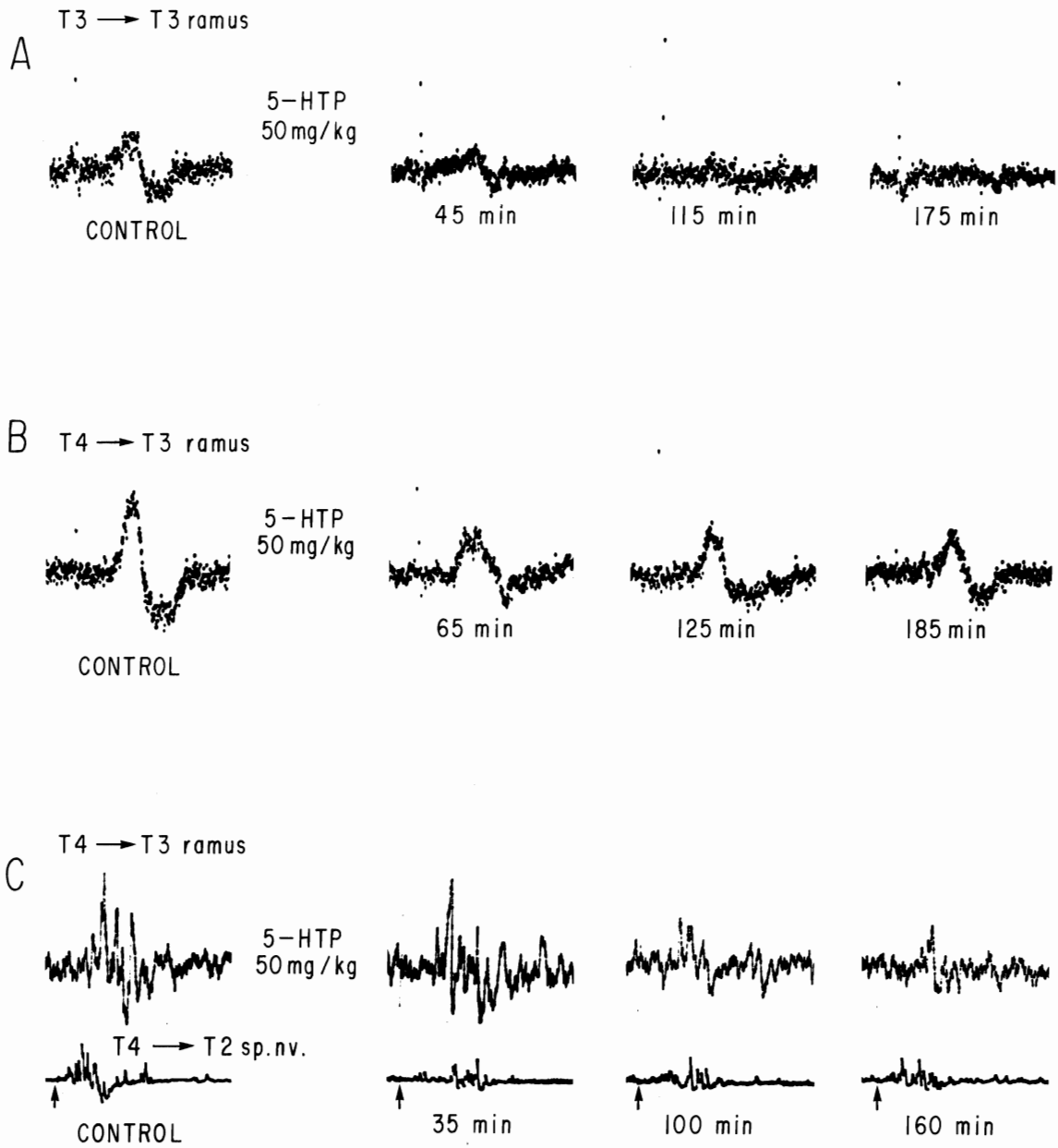

A
T4 → T2 ramus
DOPA
75 mg/kg
CONTROL
30 min
90 min
140 min
B
T4 → T2 ramus
DOPA
75 mg/kg
T4 → T3 sp.nv.
CONTROL
20 min
80 min
135 min
C
T4 → T3 ramus
DOPA
60 mg/kg
DOPA
40 mg/kg
CONTROL
45 min
60 min
130 min
D
L2 → L1 ramus
DOPA
100 mg/kg
L2 → L1 sp.nv.
CONTROL
125 min
200 min

Fig. 21. Depression of sympathetic and somatic reflex responses by 5-HTP.

A. Progressive depression of submaximal sympathetic reflex responses in T3 preganglionic ramus at the indicated times after administration of 50 mg/kg of 5-HTP. Responses were evoked by stimulation of the T3 spinal nerve. Each record is the average of 96 evoked responses.

B. Progressive depression of maximal sympathetic reflex responses in T3 preganglionic ramus by 50 mg/kg of 5-HTP. Responses were evoked by stimulation of the T4 spinal nerve. Each record is the average of 96 evoked responses.

C. Same experiment as in B. Depression of single, maximal reflex responses in T3 preganglionic ramus and T2 spinal nerve by 50 mg/kg of 5-HTP. Some recovery of the somatic response is evident in the last record. Gain of preganglionic records was ten times that of the somatic records. Arrows below each record denote stimulus artifact. Time marker: 10 msec.


A
T3 → T3 ramus
5-HTP
50 mg/kg
CONTROL
45 min
115 min
175 min
B
T4 → T3 ramus
5-HTP
50 mg/kg
CONTROL
65 min
125 min
185 min
C
T4 → T3 ramus
5-HTP
50 mg/kg
T4 → T2 sp.nv.
CONTROL
35 min
100 min
160 min

FUTURE RESEARCH PROPOSALS

Donald N. Franz

August, 1966

1. Pharmacological blockade or enhancement of the effects of dihydroxyphenylalamine (DOPA) and 5-hydroxytryptophan (5-HTP) on sympathetic reflexes in the spinal cat.

It has recently been demonstrated that spinal sympathetic reflexes are differentially influenced by the monoamine precursors, DOPA and 5-HTP (Franz, 1966). Sympathetic reflex responses recorded from preganglionic rami and evoked by stimulation of spinal nerves were enhanced by d,l-DOPA (50-100 mg/kg) and were depressed by d,l-5-HTP (25-50 mg/kg). In addition, spontaneous preganglionic activity was enhanced by DOPA. These responses were slowly progressive with time which suggested that these agents did not act directly but through the formation of their metabolites, norepinephrine (NE) or 5-hydroxytryptamine (5-HT).

In similar studies of somatic reflexes, Anden, Jukes and Lundberg (1964) and Lundberg (1965) found that both DOPA and 5-HTP depress short latency transmission from the flexor reflex afferents to primary afferents, motoneurons, and to ascending pathways. In addition, 5-HTP increased excitability of flexor and extensor motoneurons so that monosynaptic reflexes are increased and spontaneous discharges appear in ventral roots. Inhibition of decarboxylase, an enzyme necessary for the conversion of the precursors to the amines, prevents the effects of DOPA and 5-HTP, which indicates that the precursors act only after conversion to NE and 5-HT and not directly. Inhibition of monoamine oxidase, an inactivating enzyme of NE & 5-HT, potentiates the effect of both DOPA and 5-HTP on spinal somatic reflexes. Finally, the alpha adrenergic

blocking agent, phenoxybenzamine blocks the effects of DOPA but not those of 5-HTP. The latter's effects are, however, partially blocked by the 5-HT antagonist, 2-bromo d-lysergic acid diethylamide (BOL).

Determination of the ability of such agents (a decarboxylase inhibitor, a monoamine oxidase inhibitor, and phenoxybenzamine and BOL) to alter the effects of DOPA and 5-HTP on spinal sympathetic reflexes would add support to the view that these effects are mediated by NE or 5-HT.

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Lundberg, A. Monoamines and spinal reflexes. In, Studies in Physiology. (Curtis, D. R. and A. K. McIntyre, Editors). Springer-Verlag, New York, 1965, 186-190.

2. The effect of DOPA and 5-HTP upon the supraspinal control of preganglionic neurons.

The use of a specific and sensitive histochemical fluorescence method for the cellular localization of catecholamines and 5-HT has recently demonstrated that sympathetic preganglionic neurons receive a direct and very dense, terminal innervation from NE- and 5-HT- containing fibers that descend from medullary neurons via bulbospinal tracts (Carlsson et al., 1964; Dahlström and Fuxe, 1965). The effects of the precursors of NE and 5-HT, DOPA and 5-HTP, respectively, on spontaneous and reflex activity of preganglionic neurons in the spinal cat have led to the suggestion that NE serves

an excitatory transmitter function and 5-HT serves an inhibitory transmitter function for the supraspinal control of preganglionic neurons (Franz, 1966).

The purpose of this proposed investigation is to determine the effect of DOPA and 5-HTP on sympathetic reflexes evoked by discrete stimulation of supraspinal centers in the medulla or of their descending tracts in the spinal cord. This approach is more direct than the one confined to sympathetic reflex arcs, since the bulbospinal pathways that contain NE or 5-HT appear to make synaptic contact with the preganglionic neurons. Both vasodepressor and vasopressor areas in the medulla, where these neurons originate, have been described repeatedly.

As an extension of this study, the effects of monoamine oxidase inhibitors, blocking agents of NE and 5-HT, and decarboxylase inhibitors on bulbospinal-preganglionic transmission, with and without the influence of DOPA and 5-HTP, would provide additional evidence to test the credibility of the proposal that NE and 5-HT function as transmitters between bulbospinal and preganglionic neurons.

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3. The effects of strychnine and picrotoxin on spinal sudomotor neurons.

Strychnine and picrotoxin produce intense, spontaneous discharges of vasomotor neurons in the cat within several hours after spinal transection (Franz, 1966). At this time, spontaneous and reflex activity of preganglionic neurons, at least a part of which is vasomotor, is also present. However, spontaneous and reflex activity of sudomotor neurons does not return for several days after spinal transection, according to electrodermal studies in the cat (Wang, 1964).

The purpose of this proposed investigation is to determine the sensitivity of sudomotor neurons to strychnine and picrotoxin within hours after spinal transection. Electrodermal recording from the four footpads would provide a reliable measurement of sudomotor activity and the syncronization of spontaneous discharges. The cat is ideally suited for this study because it has sweat glands only in the footpads, and the sudomotor neurons for the forelimbs are confined to the 4th to the 9th thoracic segments and those for the hindlimbs, from the 11th thoracic to the 2nd lumbar segments.

If the drugs prove to be ineffective in causing spontaneous sudomotor discharges, the study can be extended to include chronic spinal cats.

This study would determine whether sudomotor neurons are involved in the spontaneous preganglionic discharges elicited by strychnine and picrotoxin and, if so, would provide a technically simple measurement of the syncrony spontaneous preganglionic discharges throughout the thoracolumbar spinal cord. A lack of effect in acute preparations would indicate that sudomotor neurons or their central afferent connections are more severely depressed following spinal transection than are the vasomotor neurons. Simultaneous electrodermal and blood pressure recordings would furnish an estimate of the

relative spontaneous activity in two, functionally different types of preganglionic neurons.

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Wang, G. H. The neural control of sweating. University of Wisconsin Press, Madison, 1964.

4. Interactions between nonmyelinated nerve fibers.

Nonmyelinated nerve fibers are contained within a tubular system of Schwann cell cytoplasm in which they are enveloped. The space between the axons and Schwann cells is continuous with the extracellular space, although some impediment to free exchange between the periaxonal space and the bulk of extracellular space appears to exist. Although a number of axons may be enveloped by one Schwann cell tube, the individual fibers crisscross from one Schwann cell to another throughout their course. The devious course a fiber takes through the Schwann cell network reduces the possibility of interaction (Gasser, 1955; Douglas and Ritchie, 1962).

The accumulation of potassium in the periaxonal space following activation of nonmyelinated fibers is sufficient to account for their negative after-potentials which summate to increase the size of subsequent compound action potentials (Ritchie and Straub, 1956). The large surface-to-volume of non-myelinated fibers accounts for the relatively large potassium efflux during activity.

The recent demonstration that single or repetitive activity in non-myelinated fibers can cause an appreciable depolarization of adjacent neuroglia in the optic nerve of the mud puppy (Orkand, Nicholls, and Kuffler, 1966)

suggests that repetitive activity in one group of nonmyelinated fibers may cause depolarization in a second group of nonmyelinated fibers by increasing the potassium concentration of periaxonal spaces common to both groups.

To investigate this problem, a small bundle of nerve fibers containing many nonmyelinated fibers would be separated at one end of a relatively long segment. Stimulation of each bundle would evoke action potentials in separate groups of fibers, both of which could be recorded from the common nerve bundle at the other end. Tetanization of one pathway should produce a decrease in the nonmyelinated action potentials of the other pathway if its axons were sufficiently depolarized by the increased potassium to prevent transmission. Demonstration of such an interaction may have implications in some types of inhibition in the CNS.

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Orkand, R. K., Nicholls, J. G. and S. W. Kuffler. An effect of nerve impulses on the membrane potential of vertebrate neuroglia. Fedn Proc. Fedn Am. Socs exp. Biol., 1966, 25, No.2, Part I, 395 (abstr.).

Ritchie, J. M. and R. W. Straub. The after-effects of repetitive stimulation on mammalian nonmedullated fibers. J. Physiol., Lond., 1956, 134, 698-711.

5. Intracellular acid-base and electrolyte devigations induced by methanol.

The severe metabolic acidosis that is induced by methanol poisoning cannot be adequately explained by the formation of formic acid in the body. If all the methanol in most poisoning cases were converted to formic acid, it should be easily buffered by the normal buffer systems of the body. Nevertheless, severe metabolic acidosis is produced in humans and primates by the ingestion of methanol. The relationship of the acidosis to the production of blindness is unknown. Laboratory investigation of this problem is complicated by the lack of acidosis production and retinal changes in subprimate laboratory animals (Cooper and Kini, 1962).

Measurement of intracellular and extracellular acid-base and electrolyte changes in primates may furnish some insight into the mechanism of methanol toxicity, especially the severe acidosis disturbances that are produced. Intracellular pH remains almost unchanged in other types of acid-base disturbances, with the exception of increased pCO_2. Marked changes in cell pH by methanol or its metabolites would be unusual. However, its occurrence would provide a possible clue to the mechanism of methanol toxicity. In spite of the greater cost of using primates, relatively few animals would be required to establish the potential advantage of this experimental approach.

REFERENCE

Cooper, J. R. and M. M. Kini. Biochemical aspects of methanol poisoning. Biochem. Pharmac., 1962, II, 405-416.

VITA

Donald Norbert Franz was born on September 23, 1932 in Indianapolis, Indiana where he received his elementary and secondary education. Following graduation from high school in 1950, he attended Butler University in Indianapolis for four years and received his Bachelor of Science degree in Pharmacy in 1954.

Soon after graduation from the University, Mr. Franz served for two years in the United States Army, primarily at the St. Louis Medical Depot and Ft. Carson, Colorado. After separation in October of 1956, he worked in Colorado Springs, Colorado for three years as a Registered Pharmacist and Assistant Manager, first, of the Colorado Springs Medical Center Pharmacy and then, of Pieper Drugs, Inc.

In September of 1959, Mr. Franz returned to Butler University for graduate studies and received his Master of Science degree in Pharmacology in June of 1962, after completing his requirements in November of 1961. During this time he also held a teaching assistantship in the College of Pharmacy.

Since March of 1962 he has been engaged in graduate study in the Department of Pharmacology, College of Medicine, University of Utah and expects to receive a Doctor of Philosophy degree in August of 1966. During his graduate studies, he has engaged in a variety of research endeavors, but his later work, including research projects in the Departments of Pharmacology and Physiology and his thesis research, has been in neuropharmacology and neurophysiology. One publication, "Viscerosympathetic Reflexes in the Spinal Cat" in collaboration with Dr. Martin H. Evans and Professor Edward R. Perl, has been accepted

for publication by the American Journal of Physiology, and several others are in preparation. He has presented papers at scientific meetings on four occasions. After graduation, he plans to spend two years of a postdoctoral fellowship at the University of Edinburgh, Scotland and a third year at Harvard Medical School.

Mr. Franz is married to Barbara Stiver of Morrison, Colorado. They have two children: Diane (5) and Beth (1 month).