NEURAL INHIBITION OF TRANSMISSION IN CENTRAL VASOPRESSOR PATHWAYS

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ABSTRACT

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By

David Wells Snyder

The purpose of this investigation was to study the inhibitory control of transmission in central sympathetic vasopressor pathways in the cat. Specific emphasis was placed on defining the nature of the sympathoinhibitory effect of the medial medullary depressor region stimulation and the nature of the sympathetic silent period.

Vasopressor outflow from the brain to the external carotid postganglionic nerve is distributed over two systems of pathways (Gebber et al., 1973). Postganglionic potentials evoked from the first pathway (long-latency) pressor pathway) were characterized by their long onset latencies (>50 msec) and receptivity to blockade upon baroreceptor reflex activation. Postganglionic potentials evoked from the second pathway (short-latency pressor pathway) were not inhibited by baroreceptor reflex activation and had onset latencies of less than 50 msec. Stimulation of sites in the medial medulla, which reduced blood pressure and spontaneously-occurring postganglionic discharges, always inhibited long-latency potentials evoked from midbrain, medullary and descending spinal components of the long

latency pressor pathway. In contrast, sympathetic nerve responses evoked from descending spinal components of the short-latency pressor pathway were not inhibited by depressor region stimulation. These effects of depressor region stimulation were mimicked by baroreceptor reflex activation. However, unlike baroreceptor reflex activation, stimulation of many sites in the depressor region inhibited the short-latency responses evoked from midbrain or medullary components of the short-latency pressor pathway. These results demonstrated that two distinct sympathoinhibitory systems can be activated from the depressor region of the medial medulla. The first mimics the baroreceptor reflexes, acting at a spinal level to inhibit transmission in the long-latency pressor pathway. The second inhibitory system of non-baroreceptor origin acts at a supraspinal level to inhibit transmission in the short-latency pressor pathway.

Baroreceptor reflex activation and depressor region stimulation, which reduced spontaneously-occurring discharge, produced reciprocal effects on the amplitude of the post-ganglionic potentials evoked from brain stem or spinal components of the two vasopressor pathways. Long-latency sympathetic nerve responses were inhibited, while the post-ganglionic responses evoked at submaximal intensities from the short-latency pathway were enhanced. The reciprocal effects were observed when the short- and long-latency responses were evoked from descending spinal pressor tracts

and recorded from the preganglionic cervical sympathetic nerve. Intensity-response curves of depressor region stimulation revealed the proportionality of the inverse relationship between the degree of enhancement of the short-latency responses elicited from the medulla and inhibition of spontaneously-occurring discharges and the long-latency postganglionic potentials. The data suggested that the facilitatory effect on transmission in the short-latency pathway involved the removal of a tonic spinal inhibition (i.e. disinhibition) which normally is being modulated by the level of basal sympathetic discharges in the long-latency pressor pathway.

Experiments were also designed to study the nature and site of initiation of the silent period which followed splanchnic sympathetic nervous excitation produced by stimulation of the medullary pressor region. The data support the view that true neural inhibition is the major factor involved in the genesis of the sympathetic silent period. This was indicated since it was possible to set the interval between conditioning and test stimuli applied to a medullary pressor site so that the test splanchnic nerve discharge, but not the attendant silent period, was blocked. Thus, the silent period could be dissociated from the test discharge.

The level of the neuraxis at which the inhibition responsible for the silent period occurred was studied by comparing the excitability-recovery curves of the test splanchnic discharge elicited by stimulation of medullary

and spinal pressor sites. The test discharge evoked by stimulation of descending tracts in the midcervical spinal cord was depressed following conditioning stimuli applied to the medullary pressor region or to the same spinal site. The time course (>1 sec) of depression of the test discharge evoked in either case was the same as that observed for a test discharge evoked by medullary stimulation. Transection of the spinal cord at the first cervical vertebra failed to alter the time course or degree of depression of the test discharge when conditioning and test stimuli were applied to the same midcervical spinal site. These observations indicate that the inhibition mediating the silent period occurred at a spinal synapse through a feed-forward inhibitory pathway. The experiments also suggest that sympathoinhibitory systems of the medial medulla play a subordinate role in the generation of the silent period from the medulla. The subordinate effect of the medial medulla also was exerted at a spinal locus through a second feed-forward pathway.

Gebber, G. L., D. G. Taylor and L. C. Weaver. Electrophysiological studies on organization of central vasopressor pathways. Am. J. Physiol. 224:470-481, 1973.

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to my wife, Linda

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INTRODUCTION

A. Central Organization of Sympathetic Vasopressor Systems

To study the modulating influences on sympathetic nervous discharge which govern the level of blood pressure, one must be aware of the organization of the central neural components of the sympathetic nervous system. Traditionally, the medulla has been regarded as the integrating center for central cardiovascular control. The initial evidence was obtained in 1871 when Owsjannikow (see Bard, 1960) showed that the medulla oblongata was necessary for the maintenance of resting blood pressure. He made successive, rostral to caudal, serial transections of the brain while monitoring blood pressure. He found that transection of the brain stem 1-2 mm below the border of the inferior colliculi slightly reduced resting blood pressure; however, at a level 4-5 mm above calamus scriptorius, blood pressure fell to the same level as that seen in an animal whose spinal cord was transected at the first cervical vertebra.

In 1916, Ranson discovered the importance of supraspinal neural elements in the pressor reflex arc. According to Ranson, the pressor response to brachial and sciatic nerve stimulation was similar in normal cats. However, in cats with chronic lesions placed in the posterior gray

columns and posterior funiculi of the cervical spinal cord, the pressor response to sciatic nerve stimulation was markedly reduced when compared to that evoked by brachial stimulation. The lesion, which was caudal to the origin of the brachial nerves, blocked the afferent impulses of sciatic nerve stimulation as they ascended in the spinal cord. He concluded that the pressor reflex arc evoked by afferent nerve stimulation was not complete within the spinal cord, but dependent upon a supraspinal pathway.

Ranson and Billingsley (1916) explored the surface of the fourth ventricle in an attempt to localize sites that influenced blood pressure. They found that electrical activation of the medullary surface with needle monopolar stimulating electrodes at the level of the inferior fovea produced pressor responses, whereas a fall in blood pressure was observed upon stimulation of area postema. However, with the advent of the Horsley-Clark stereotaxic technique, the depths of the brain stem could also be explored in detail. Using this three-dimensional mapping system, Wang and Ranson (1939) observed that pressor responses were obtained from electrical activation of sites confined to the dorsal lateral reticular formation and adjacent periventricular gray. In contrast, depressor responses were evoked primarily from the caudal area of the medial medullary reticular formation.

The classical study of Alexander (1946) showed that changes in postganglionic sympathetic nervous discharges

were related to changes in blood pressure, while he was mapping the lower brain stem for vasoactive sites. He defined the lateral reticular formation of the caudal pons and rostral 2/3 of the medulla as the pressor region, whereas the depressor region occupied the medial reticular formation in the caudal half of the medulla. With the use of brain stem transection, Alexander tried to locate the origin of tonic sympathetic nervous activity. He found that sectioning the brain stem rostral to the auditory tubercle produced little effect on blood pressure or spontaneously-occurring postganglionic sympathetic nervous discharge. A significant reduction in blood pressure and nerve activity was observed following section at the level of the auditory tubercle. This eliminated the rostral pressor regions without affecting the caudal depressor sites. A section slightly rostral to the obex produced a maximal fall in blood pressure and abolished sympathetic discharges recorded on the inferior cardiac nerve. This section eliminated almost all of the pressor area, but left the majority of the depressor region intact with the spinal cord. Section at the first cervical vertebra led to a return of a small, but noticeable, level of sympathetic nervous discharges. Alexander concluded that the neural elements in the pressor region of the medulla are essential for the maintenance of blood pressure through a tonic excitatory outflow to the sympathetic vasoconstrictor nerves. He further suggested that the depressor region exerted a tonic sympathoinhibition on the sympathetic

neurons of the spinal cord. However, the sympathetic discharge observed in the spinal animals may have been the result of anoxia.

The studies referenced above described regions of the brain stem which, upon activation, evoked changes in resting blood pressure. However, they offered little information regarding the organization of central sympathetic pressor pathways. As suggested by Alexander (1946) and Chai and Wang (1968), it would be difficult to decide if stimulation of these pressor sites activated efferent, afferent or association elements of the vasopressor pathway. In addition, it would be impossible to determine if more than one pressor system was activated in such experiments.

Gebber et al. (1973), have recently addressed the problem of the central organization of vasopressor pathways, using an electrophysiological approach. They demonstrated that vasoconstrictor outflow from the hypothalamus to the external carotid nerve is distributed over two systems of pathways, the short-and long-latency pressor systems. Recording sympathetic discharges from a postganglionic fiber bundle which subserves strictly vasoconstrictor function, they applied single shocks or short trains of 3 pulses to pressor sites at various levels of the neuraxis. The centrally-evoked discharges were defined on the basis of their onset latency, contours, following frequency and receptivity to blockade by baroreceptor reflex activation. The responses evoked by medullary stimulation were obtained

from the periventricular gray, underlying dorsolateral reticular formation and lateral portions of nucleus reticularis ventralis. These structures correspond to the pressor region described by Alexander (1946). The medullary-evoked responses could be divided into two groups: 1) those with latencies greater than 50 msec were always blocked by baroreceptor reflex activation, and 2) those with shorter latencies (less than 50 msec) were unaffected by baroreceptor reflex activation.

Postganglionic potentials evoked by stimulation of spinal pressor sites could be divided into three groups: 1) those with long onset latencies (60-120 msec) which were blocked by baroreceptor reflex activation and eliminated by C-1 transection and, therefore, assumed to be evoked by stimulation of ascending afferent pathways; 2) those with latencies of 36-52 msec that were partially blocked by baroreceptor reflex activation; and 3) those with onset latencies of 26-42 msec that were not inhibited by baroreceptor reflex activation. The potentials in groups 2 and 3 were unaffected by transection of the spinal cord at C-1 and thus, presumably, were evoked by activation of two different systems of descending spinal tracts.

Taylor and Gebber (1973) extended this investigation further by recording from single cervical preganglionic sympathetic neurons of the cat thoracic spinal cord while stimulating medullary pressor sites. They observed that individual preganglionic sympathetic neurons are influenced

by neural elements located throughout the medullary pressor region. They described two types of unitary response patterns evoked from various medullary pressor sites. The first type showed a variable onset latency discharge when activated from one pressor site in the medulla. These responses were always blocked by baroreceptor reflex activation. The second type showed a relatively fixed latency discharge and was not amenable to baroreceptor reflex induced inhibition. The authors concluded that these two patterns, 1) the variable onset latency pattern, and 2) the relatively fixed onset latency pattern, represented the activation of the medullary components of the two systems of vasopressor pathways previously described (Gebber et al., 1973).

B. Neural Control Mechanisms

1. Nature of Synaptic Inhibition

Neural inhibition within the mammalian central nervous system is either of a postsynaptic or of a presynaptic nature. Most of our knowledge about inhibitory synaptic processes stems from the ideas of Sherrington (1906), which were elaborated upon by Eccles' group with the use of sophisticated electrophysiological techniques performed on alpha (α) motoneurons. Only recently have workers employed similar techniques to study the control mechanisms of the autonomic reflex pathways (Fernandez DeMolina et al., 1965a,b).

The initial investigation describing a postsynaptic inhibitory mechanism was made by Brock et al. (1952). Recording intracellularly from cat α extensor motoneurons, they observed that stimulation of a group Ia afferent pathway from a flexor muscle produced a brief transmembrane hyperpolarization. Coombs et al. (1955) termed this hyperpolarization the "inhibitory postsynaptic potential" (IPSP). For a detailed description of the characteristics and possible mechanisms involved in the generation of the IPSP, see Eccles (1964, 1969). According to Eccles, the IPSP is induced primarily by a change in potassium (K^+) and chloride (Cl^-) conductances across the cell membrane through the action of an unknown inhibitory neurotransmitter released from the presynaptic endings of an inhibitory interneuron. It has been shown that strychnine is a highly effective depressant of postsynaptic inhibition in the spinal cord (Bradley et al., 1953; and Eccles et al., 1954a).

The second type of inhibitory mechanism, presynaptic inhibition, has also been most extensively studied in the α motoneuron. Frank and Fuortes (1957) showed that stimulation of a group Ia afferent could reduce the size of a monosynaptically-evoked excitatory postsynaptic potential (EPSP). Stimulation of the same Ia afferent alone produced no change in the resting membrane potential of the motoneuron. The antidromic spike evoked in the motoneuron by stimulation of the ventral root was not altered by

stimulation of the Ia inhibitory afferent. This process was further investigated by Eccles et al.(1961), who suggested that the depression of the EPSP by the inhibitory volley might be the result of a partial depolarization of the terminals of the presynaptic fibers in the spinal cord which mediates the EPSP. This was based on the fact that the time course of depression of the evoked EPSP corresponded to the time course of a negative potential evoked by the inhibitory volley and recorded from the dorsal root of the afferent pathway mediating the EPSP. Using intracellular recording techniques, Eccles et al.(1962) demonstrated presynaptic depolarization of excitatory Group I afferent fibers. The partial depolarization of single afferent Group I fibers produced by an inhibitory volley was shown to correspond closely with the depression of the EPSP in both its time course and its summation with repetitive stimulation. It was postulated that inhibitory interneurons make synaptic connections on either the terminal or axon of the excitatory presynaptic fiber. The excitatory presynaptic fiber would be depolarized by the mediator released as the result of the inhibitory volley.

Eccles (1961) has calculated that a small depolarization of the excitatory presynaptic terminal by an inhibitory volley would be sufficient to reduce the amount of excitatory mediator released upon subsequent activation. Thus, a subnormal EPSP would be evoked during the activation of an inhibitory pathway mediating presynaptic inhibition.

Wall (1964) has proposed an alternative mechanism of presynaptic inhibition. According to Wall, the reduced EPSP may be the result of a complete blockade of impulse propagation into a few individual terminals of the presynaptic neuron so that the total amount of transmitter released is reduced. Depolarization of the individual terminals, due to the activation of the inhibitory pathway, would result in the block of conduction of the impulse into the terminal, and thereby block the release of the transmitter.

2. Types of Inhibitory Circuits

Eccles (1969) has defined two elementary types of neuronal inhibitory circuits, feed-back and feed-forward. In the former type, the inhibitory action is fed back to the previously excited neurons or other neurons of the same kind. Renshaw cell inhibition of the α motoneuron would be an example of a recurrent collateral control system (Renshaw, 1941; Eccles et al.,1954a). Eccles (1964, 1969) summarized this type of feed-back inhibition in the following manner. The Renshaw cell, a spinal inhibitory interneuron which makes synaptic connections with α motoneurons, is activated by a collateral pathway arising from the axon of the same kind of motoneuron, i.e. recurrent collateral inhibition. With intracellular recording techniques, a hyperpolarization or IPSP (indicating postsynaptic inhibition) has been demonstrated in an α motoneuron following activation of the collateral pathway. Strychnine and

dihydro-β-erythroidine (DHβE) have been shown to be effective in blocking this feed-back inhibition (Eccles et al, 1954a). The former depressed the evoked IPSP, while the latter blocked the collateral activation of the Renshaw cell.

Besides the α motoneuron system, this type of feed-back inhibition has been identified at various levels of the central nervous system (see Eccles, 1969). In the thalamus, a feed-back recurrent inhibitory system controls the level of activity in the somato-sensory pathway as it projects to the motor cortex. In the neocortex, a recurrent collateral system controls the discharge of pyramidal cells. Recently, an analogous system has been identified with the autonomic nervous system as a control mechanism of bladder function (DeGroat and Ryall, 1968).

The second type of neuronal inhibitory circuit, feed-forward inhibition, has been defined as inhibitory action exerted in the forward direction relative to the excitatory lines of action. An example of this would be the inhibition of α extensor motoneurons induced by inhibitory interneurons excited by Ia afferent fibers emanating from skeletal muscle (Eccles et al., 1954b, 1956, 1960). Activation of the Ia afferent fiber inhibits α extensor motoneurons and at the same time excites α flexor motoneurons. As stated above, this inhibitory pathway has a postsynaptic site of action and can be blocked by strychnine.

A feed-forward type of inhibition has been identified in the cuneate nucleus controlling transmission in the somato-sensory pathway (see Eccles, 1969). The sympatho-inhibition mediated by the baroreceptor afferents may be classified as feed-forward since there is a corresponding excitation of the cardiovagal pathway.

Another control system which governs recurrent feed-back inhibition has been established by Wilson and Burgess (1962). Using intracellular recording techniques, they reported a small depolarization of the motoneuron following antidromic conditioning stimuli applied to the ventral root. The depolarization did not appear to be the result of an EPSP, since hyperpolarization of the motoneuron induced by anodal current injection changed the depolarization to hyperpolarization. The antidromic conditioning volley also increased the magnitude of a subthreshold EPSP evoked by stimulation of excitatory orthodromic pathways. It was suggested that the small depolarization which enhanced the responsiveness of the motoneuron to excitatory synaptic activation was the result of the removal of a tonic inhibitory impingement. This phenomenon was termed disinhibition. The function of disinhibition is to control the discharge pattern of inhibitory interneurons. Disinhibition has been recognized in the cerebellum, as well as at α motoneurons (Eccles, 1969).

C. Types of Sympathoinhibition

1. Sympathoinhibition of Baroreceptor Origin

a. Role of the Baroreceptors in Regulation of Blood Pressure

Reflex inhibitory regulation of the cardiovascular system was initiated with the experiments of Cyon and Ludwig (1866; see Heymans & Neil, 1958). They observed that stimulation of the central end of the aortic depressor nerve caused a decrease in heart rate and blood pressure. The basic understanding of the baroreceptor reflexes, however, must be credited to Hering (1923; see Heymans & Neil, 1958). Hering's experiments can be summarized as follows: Mechanical stimulation of the carotid sinus area by means of a small clip applied to the bifurcation of the common carotid artery produced a fall in blood pressure and slowing of heart rate. Similar results were obtained by stimulation of the central end of the sinus nerve or by stretching the carotid sinus area. Changing the level of intrasinus pressure had pronounced effects on blood pressure and heart rate. Reducing intrasinus pressure by occluding the common carotid artery caused an increase in blood pressure and heart rate. Increasing pressure within the carotid sinus region led to a fall in blood pressure with a corresponding bradycardia. Hering showed that the bradycardia and hypotensive response could be dissociated by the administration of atropine, which reduced the bradycardia. Sectioning both

sinus nerves abolished the cardiovascular responses to changes in carotid sinus pressure and produced a marked hypertension. Thus, Hering noted that the sinus nerves are tonically active.

Bronk and Stella (1934) have demonstrated a relationship between mean blood pressure and the level of activity in the carotid sinus nerve. They reported that the rate of impulse discharge was closely related to the level of pressure within the carotid sinus. Bronk (1933-1934) also showed that sympathetic activity recorded from the cardiac and cervical sympathetic nerves was increased or decreased following respective decreases or increases in carotid sinus pressures. They also reported that sympathetic nerve activity was enhanced following bilateral section of the carotid sinus nerves.

Thus, the baroreceptor reflex arc serves as a homeostatic mechanism for the maintenance of resting blood pressure. The baroreceptor afferent fibers are tonically active at normal levels of systemic blood pressure. The tonic discharge exerts an inhibitory action on sympathetic vasomotor activity. A rise in systemic blood pressure increases the discharge of the baroreceptor afferent nerves, resulting in an inhibition of sympathetic vasoconstrictor activity and an eventual fall in blood pressure. Conversely, a fall in systemic blood pressure reduces baroreceptor discharge, thus reducing sympathetic inhibition, resulting in an eventual rise in blood pressure. Thus, this reflex

essentially functions as a negative control feed-forward system.

b. Brain Stem Areas Necessary for the Integration of Baroreceptor Reflex

The early studies of Owsjannikow (1871; see Bard, 1960) and Alexander (1946) showed that the neural elements involved in maintaining the "resting" level of blood pressure were located within the caudal brain stem below the inferior colliculi. Thus, it appeared that the forebrain played little or no role in sustaining resting levels or reflex changes in sympathetic vasoconstrictor activity. This view was supported by the investigations of Chai et al.(1963) and Wang and Chai (1962). They showed that in vagotomized cats, the reflex increase in blood pressure and heart rate produced by bilateral carotid occlusion, stimulation of the dorsal medulla or sciatic nerve was not significantly reduced following midcollicular decerebration. It was suggested that the integrity of suprabulbar structures was not necessary to maintain baroreceptor-induced changes in sympathetic activity.

According to Manning (1965), however, the forebrain can exert tonic as well as phasic influences on vascular and cardiac activity which are independent of the medullary vasomotor area. He showed that extensive lesions throughout the dorsolateral pressor region of the medulla did not significantly alter the cardiovascular response evoked by bilateral carotid occlusion or stimulation of the sciatic

nerve. Under these conditions, midcollicular decerebration markedly reduced the hypertensive effects produced by bilateral carotid occlusion or sciatic nerve stimulation. He suggested that the integration of the reflex cardiovascular response occurred at a supramedullary locus manifested through an ascending afferent pathway. However, these results have been questioned (Wang and Chai, 1967) because Manning did not use vagotomized cats.

Other investigators (Peiss, 1960; Hilton and Spyer, 1969, 1971; Doba and Reis, 1973) have demonstrated the importance of the forebrain in mediating the sympathetic component of the baroreceptor reflex. Hilton and Spyer (1969, 1971) have reported that electrical stimulation of a discrete area of the anterior hypothalamus results in a depressor response indistinguishable from that obtained by baroreceptor afferent stimulation. Hilton and Spyer (1971) demonstrated that bilateral lesions, restricted to the same depressor area of the anterior hypothalamus, reduced the response to baroreceptor afferent stimulation. They further showed that lesions in the medullary depressor area which spared the nucleus of the tractus solitarius also reduced the baroreceptor reflex response. The reflex was abolished when the two lesions were combined. Thus, Hilton and Spyer suggested that specific regions of the hypothalamus are normally involved in the baroreceptor reflex.

In unanesthetized rats, Doba and Reis (1973) recently showed that the acute neurogenically mediated hypertensive

response following central de-afferentation of the baroreceptor reflexes could be prevented by midcollicular decerebration. They suggested that the baroreceptors, after terminating the medulla, projected in long loop pathways to higher brain areas to mediate reflex changes in cardiovascular activity. However, these results could also be explained as a removal of a baroreceptor-activated inhibitory system mediated at a medullary or spinal level which modulated the tonic influence exerted by the forebrain on vasoconstrictor sympathetic activity.

Several investigators have suggested that the central integration of the baroreceptor reflex lies within the medulla (Illert and Seller, 1969; Illert and Gabriel, 1972; and Koizumi et al.,1971). Koizumi et al. (1971) presented the most direct evidence on an electrophysiological basis. Recording from sympathetic white rami, they reported that spinal reflex discharges evoked by stimulation of somatic afferents were not affected during carotid sinus distension. However, reflex discharges mediated over supraspinal pathways were inhibited by baroreceptor reflex activation. They concluded that baroreceptor-induced inhibition of sympathetic nervous discharge occurred at a medullary level.

The results of Kirchner et al. (1970) and Gebber et al. (1973) are not in agreement with the conclusion of Koizumi et al. (1971). Recording from the renal postganglionic nerve and cervical preganglionic trunk, Kirchner et al. (1970) demonstrated that spinal reflex discharges evoked by

somatic afferent nerve stimulation were reduced by baroreceptor reflex activation. Sympathetic reflex discharges mediated over supraspinal pathways were completely abolished. This suggests a spinal, as well as supraspinal site of baroreceptor reflex induced inhibition. Gebber et al. (1973) came to a similar conclusion when they reported that postganglionic sympathetic discharges evoked from descending tracts of the midcervical spinal cord were reduced 45% by baroreceptor reflex activation. These studies did not rule out the medulla or forebrain as a site of action for baroreceptor-induced inhibition; they merely add that the inhibition of baroreceptor origin also has a spinal locus. Thus, all three levels of the neuraxis appear to play a role in the integration of the baroreceptor reflexes.

c. Suprabulbar Modulation of Baroreceptor Reflexes

Electrical activation of various suprabulbar structures is known to produce cardiovascular responses (see Bard, 1960; Peiss, 1965). Evidence offered by Chai and Wang (1968), Smith (1965) and Peiss (1965) suggest that two parallel pressor pathways may descend from rostral brain structures to the spinal cord: one pathway being interrupted at a bulbar level, while the other having a direct projection to the spinal cord. Since integration of the baroreceptor reflex lies, in part, within the medulla (Illert and Seller, 1969; Illert and Gabriel, 1972; and Koizumi et al.,1971), it is conceivable that activation

of descending pathways which synapse in the medulla could exert a modulating influence on baroreceptor input.

Moruzzi (1940) first demonstrated a supramedullary influence on the baroreceptor reflex arc. He showed that electrical stimulation of the anterior cerebellum antagonized the hypertensive response produced by bilateral carotid occlusion in vagotomized cats. In 1960, Bard postulated that the hypothalamus influences baroreceptor reflex responsiveness. He recognized that the hypertension associated with muscular exercise does not antagonize the concomitant tachycardia. Reis and Cuenod (1962, 1964, 1965) suggested that suprabulbar regions may, in fact, exert a tonic influence on the integration of baroreceptor reflexes. Reis and Cuenod (1965) demonstrated that, after midcollicular decerebration in vagotomized cats, the hypertensive response produced by bilateral carotid occlusion was reduced and the depressor response produced by carotid sinus stretch augmented. It was the contention of these authors and supported by the study of Doba and Reis (1973, see above) that the baroreceptor reflexes are tonically influenced from structures rostral to the lower brain.

In 1963, Hilton was the first to experimentally test Bard's hypothesis. He reported that the depressor response and bradycardia evoked by raising carotid sinus pressure in the cat were blocked during the pressor response produced by hypothalamic stimulation. He concluded that hypothalamic stimulation inhibited the central actions of

the baroreceptor reflex arc. However, Hilton did not determine whether the effect of hypothalamic stimulation was true neural inhibition or a simple summation of opposing peripheral effects. Further, Hilton did not examine a possible differential effect of hypothalamic stimulation on sympathoinhibition and vagal excitation induced by baroreceptor activation.

It has been suggested by several investigators that the inhibitory effects of hypothalamic stimulation on the baroreceptor reflex are of central origin. Weiss and Crill (1969) provided electrophysiological evidence that true neural inhibition was involved. They demonstrated primary afferent depolarization of the carotid sinus nerve following a conditioning stimulus to a discrete area of the hypothalamus. They concluded that presynaptic inhibition may be, in part, responsible for hypothalamic-induced inhibition of the baroreceptor reflexes. Gebber and Snyder (1970) showed that activation of hypothalamic sites which increased heart rate and blood pressure in intact cats inhibited bradycardia evoked by stimulation of the carotid sinus nerve in the high spinal cat. This suggested that hypothalamic stimulation resulted in a true central neural inhibition of the vagal component of the baroreceptor reflexes. However, in contrast to Hilton's observation, Gebber and Snyder demonstrated that hypothalamic stimulation had little effect on the depressor response produced by carotid sinus pulsation in the vagotomized cat.

Further, pre- or postganglionic sympathetic nerve activity evoked by hypothalamic stimulation was attenuated during the pressor response produced by the i.v. injection of norepinephrine. Lisander (1970) also showed that hypothalamic stimulation blocked the reflex bradycardia, but did not alter the concomitant reflex hypotension.

Klevans and Gebber (1970) demonstrated that excitation of certain rostral brain structures enhanced vagal bradycardia induced by baroreceptor activation. They showed that stimulation of septal, preoptic or anterior hypothalamic areas produced little change in heart rate or blood pressure; however, reflex bradycardia evoked by sinus stretch and i.v. norepinephrine was markedly facilitated. Thus, certain suprabulbar structures appear to exert a facilitatory modulating effect on the baroreceptor reflex arc.

d. Baroreceptor Afferent Termination

The anatomical evidence for the central termination of baroreceptor afferents has been derived from degeneration studies following selective lesions of the rootlets of cranial nerves, IX and X. It has been proposed that the principle site of termination of baroreceptor afferents is in the middle third of the nucleus of the tractus solitarius (NTS) near the obex (Cottle, 1964; Kerr, 1962).

Electrophysiological studies have been undertaken to determine the central termination of afferents from the

baroreceptor nerves. Crill and Reis (1968) located baroreceptor and/or chemoreceptor afferent fibers by stimulation of the medulla and recording antidromic action potentials from the carotid sinus and aortic depressor nerves. Antidromic action potentials were evoked by stimulation of medullary sites located primarily in the medial portion of nucleus tractus solitarius (NTS) adjacent to and slightly rostral to the obex. The evoked short latency responses followed high frequency stimulation (>900 Hz), indicating the potentials were antidromically activated. Repetitive stimulation of these medullary areas resulted in a fall in blood pressure. This suggested that the antidromic potentials mediated the activation of baroreceptor afferent fibers. Antidromic action potentials were also evoked in the carotid sinus and aortic depressor nerves by stimulation of the paramedian reticular nucleus (PRN), indicating a direct projection into the medial medullary reticular formation for these buffer nerves.

Miura and Reis (1968, 1969) reversed the techniques of Crill and Reis (1968) by recording medullary extracellular negative potentials evoked by electrical stimulation of the carotid sinus nerve. Short-latency responses were recorded from units within the intermediate portions of NTS and within the PRN upon activation of the carotid sinus nerve. The unit responses had mean peak latencies of less than 2 msec and failed at much lower frequencies of stimulation than the antidromic action potentials recorded from the carotid sinus nerve (Crill and Reis, 1968). Thus, these

investigators suggested that the short-latency potentials evoked within NTS and PRN were conducted through a monosynaptic pathway from the baroreceptor nerves.

Further evidence that afferent fibers arising from baro- and chemoreceptors project directly into the medial medullary reticular formation was offered by Homma et al. (1970). Using intracellular recording techniques, they observed short-latency (0.7 msec) monosynaptic excitatory postsynaptic potentials within PRN upon activation of the carotid sinus nerve. In contrast, most of the neurons in the area of the paramedian nucleus which were activated by carotid sinus nerve stimulation responded with long-latency potentials, indicating a polysynaptic pathway.

It should be pointed out that other investigators (Spyer and Wolstencroft, 1971; Lipski et al.,1972; McAllen and Spyer, 1972; Biscoe and Sampson, 1970a,b) have failed to confirm a direct projection of the carotid sinus afferents to the PRN. However, Miura and Reis (1972) recently demonstrated the importance of the paramedian reticular area in the integration of the baroreceptor reflex. They showed that bilateral lesions of the paramedian reticular area of the medulla which destroyed the PRN abolished the depressor response to electrical stimulation of myelinated fibers of the carotid sinus nerve. Lesions placed in this area also attenuated the depressor response to carotid sinus stretch and augmented the chemoreceptor response induced by lobeline. In contrast,

bilateral ablation of the medial portion of NTS abolished all reflex cardiovascular responses to electrical stimulation of NTS, carotid sinus stretch, following administration of lobeline. They concluded that the PRN serves as a specific site for the integration of cardiovascular reflexes. In regard to this, other studies have shown that the integrity of this medial reticular nucleus is necessary for sustaining the cardiovascular responses elicited by activation of the forebrain (Lofving, 1961a) or cerebellum (Miura and Reis, 1970). Thus, supramedullary modulation of baroreceptor activity may be integrated within the PRN.

Electrophysiological studies have suggested that secondary projections of baroreceptor afferents are localized in both the medial and lateral regions of the brain stem. Several investigators have reported that the changes in baroreceptor input influenced the rate of firing of spontaneous discharging single units recorded from the medial and lateral reticular formation of the medulla and pons (Biscoe and Sampson, 1970b; Przybyla and Wang, 1967; Salmoiraghi, 1962). Long-latency field potentials and single unit responses evoked by electrical activation of the buffer nerves have been recorded from the medial and lateral areas of the reticular formation of the lower brain stem (Humphrey, 1967; Miura and Reis, 1969; Seller and Illert, 1969; Biscoe and Sampson, 1970a,b). Miura and Reis (1969) suggested that the late response (latency

>5 msec) evoked by carotid sinus nerve stimulation monitored the activation of a polysynaptic pathway. In agreement with Humphrey (1967), they recorded this late response from three regions of the medulla: the region of the PRN, the dorsolateral reticular formation comprising the nucleus medulla oblongata centralis, and the nuclei of the raphé, as well as the central tegmental area of the pons which comprise the nucleus gigantocellularis, nucleus pontis centralis caudalis and nucleus parvocellularis.

Biscoe and Sampson (1970b) recorded from spontaneously active single units in nucleus gigantocellularis and nucleus parvocellularis of the lower brain stem. An increase in carotid sinus pressure or electrical stimulation of carotid sinus, glossopharyngeal, aortic and superior laryngeal nerves would alter the discharge rate of these spontaneously active reticular neurons. Some cells which were inhibited by buffer nerve stimulation were also inhibited during a rise in pressure in the carotid sinus region. On several occasions, cells that responded in this manner were impaled with a microelectrode, and hyperpolarizing postsynaptic potentials were observed following buffer nerve stimulation. The time course of the hyperpolarizing potential corresponded to the inhibition of the spontaneous discharge. This suggested that the site of baroreceptor reflex inhibition lies within the medial region of the reticular formation in or near nucleus gigantocellularis.

In addition, Spyer (1972) has presented electrophysiological evidence that secondary connections of baroreceptor afferents project to neurons in the anterior hypothalamus. He recorded from spontaneously active single units in the area of the hypothalamus that, upon electrical activation, produce a depressor response (Hilton and Spyer, 1969, 1971). Spyer showed that 15 of 21 units increased their rate of discharge following a rise in carotid sinus pressure. He suggested that the anterior hypothalamus represents the rostral extension of the integrative "center" for the baroreceptor reflex. Thomas and Calaresu (1972) have also shown that unit activity recorded from a region in the posterior medial hypothalamus can be modified by electrical activation of systemic baroreceptors. Thus, electrophysiological evidence was presented for the existence of a long ascending loop from the baroreceptor afferents, as suggested by Manning (1965) and Doba and Reis (1973).

2. Sympathoinhibition of Non-Baroreceptor Origin Evoked from the Medial Medulla - A Bulbar Inhibitory Area

a. Sympathoinhibition of Vasomotor Activity

The medial medulla appears to be a generalized bulbar inhibitory region, and not strictly involved with the central neural connections of the baroreceptor afferents. Coote et al. (1969) compared the effects of baroreceptor reflex activation and medial medullary stimulation on reflex discharges of spinal origin, which were recorded

from sympathetic white rami. These investigators reported that distension of the carotid sinus failed to block the spinal sympathetic reflex discharge. However, stimulation of the ventromedial medullary depressor area did block the spinal reflex. It was suggested that stimulation of the ventromedial medulla activated a sympathoinhibitory system which was distinct from inhibition mediated by the baroreceptors. Recording from "vasoconstrictor" efferent sympathetic fibers to the kidney, Kirchner et al. (1971) confirmed the findings of Coote et al. (1969).

b. Sympathoinhibition of Non-Vasomotor Activity

Wang and Brown (1956) demonstrated that electrical activation of the ventromedial bulbar reticular formation inhibited the galvanic skin reflex in the cat. The galvanic skin reflex monitors the activity of sweat glands which are innervated by sympathetic fibers. Yet, the effects of baroreceptor reflex activation were not tested on this reflex. Thus, it was not possible to determine whether this inhibition of the galvanic skin reflex by stimulation of the medial medulla resulted in the activation of an inhibitory system of baroreceptor or of non-baroreceptor origin. In regard to this, it should be pointed out that other physiological functions are influenced during baroreceptor reflex activation. Zanchetti et al. (1960) demonstrated that baroreceptor activation has a modulating influence on spontaneously-occurring outbursts of sham rage. Increasing carotid sinus

pressure reduced the spontaneously-occurring sham rage response. In contrast, bilateral carotid occlusion induced sham rage outburst. Bonvallet et al.(1954) showed that EEG activity was influenced by baroreceptor reflex activation. An increase in carotid sinus pressure resulted in a synchronization of the EEG. It was suggested that baroreceptor reflex activation resulted in an inhibition of the ascending reticular neurons, which led to the synchronized EEG.

c. Inhibition of Somatic Nervous Activity

Magoun and Rhines (1946) demonstrated that electrical stimulation of the medial bulbar reticular formation had an inhibitory effect on somatic motor activity evoked reflexly or by stimulation of the motor cortex. They also reported that stimulation of the medial medulla abolished decerebrate rigidity for the duration of the stimulation. In this case, activation of the medial medulla resulted, in part, in an inhibition of α motoneurons which innervate extensor muscle fibers. Llinas and Terzuolo (1964) studied this mechanism in detail and concluded that the inhibition produced by medial medullary stimulation was the result of a direct and continuous hyperpolarization of the α extensor motoneuron. This was based on the fact that stimulation of the bulbar reticular formation depressed the excitability of the α extensor motoneuron, blocked the monosynaptic reflex to this cell and increased the transmembrane potential of the cell, i.e. hyperpolarization.

Recently, Chan and Barnes (1972) demonstrated another inhibitory mechanism evoked by stimulation of the medial reticular formation of the cat brain stem. Recording from a dorsal root in the lumbar region, they evoked the electrophysiological correlate to presynaptic inhibition (a negative dorsal root potential) during stimulation of the bulbar reticular formation. Since the decrease in the monosynaptic reflex potential recorded from a ventral root and enhancement of the antidromic potentials followed the time course of the dorsal root potential, the authors suggested that the inhibitory effect of medial medullary stimulation was, in part, presynaptically mediated. Thus, stimulation of the medial medulla modulates somatic reflex discharges at a spinal level through postsynaptic and presynaptic inhibitory processes.

3. Silent Period

The phenomenon commonly referred to as the silent period (Sell et al., 1958; Koizumi and Brooks, 1972), or postexcitatory depression (Pitts and Bronk, 1942), can be defined as the period of depression of spontaneously-occurring sympathetic nervous discharge which follows excitation of supraspinal origin. This phenomenon undoubtedly is important in determining the discharge pattern of sympathetic neurons by acting possibly as a low pass filter (see Koizumi and Brooks, 1972) and, therefore, should be included in a study of the influences which govern sympathetic nervous discharge. The silent period has been

studied by measuring the duration of the depression of spontaneously-occurring discharges following sympathetic excitation (Pitts et al., 1941; Pitts and Bronk, 1942; Scherrer, 1963; Koizumi et al., 1968; Polosa, 1967; Wyszogrodski and Polosa, 1973) or by measuring the time course of the depression of the test sympathetic reflex discharge using condition-test techniques (Coote and Perez-Gonzalez, 1970; Iwamura et al., 1969a; Koizumi et al., 1968; Sato et al., 1967; Sato and Schmidt, 1971; Schmidt and Schonfuss, 1970). The latter group of investigators have demonstrated that the duration of the silent period following the reflex discharge corresponds to the time course of depression of the test sympathetic reflex discharge in experiments in which paired shocks are applied to an afferent nerve.

a. Nature of the Silent Period

Pitts et al. (1941) were the first to report that sympathetic nervous excitation evoked by high frequency stimulation of the hypothalamus was followed by a long-lasting (several seconds) depression of spontaneously-occurring discharge. They observed this while recording preganglionic or postganglionic sympathetic activity. High frequency stimulation of the hypothalamus evoked a pressor response. However, the long-lasting inhibition of spontaneous activity was not the result of baroreceptor reflex-induced inhibition since the postexcitatory depression was observed in debuffered animals. Scherrer (1963), using a

similar approach, reported that, as a consequence of baroreceptor denervation, the postexcitatory depression was markedly reduced. However, the duration of the remaining inhibition was more than 1 sec, which he concluded to be the central component.

(1) Recovery Process

Recording from single preganglionic fibers of the cervical sympathetic trunk, Pitts and Bronk (1942) studied the postexcitatory depression following hypothalamic stimulation. They observed that, following a period of repetitive stimulation, nervous activity of the preganglionic neuron was depressed in direct proportion to the frequency, intensity, and duration of stimulation. Since the recovery of excitability of peripheral nerves (Erlanger and Gasser, 1937) and sympathetic ganglia (Bronk, 1939) was influenced by the frequency and intensity of stimulation, Pitts and Bronk suggested that a similar recovery process was responsible for the silent period. They also reported that the silent period of preganglionic sympathetic neurons which followed their antidromic activation was of shorter duration than that produced following orthodromic activation from the hypothalamus with the same number of stimuli. This prompted Pitts and Bronk to conclude that the silent period was due to a recovery process which was initiated primarily at a site central to the preganglionic neuron.

Polosa (1967) employed more sophisticated recording techniques to determine the involvement of the sympathetic

preganglionic neuron in the generation of the silent period. Recording extracellularly from single preganglionic neurons in the cat thoracic spinal cord, Polosa found that repetitive antidromic activation of spontaneously-discharging units was followed by a long-lasting depression, or silent period. He concluded that the silent period of sympathetic preganglionic neurons is generated in the spinal cord and due to a subnormality of the spinal sympathetic neurons following intense activation.

(2) True Neural Inhibition

It is generally accepted, however, that the silent period which follows the reflex discharge of supraspinal origin evoked by afferent nerve stimulation represents, in part, true neural inhibition. The best evidence was presented by Koizumi et al. (1968) in experiments in which double shocks were applied to afferent nerves. Recording from lumbar white rami, they showed that the test response was blocked when evoked during the silent period produced by the conditioning stimulus. However, the duration of the silent period produced by the combination of conditioning and test stimuli was considerably increased when compared to that produced by the conditioning stimulus alone. This illustrated that the inhibition of spontaneously-occurring discharges was not a recovery process since no preceding excitation had occurred. They proposed that independent mechanisms were involved in excitation and inhibition of sympathetic efferent discharge.

Iwamura et al. (1969a,b) used similar techniques to confirm the findings of Koizumi et al. (1968). Recording postganglionic sympathetic discharge from the renal nerve, Iwamura and co-workers studied other characteristics of the depression of spontaneous activity following the supraspinal reflex discharge evoked by stimulation of somatic afferent nerves. They observed that the silent period evoked by afferent nerve stimulation was prolonged during REM sleep, while the preceding sympathetic discharge was reduced. Following single shocks applied to afferent nerves, they reported that the amplitude of the evoked supraspinal reflex discharge was not related to the duration of the following silent period.

In addition, inhibition of spontaneously-occurring preganglionic activity produced by somatic afferent nerve stimulation is not always preceded by a reflex discharge (Iwamura et al., 1966/1967, 1969b; Koizumi et al., 1968; Koizumi and Sato, 1972; Wyszogrodski and Polosa, 1973). Recording from single postganglionic sympathetic fibers which innervate skeletal muscle, Koizumi and Sato (1972) demonstrated that stimulation of Group II afferents evoked a reflex depression of spontaneous activity. The onset latency was 400 msec and lasted for 400-500 msec. In other postganglionic fibers, a reflex discharge believed to be of supraspinal origin was observed prior to the depression of spontaneous activity. When the intensity of stimulation was raised to include Group II and III

afferents, a reflex discharge (onset latency, 340 msec) and subsequent silent period was evoked. This depression was reported to be 100-150 msec longer than that observed when only Group II afferents were activated. This would also suggest that separate mechanisms are involved in the generation of the supraspinal reflex and subsequent silent period and, therefore, the silent period is not the result of a recovery process.

b. Possible Mechanisms Involved in the Sympatho-inhibition Representing the Silent Period

It is believed that the long-lasting depression of spontaneously-occurring sympathetic discharge following the supraspinal discharge is mediated through the medulla (Iwamura et al., 1969a; Koizumi et al., 1968; Sato, 1972a,b; Sato and Schmidt, 1973; Sell et al., 1958; Weidinger et al., 1961). In regard to this, Koizumi et al. (1968) compared the time course of depression of a test sympathetic discharge of supraspinal origin prior to transection of the spinal cord with that of a test discharge of spinal origin after cord section. They reported that the test sympathetic spinal reflex elicited by stimulation of a somatic afferent nerve was little affected by a conditioning shock applied to the same nerve. In contrast, in intact preparations, the supraspinal test reflex discharge was depressed for over 700 msec by a conditioning shock applied to the same nerve. Since the reflex discharge of spinal and supraspinal origin were of similar amplitude, they concluded

that the supraspinal pathway must initiate the silent period.

Further, Iwamura et al. (1969a) reported that lesions placed in different areas of the lower brain stem of the cat could selectively abolish either the sympathetic reflex discharge or its attendant silent period. Recording post-ganglionic sympathetic nervous discharges on the renal nerve, they evoked the supraspinal reflex response and following silent period with single shocks applied to afferent nerves. They reported that lesions in the dorso-lateral medulla selectively abolished the supraspinal reflex discharge without altering the depression of spontaneous activity. Other lesions placed in the ventro-lateral area of the medulla eliminated the silent period with little or no change in the supraspinal discharge. In regard to this, Iriuchijima (1959) had previously reported that transection of the medulla at the calamus scriptorius blocked the following silent period with little change in the preceding splanchnic discharge evoked by afferent nerve stimulation in the toad. Thus, it was concluded that the silent period depends on an inhibitory pathway through the medulla.

Beacham and Perl (1964a,b) had previously studied in detail the sympathetic preganglionic discharge evoked by afferent nerve stimulation in spinal cats. Using condition-test techniques, they reported a relatively short (50-100 msec) period of depression of the test reflex discharge of

spinal origin. In addition, they observed a diphasic recovery cycle for the spinal sympathetic reflex discharge. They suggested that the change in slope in the recovery curve 20 msec after the conditioning stimulus was the result of an afferent inhibitory process which began at that time. Complete recovery of the spinal reflex occurred within 100 msec after the conditioning stimulus. Beacham and Perl (1964b) also reported that the threshold for the brief spinal reflex inhibition was less than that for the spinal reflex discharge. This afferent spinal inhibitory phenomenon has since been described by others (Janig and Schmidt, 1970; Koizumi and Sato, 1972; Sato, 1972a,b; Wyszogrodski and Polosa, 1973).

In single spontaneously active postganglionic sympathetic fibers which innervate skeletal muscle, Koizumi and Sato (1972) have described a "pre-excitatory depression" evoked by afferent nerve stimulation. This brief (20-60 msec) reflex depression of spontaneously-occurring discharge preceded the supraspinal reflex discharge. They assumed that this inhibitory phenomenon occurred through a spinal reflex pathway. Sato (1972a) reported a similar early depression of spontaneous sympathetic activity in single preganglionic fibers from lumbar white rami. The early inhibition occurred alone or following the spinal reflex discharge, yet prior to the reflex discharge of supraspinal origin.

Recording from single preganglionic fibers in the lumbar white rami, Sato (1972b) made an extensive investigation of this early reflex inhibition. He showed that the early spinal reflex discharge evoked by a test shock applied to an afferent nerve was inhibited by a prior conditioning shock applied to the same or a different afferent nerve. The test shock was depressed for 150-300 msec after the conditioning stimulus. He also observed that the degree of depression of the test spinal reflex discharge was reduced after spinal transection. Sato concluded that the brief inhibition of the spinal discharge was derived from three components: 1) postexcitatory depression, 2) afferent inhibitory process in the spinal cord, and 3) descending inhibitory effect from the supraspinal level. The onset latency of this spinal inhibition, mediated in part by a supraspinal pathway, was 20-30 msec, which was faster than the onset latency of the supraspinal reflex discharge (60 msec). In regard to this, it has been shown that electrical stimulation of a descending inhibitory pathway from the medullary depressor area inhibits activity in the spinal sympathetic reflex pathway (Coote et al., 1969; Kirchner et al., 1971).

Gootman and Cohen (1971) compared the effects of electrically activating the medullary pressor and depressor regions with short trains of stimuli, while monitoring preganglionic splanchnic nervous discharges. They found that a descending inhibition evoked from the depressor region had an onset latency 10 msec shorter than that of the

descending excitation evoked from the pressor region. Thus, as suggested by Sato (1972a), the supraspinal component may be mediated through the medullary depressor region. However, it would appear that the early spinal inhibition, which is mediated in part over a supraspinal pathway, is not directly responsible for the silent period which follows the supraspinal reflex discharge. This was evident by the fact that the test reflex discharge of supraspinal origin had a long time course of depression (600-800 msec) when tested after the supraspinal reflex discharge evoked by the conditioning stimulus (Sato, 1972b). This was more than twice the duration for the depression of the test reflex discharge of spinal origin, which had returned to control amplitude within 300 msec following the conditioning stimulus. In a recent review, Sato and Schmidt (1973) concluded that the inhibition of the reflex discharge of supraspinal origin occurred at a medullary synapse.

The exact mechanisms underlying the true neural inhibition which is represented by the sympathetic silent period are unknown. Yet, several hypotheses dealing with this problem have been presented. One possibility, presented by Okada et al. (1960), is that the resting nervous activity of some sympathetic neurons in the medullary pressor region may be inhibited directly by afferent nerve impulses. Alternatively, they have proposed that afferent nerve impulses activate a sympathoinhibitory system in the medulla which, in turn, inhibits sympathetic

nervous discharge. The medial medullary depressor region is believed to be involved in sympathoinhibition of baroreceptor, as well as of non-baroreceptor origin (Alexander, 1946; Biscoe and Sampson, 1970a; Chai and Wang, 1968; Coote et al., 1969; Crill and Reis, 1968; Humphrey, 1967; Johansson, 1962; Kirchner et al., 1971; Lofving, 1961a; Miura and Reis, 1969, 1972; Wang and Chai, 1967). The evidence presented above by Iwamura (1969a), Koizumi et al. (1968), Koizumi and Sato (1972), and Sato (1972a,b) would favor the latter alternative of Okada et al. (1960). The silent period and the supraspinal discharge evoked by afferent nerve stimulation may result from the asynchronous activation of the pressor and depressor regions of the medulla.

Further studies indicated that stimulation of the hypothalamus or medullary pressor region produced a sympathetic discharge which was followed by a silent period (Coote and Downman, 1969; Koizumi et al., 1968; Pitts and Bronk, 1942; Scherrer, 1963). Coote and Downman (1969) have reported that the silent period evoked by central stimulation is similar to that which follows the supraspinal discharge evoked by afferent nerve stimulation. Thus, it would appear that some interaction between the pressor and depressor region results in the sympathetic discharge of supraspinal origin and the subsequent silent period. In regard to this, collateral fibers from axons of reticulospinal neurons have been

identified in the medulla (Brodal, 1957; Scheibel and Scheibel, 1967). Thus, an interaction between the pressor and depressor region of the medulla may occur through the axon collateral branches of the reticulospinal neurons.

No direct evidence has been offered to define the site or sites at which impulse transmission is inhibited in generating the sympathetic silent period. Schmidt and Weller (1970) have proposed that the silent period results from a medullary inhibition of reticulospinal neurons, which convey excitatory impulses to the spinal preganglionic neurons. This was suggested because activation of C fibers in an afferent nerve evoked a reflex discharge which shortened the duration of the silent period. The excitation produced by C fiber stimulation bypassed the medullary relay neurons which were inhibited and thereby used a different reflex pathway to excite the preganglionic neurons at the peak of the postexcitatory depression.

It has also been suggested that inhibitory impulses which originate in the medulla act on sympathetic neurons in the spinal cord to produce the silent period (Koizumi et al., 1968; Okada et al., 1960). However, definitive evidence for the existence of such a mechanism, which mediates the silent period following sympathetic discharge of supraspinal origin, has not been presented. This system would, however, be similar to the supraspinal component of the early spinal inhibition which controls the reflex sympathetic discharge of spinal origin (Sato, 1972b).

In a recent review, Sato and Schmidt (1973) contradicted themselves in discussing the site at which sympathoinhibition responsible for the silent period occurs. On page 935 of their review, they concluded that the inhibition of the reflex discharge of supraspinal origin occurred at a medullary synapse when evoked during the silent period produced by the conditioning stimulus. On the following page, they suggested that the depression of the supraspinal reflex discharge by the conditioning stimulus occurred at a spinal site. Thus, this question has not been resolved by studying the silent period following the supraspinal sympathetic reflex discharge evoked by afferent nerve stimulation.

D. Statement of Problem

In the cat, sympathetic vasoconstrictor outflow from the hypothalamus is distributed over two distinct systems of parallel pathways (Gebber et al., 1973). Characteristic postganglionic potentials evoked from these pressor systems are differentially affected by baroreceptor reflex activation. Postganglionic potentials with onset latencies greater than 50 msec (long-latency pressor pathway) were blocked by baroreceptor reflex activation. In contrast, potentials with onset latencies less than 50 msec (short-latency pressor pathway) were not blocked by baroreceptor reflex activation. Thus, the short-latency pressor system is not under the inhibitory influence of the baroreceptor reflex.

Stimulation of the medial medulla has been shown to elicit sympathoinhibition of baroreceptor, as well as of non-baroreceptor origin (Alexander, 1946; Coote et al., 1969; Crill and Reis, 1968; Johansson, 1962; Kirchner et al., 1971; Lofving, 1961a; Wang and Chai, 1967). It is conceivable that sympathoinhibition evoked from the medial medulla may be involved in controlling transmission in the short latency pressor pathway.

The silent period which follows sympathetic excitation appears to be important in determining the discharge pattern of sympathetic neurons (see Koizumi and Brooks, 1972). However, controversy exists concerning both the mechanism of and the site at which inhibition representing the silent period is exerted.

The purpose of this investigation was to study the inhibitory control of transmission in these two excitatory vasopressor pathways. Specifically, it was decided to study: 1) the sympathoinhibition evoked from the medial medullary depressor region, and 2) the silent period which follows sympathetic excitation. In the first part of this dissertation, the effects of depressor region stimulation were tested on postganglionic potentials evoked from the two pressor systems at different levels of the neuraxis. In this way, it was possible to: 1) identify the selective nature of the inhibitory effect evoked by medial medullary stimulation, and 2) determine the level of the neuraxis that transmission in each pressor pathway is interrupted.

With regards to the silent period, specific emphasis was placed on: 1) determining at what level of the neuraxis the inhibition of sympathetic activity, representing the silent period, is exerted; and 2) identifying the mechanism by which this inhibition is mediated. Using condition-test techniques, excitability-recovery curves were employed to study the true silent period.

METHODS

Cats of either sex, weighing between 2.0 and 3.5 kg, were used in this study. The cats were anesthetized by intraperitoneal administration of Dial-urethane (0.6 ml/kg), which consisted of a mixture of sodium diallylbarbiturate (116 mg/ml), urethane (466 mg/ml), and monoethylurea (466 mg/ml). Rectal temperature was maintained at 37-38°C with the use of a heating pad and a 200-watt incandescent light bulb.

Blood pressure was recorded from a femoral artery with a Statham P-23AC series pressure transducer and displayed on a Grass polygraph. Drugs were administered through a cannula inserted into the femoral vein.

The anesthetized cats were immobilized with gallamine triethiodide (4 mg/kg, i.v.) and placed on artificial respiration (Harvard Respirator, Model 607). The tidal volume on the respirator was adjusted for 30-60 ml/stroke at 14-16 strokes/min. Gallamine was used to prevent skeletal muscle movements, which were often associated with brain stimulation. Supplemental doses (1-2 mg/kg, i.v.) were administered as required during the course of the experiment. The doses of gallamine employed failed to affect

spontaneously-occurring or electrically evoked pre- or post-ganglionic sympathetic nerve responses.

A. Depressor Region Stimulation Experiments

These experiments refer to preparations in which centrally evoked sympathetic nerve responses were recorded from the postganglionic branch of the superior cervical ganglion or the cervical sympathetic preganglionic trunk.

1. Placement of Stimulating Electrodes

a. Brain Stem

The head of the cat was placed in a stereotaxic apparatus (David Kopf, Model 1404). The frame of the stereotaxic was rotated 180° so as to position the animal on its back. The rostral portion of the trachea and esophagus was separated from the surrounding tissue and then retracted into the mouth in order to approach the ventral surface of the brain stem. The muscle and bone covering the ventral surface of the brain stem was removed. The dura and pia mater were opened without damage to the vertebral or basilar arteries. Using the stereotaxic coordinates of Berman (1968), the stimulating electrodes were positioned into the midbrain or medullary region. The anterior-posterior (AP) level was set stereotaxically. The ventral median fissure and ventral surface of the brain stem were used as reference points for lateral and dorsoventral orientation, respectively. The electrodes were lowered from the ventral to the dorsal surface of the brain stem,

and then retracted toward the ventral surface in 0.5-1 mm steps in search of the area in question.

b. Spinal Cord

The spinal cord was also approached from the ventral aspect at the level of the fourth cervical vertebra. The overlying muscle and base of the vertebra were removed, exposing the ventral surface of the spinal cord. The dura and pia mater were opened without damage to the vascular supply. The surface of the spinal cord and the ventral median fissure were used as landmarks for the dorsoventral and lateral orientation, respectively.

2. Stimulation Parameters

Stimuli were applied to the brain stem and spinal cord by means of a square-wave stimulator (Grass, Model S-8 or S-48), the output of which was passed through a stimulus isolation unit to concentric stainless steel electrodes (David Kopf Instruments, Model SNE-100). The center lead of the electrode and the shaft (outer contact) were exposed 0.25 mm. The distance between the two leads was approximately 0.5 mm. Ten millisecond trains of 3 or 4 pulses (3-15v, 0.5 msec, 300-400 Hz) were applied to pressor sites in the brain stem and spinal cord 1-4 times each second. Sites within the medullary depressor region were stimulated continuously for 15-30 sec at 2-10v, 0.5 msec and 50-100 Hz. Stimulation was performed on the side ipsilateral to the recording electrode. To verify the

central site of activation, a lesion (2 mA/10 sec) was made in the brain or spinal cord at the end of each experiment.

3. Electrical Recording

The superior cervical ganglion and the external carotid postganglionic nerve were prepared for recording following removal of the surrounding connective tissue. Care was taken so that small blood vessels supplying the ganglion were not disturbed. A silk ligature, soaked in saline (0.9% NaCl), was tied to the postganglionic nerve near its junction with the external carotid artery. The nerve was then sectioned between the silk tie and the artery and the portion of the nerve attached to the ganglion was placed on bipolar platinum electrodes. One pole of the electrode was placed on the loop of silk string so as to record monophasically. In a few experiments, the cervical preganglionic trunk was used to monitor sympathetic nervous discharge. The cervical preganglionic trunk was separated from the carotid sheath, vagus nerve and the aortic depressor nerve, and the connective tissue was stripped from the nerve. The preganglionic trunk was cut about 4 cm from the superior cervical ganglion and the central end placed on a bipolar platinum electrode. The indifferent pole of the electrode was placed on a loop of string that was tied to the central end of the cut nerve. The nerves were then immersed in mineral oil. The

sympathetic nerve responses were amplified with a capacitance coupled preamplifier (Grass, Model P511). The high and low filter settings were 1000 and 30 Hz, respectively. The output of the preamplifier was directed to a Tektronix oscilloscope (Model 502A) and a Nicolet signal averaging computer (Model 1070). The memory content of the computer was displayed on a second oscilloscope in analog form and photographed with a Grass camera (Model C4M). Pulses from the square-wave stimulator triggered the oscilloscope and the computer. At times, superimposed traces of nerve activity were displayed on a Tektronix storage oscilloscope (Model 564). Spontaneously-occurring pre- and postganglionic discharges were also amplified with a Grass 7P3A capacitance-coupled preamplifier and displayed on a polygraph with half-amplitude responses at 10 and 75 Hz. The experimental preparation and electrical equipment was housed in a radio frequency shielded room. The animal and all electronic devices were grounded to a common pole.

4. Data Processing

The Nicolet (Model 1070) computer was used to compute the sum of the sympathetic potentials evoked 8, 16 or 32 times at a frequency of 0.2-4 Hz. The process of signal averaging improves the signal to noise ratio, thus allowing the nerve response evoked by the stimulus to be more accurately analyzed. The evoked sympathetic nerve response is time-locked to the stimulus artifact which

triggers the computer. The time-locked response is summed while the randomly-occurring background activity (noise and spontaneously-occurring discharges) is reduced in proportion to the square root of the number of trials measured.

B. Silent Period Experiments

These experiments generally refer to preparations in which centrally-evoked sympathetic nerve responses were recorded from the splanchnic preganglionic trunk. In a few instances, however, nerve activity was monitored from external carotid nerve or cervical preganglionic trunk.

1. Placement of Central Stimulating Electrodes

a. Brain Stem

The cat was placed in the normal stereotaxic position. The midline incision was made through the scalp and the underlying muscles were retracted in order to expose the cranium. A portion of bone and underlying dura was removed to expose the dorsal surface of the brain. The stereotaxic coordinates of Berman (1968) were used to position the concentric stimulating electrodes from the dorsal surface. The dorsoventral reference point (Ho) was considered to be 10 mm above the intra-aural line.

b. Spinal Cord

When the cervical region of the spinal cord was stimulated, the midline incision was extended from the head to the first thoracic vertebra. The skeletal muscles

covering the surface of the first and fourth cervical vertebrae were removed. The dorsal surface of the first and fourth vertebra and the underlying dura were removed to expose the spinal cord. At the level of the fourth cervical vertebra, a concentric stimulating electrode was positioned so as to approach the dorsal surface of the spinal cord at right angles. The dorsal medial sulcus and dorsal surface of the cord were used as the reference points for the lateral and dorsoventral orientation. In some experiments, the spinal cord was transected at the level of the first cervical vertebra. The exposed areas of the brain and spinal cord were covered with mineral oil. The concentric stimulating electrodes were positioned at selected pressor sites in the medulla and spinal cord. Ten millisecond trains of 3 pulses (10v, 0.5 msec) were applied once every 4 sec to the pressor sites in the brain and spinal cord on the side ipsilateral to the recording electrode. Two Grass stimulators (Models S48 and S8) were linked in series to perform condition-test techniques. The delay between the two stimuli was set by the S2 delay setting on the S8 stimulator.

2. Sympathetic Nerve Recording

The left greater splanchnic nerve was exposed in the area of the costovertebral triangle and dissected free from the surrounding connective tissue. A silk ligature, soaked in saline (0.9% NaCl), was tied to the preganglionic

nerve near its junction with the celiac ganglion. The nerve was then sectioned between the tie and the ganglion and its central end placed on bipolar platinum electrodes. The indifferent pole of the electrode was placed on the loop of silk string so as to record monophasically. A pneumothoracotomy was performed to minimize respiratory-induced thoracic movement artifacts on the nerve recordings. The nerve was immersed in mineral oil. Splanchnic nerve activity was amplified with a capacitance-coupled preamplifier (Grass, Model P511) and processed in two ways: 1) superimposed traces of nerve activity were displayed on a storage oscilloscope (Tektronix, Model 564) with low and high half-amplitude responses at 30 and 1000 Hz, respectively; and 2) activity was summed by an average response computer with low and high half-amplitude responses at 10 and 1000 Hz, respectively. The memory content of the computer was displayed on an oscilloscope and photographed. Excitability recovery curves of test splanchnic nerve responses were constructed from the records of summed activity.

C. Histology

At the end of most experiments, the brain stem and/or cervical region of the spinal cord was removed. The portion of the tissue that had housed the electrode was fixed in Formalin for at least a week. The brain stem or spinal cord was cut on a frontal plane at a 30 μ

thickness on a freezing microtome (Lipshaw Cryotome, Model 1500). The brain sections were then stained with cresyl violet for cell bodies, using a modified method of Powers and Clark (1955). The electrode tracks and lesions were identified with a binocular microscope and plotted on a frontal map of the brain stem. Shrinkage (15%) was taken into account before the sites of stimulation were plotted.

D. Drugs

The following drugs were used: dihydro-β-erythroidine hydrochloride, gallamine triethiodide, hexamethonium chloride, mecamylamine hydrochloride, norepinephrine bitartrate and strychnine sulfate. All doses are expressed in terms of the salts.

E. Data Analysis

Statistical analysis was performed using Student's *t*-test for unpaired data, described by Snedecor (1956). A P-value <0.05 was considered to indicate statistical significance. Values presented for each series of experiments are means ± SE.

RESULTS

A. The Effects of Medial Medullary Depressor Region Stimulation on Postganglionic Sympathetic Nerve Responses Evoked from the Medulla

1. High Frequency Stimulation Applied to Depressor Region

The typical effects produced by high frequency stimulation (50-100 Hz) of sites within the medial medullary depressor region on sympathetic nerve discharges recorded from the postganglionic external carotid nerve are illustrated in Figure 1. This branch of the superior cervical ganglion is innervated exclusively by the S_2 component of the preganglionic cervical sympathetic nerve (Gebber et al., 1973). The S_2 preganglionic fiber group is known to innervate ganglion cells which subserve a vasoconstrictor function (Bishop and Heinbecker, 1932). Stimulation of sites distributed diffusely throughout the dorsomedial and ventromedial medullary reticular formation decreased blood pressure and spontaneously-occurring postganglionic discharges (Fig. 1A). This has been previously reported by Alexander (1946), Coote and Downman (1969), Coote et al. (1969), Kahn and Mills (1967). Maximal changes in blood pressure and sympathetic nerve activity were noted when the intensity of stimulation was 8-10v.

Figure 1. Effects of high frequency stimulation of the depressor region on postganglionic discharges recorded from external carotid sympathetic nerve

A: top trace is blood pressure (mmHg). Middle trace is time base (1 sec/division). Bottom trace is spontaneously-occurring nerve activity. A site in the medial medulla was stimulated (10v, 0.5 msec, 100 Hz) during downward deflection of time base. B: summed long-latency postganglionic response (8 trials) evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied to medullary pressor site once each sec. B1 - control response. B2 - response during depressor region stimulation. B3 - response after depressor region stimulation. C: summed short-latency postganglionic response (32 trials) evoked by 10 msec trains of 3 pulses (7v, 0.5 msec, 300 Hz) applied to medullary pressor site 2 times each sec. C1-C3 - same sequence as described for B1-B3. Horizontal calibrations are 100 msec in B and 50 msec in C. Vertical calibrations are 20 μV in A and 106 μV in B and C.

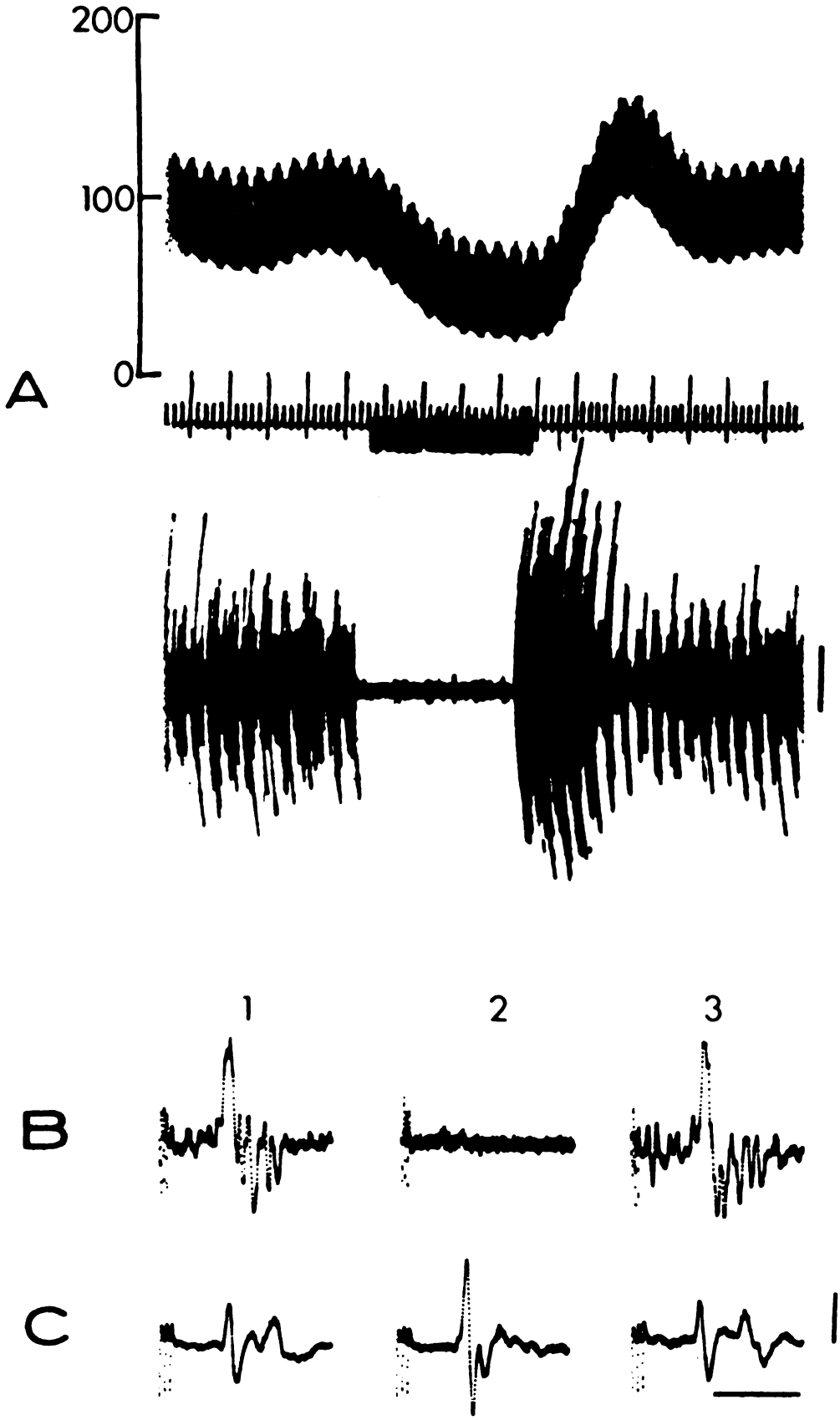


Figure 1

The degree of inhibition of spontaneously-occurring discharges appeared to be directly related to the magnitude of the depressor response (10-80 mmHg) elicited from any particular site of stimulation.

During high frequency stimulation of the depressor region, 8-32 postganglionic responses, evoked by stimulation of the medullary pressor region, were summed. These responses were elicited by 10 msec trains of 3 or 4 pulses (3-15v, 0.5 msec, 300-400 Hz) applied to medullary pressor sites 1-4 times each second. The peak amplitude of the summed potentials was compared with that of control responses, which was obtained in the absence of medullary depressor region stimulation. Although a train of 3 pulses, applied to pressor sites, failed to change blood pressure, stimulation at a frequency of 50 Hz for 15 sec raised mean arterial pressure in excess of 40 mmHg. Postganglionic sympathetic nerve responses, with onset latencies of 50-100 msec, were inhibited during stimulation of the medullary depressor region (Fig. 1B). In confirmation of an earlier report (Gebber et al., 1973), these long-latency potentials were evoked from pressor sites distributed diffusely throughout the periventricular gray, dorsolateral reticular formation and lateral portions of nucleus reticularis ventralis (R.v.) of the caudal medulla. Postganglionic potentials, with onset latencies less than 50 msec, were enhanced in amplitude when elicited with pulses of submaximal intensity (<8v) during depressor

region stimulation (Fig. 1C). The short-latency responses were unchanged by depressor region stimulation when they were elicited with pulses of supramaximal intensity (>8v). These potentials were evoked almost exclusively from pressor sites in R.v., as has been previously reported (Gebber et al.,1973).

2. Short Trains of Stimuli Applied to Depressor Region

Short trains of stimuli applied to the same sites in the depressor region also were effective in producing reciprocal effects on the short-and long-latency potentials evoked from the pressor region of the medulla (Fig. 2). A 10 msec train of 3 pulses (10v, 0.5 msec, 300 Hz), applied to a site in the depressor region, produced a depression of spontaneously-occurring postganglionic discharges without a preceding excitation (Fig. 2A). During the interval of peak depression of spontaneously-occurring sympathetic activity, the long-latency potential evoked from a medullary pressor site was inhibited (Fig. 2B). In contrast, the amplitude of the short-latency potential, evoked from a medullary pressor site at submaximal intensity (<8v), was enhanced (Fig. 2C).

A summary of the effects of depressor region stimulation on the short-and long-latency postganglionic responses evoked from the medulla is presented in Table 1A. The distribution of 99 depressor sites in 26 cats, from which these effects were elicited, is shown in Figure 3. The

Figure 2. Effects of short trains of stimuli applied to the depressor region on postganglionic discharges recorded from external carotid sympathetic nerve

A: 16 superimposed traces showing inhibition of spontaneously-occurring discharges produced by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied once every sec to a medial medullary site. B: summed long-latency post-ganglionic response (16 trials) evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied to medullary pressor site once every sec. B1 - control response. B2 - response during peak interval (200 msec) of depression of spontaneous nerve activity produced by depressor region stimulation. C: summed short-latency postganglionic response (16 trials) evoked by 10 msec trains of 3 pulses (7v, 0.5 msec, 300 Hz) applied to medullary site once each sec. C1-C3 - same sequence as described for B1-B3. Horizontal calibrations are 100 msec each division in A and 50 msec in B and C. Vertical calibrations are 20 μV in A and 106 μV in B and C.

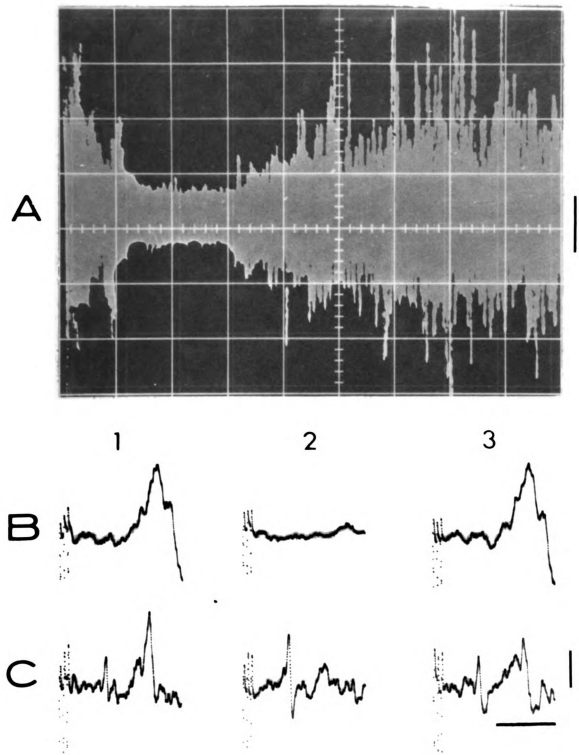


Figure 2

Table 1 - Reciprocal effects of depressor region stimulation on postganglionic sympathetic nerve responses evoked from medulla and spinal cord

A. Medulla

Potential Type, % Change from Control Amplitude

Short-latency (<50 msec)	Long-latency (>50 msec)
+115 ± 10 (79)	-77 ± 4 (32)

Values for % change are mean ± SE with N given in parenthesis.

B. Spinal Cord

Potential Type, % Change from Control Amplitude

Baroreceptor - nonblocked	Baroreceptor - blocked
+72 ± 13 (34)	-79 ± 9 (10)

Values for % change are mean ± SE with N given in parenthesis.

Baroreceptor-nonblocked - potentials with onset latencies of 28-42 msec which are not inhibited during pressor action of norepinephrine.

Baroreceptor-blocked - potentials with onset latencies of 34-39 msec which were inhibited during pressor action of norepinephrine.

Figure 3. Distribution of 99 medullary sites in 26 cats from which the effects of stimulation depicted in Figs. 1 and 2 were produced.

A and B: represent a frontal section about 2 mm rostral to the obex. Abbreviations are those of Brodal (1957). d.: dorsal group of paramedian reticular nucleus. N.c.e.: external cuneate nucleus. N.f.c.: nucleus cuneatus. N.r.l.: lateral reticular nucleus. N.tr.sp.V.: spinal nucleus of trigeminal nerve. Ol.i.: inferior olive. R.pc.: nucleus reticularis parvocellularis. R.v.: nucleus reticularis ventralis. T.s.: tractus solitarius. Tr.sp.V.: spinal tract of trigeminal nerve. v.: ventral group of paramedian nucleus. XII: motor nucleus of hypoglossal nerve.

A-P levels of medial medullary stimulation were sufficiently close so that the data could be plotted on a frontal section of the medulla at a level approximately 2 mm rostral to the obex. The majority of histologically-verified sites were located in the regions of the parahypoglossal area, paramedian reticular nucleus, inferior olive and medial portions of R.v. This map is similar to those of Alexander (1946), Chai and Wang (1962) and Wang and Ranson (1939) which depicted the depressor region of the lower brain stem.

3. Intensity-Response Relationships for the Reciprocal Effects of Depressor Region Stimulation

The purpose of these experiments was to determine if the inhibition of the long-latency potential and spontaneously-occurring discharges could be dissociated from the enhancement of the short-latency potential during depressor region stimulation. Figure 4 is typical of the results observed in five experiments in which intensity-response relationships were established for the effects of depressor region stimulation on spontaneously-occurring and centrally-evoked postganglionic sympathetic nervous discharges. Parallel reductions of the peak amplitude of spontaneously-occurring discharges and the long-latency responses evoked from the medulla were observed as the intensity of depressor region stimulation was raised from 2-10v (Fig. 4A-B). These effects were inversely related

Figure 4. Relationships between intensity of high frequency (50 Hz) depressor region stimulation and inhibition of spontaneously-occurring postganglionic discharges (A), inhibition of long-latency potential (B), and enhancement of short-latency potential (C) in a cat

The short-and long-latency postganglionic responses were evoked with trains of 3 pulses applied to medullary pressor sites. Values are expressed as a percent of control amplitude.

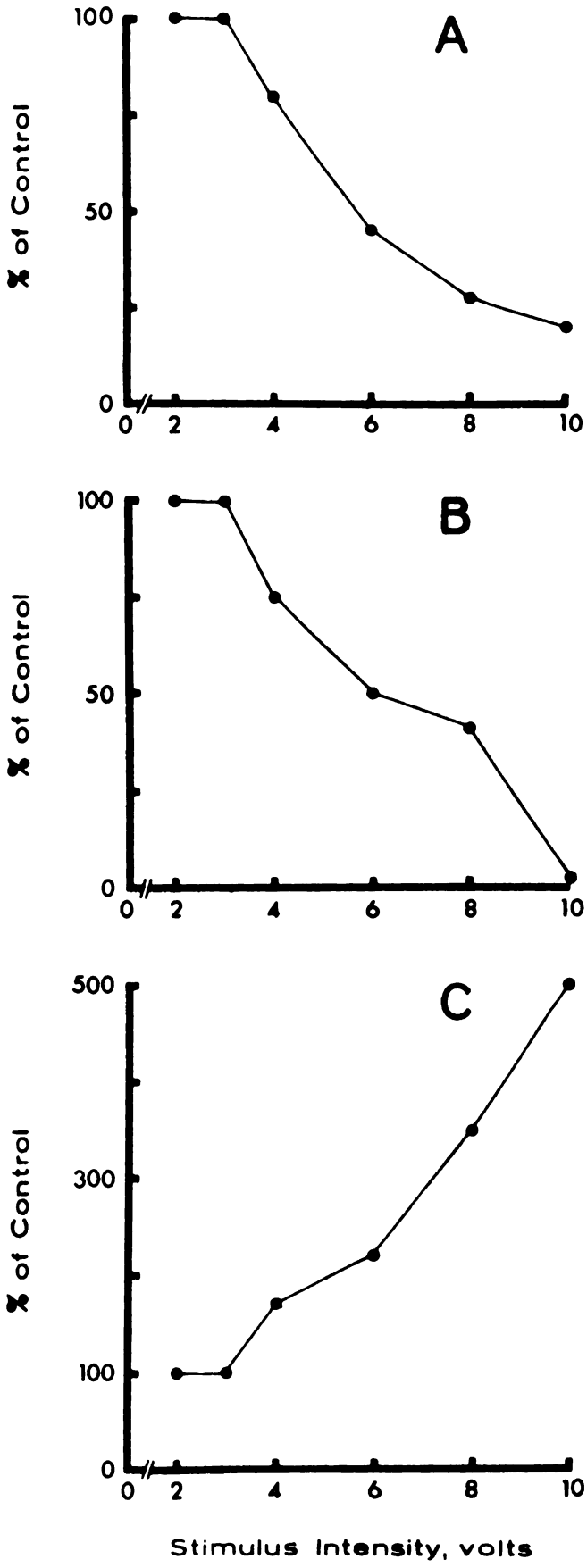

A
B
C
% of Control
100
50
0
500
300
0
2
4
6
8
10
Stimulus Intensity, volts

Figure 4

to the degree of enhancement of the short-latency postganglionic responses evoked from medullary pressor sites in R.v. (Fig. 4C). The intensity of stimulation (3-5v) required to produce threshold effects was the same for each of the three parameters measured in any particular experiment. Thus, the enhancement of the short-latency potential could not be dissociated from the inhibitory effects produced by depressor region stimulation.

B. The Effects of Baroreceptor Reflex Activation on Postganglionic Sympathetic Nerve Responses Evoked from the Medulla

The medial medullary depressor region is believed to mediate sympathoinhibition of baroreceptor, as well as of non-baroreceptor origin (Alexander, 1946; Coote et al., 1969; Johansson, 1962; Kirchner et al., 1971; Lofving, 1961a; Wang and Chai, 1967). To determine the nature of the response to depressor region stimulation, the effects of baroreceptor reflex activation were tested upon the short- and long-latency postganglionic responses evoked from the medulla.

Baroreceptor reflex activation mimicked the effects of depressor region stimulation, which were illustrated in Figure 1. The results shown in Figure 5 are typical of those obtained in 26 cats. A rise in blood pressure to a level in excess of 200 mmHg was produced by the intravenous injection of 1-2 μg/kg of norepinephrine bitartrate

Figure 5. Effects of hypertensive action of norepinephrine on postganglionic discharges recorded from external carotid sympathetic nerve

A: sequence of tracings is same as in Fig. 1A. Norepinephrine (1 μg/kg, i.v.) was injected at downward deflection of time base. B: summed long-latency postganglionic response (8 trials) evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied to medullary pressor site once each sec. B1 - control response. B2 - response during inhibition of spontaneously-occurring postganglionic discharges accompanying pressor action of norepinephrine. B3 - response following dissipation of pressor action of norepinephrine. C: summed short-latency postganglionic response (16 trials) evoked by 10 msec trains of 3 pulses (6v, 0.5 msec, 300 Hz) applied to medullary pressor site 4 times each sec. C1-C3 - same sequence as described for B1-B3. Horizontal calibration is 100 msec. Vertical calibrations are 20 μV in A and 53 μV in B and C.

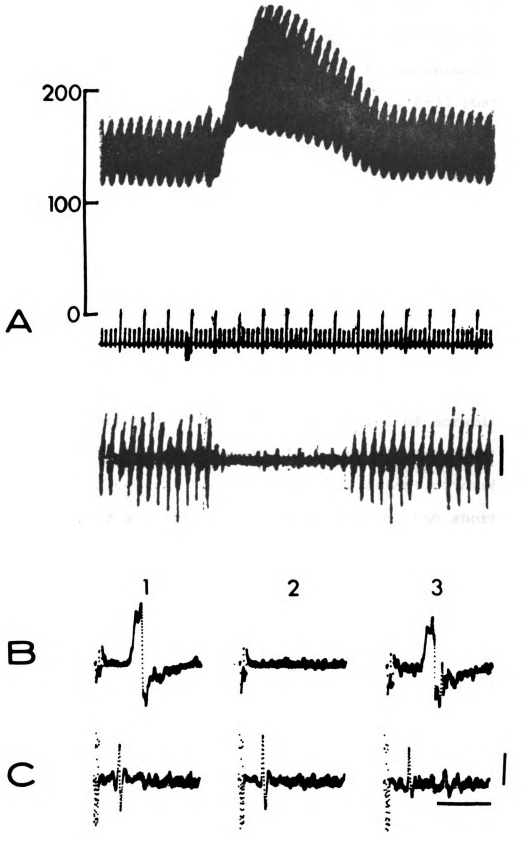


Figure 5

(norepinephrine). The period of maximum inhibition of spontaneously-occurring postganglionic discharges associated with the pressor response induced by norepinephrine was 15-40 sec (Fig. 5A). During this time, 8-32 postganglionic responses, which were elicited by 10 msec trains of 3 pulses applied to the medullary pressor sites once every second, were summed. The peak amplitude of the summed potentials was compared with that of control responses, which was obtained in the absence of exogenously-administered norepinephrine. The pressor action of norepinephrine abolished the long-latency responses evoked from the medulla (Fig. 5B). Gebber et al., (1973) have previously reported that the sympathoinhibitory effects observed during the pressor action of norepinephrine (Fig. 5A-B) are prevented by bilateral section of the baroreceptor nerves. The hypertensive effect of norepinephrine also was accompanied by enhancement of the short latency responses elicited with pulses of submaximal intensity (Fig. 5C). The amplitude of the short-latency postganglionic potentials, evoked with pulses of supramaximal intensity (>8v), was unchanged during the rise in blood pressure produced by norepinephrine. It is not surprising that baroreceptor reflex-like effects were observed upon depressor region stimulation, since it has been demonstrated that baroreceptor afferents make primary and secondary connections with neurons of the medial medulla

(Biscoe and Sampson, 1970a; Crill and Reis, 1968; Homma et al.,1970; Humphrey, 1967; Miura and Reis, 1969, 1972).

C. The Effects of Depressor Region Stimulation on Preganglionic Sympathetic Nerve Responses Evoked from the Medulla

The effects of depressor region stimulation were tested on the responses evoked in the cervical sympathetic preganglionic trunk by electrical activation of the medullary pressor region in five cats. The purpose of these experiments was to determine whether the reciprocal actions of depressor region stimulation evoked on the external carotid nerve were of ganglionic or central origin.

Because of the functionally heterogeneous nature of the cervical sympathetic preganglionic nerve (Bishop and Heinbecker, 1932), the short- and long-latency responses were first identified on the postganglionic "vasoconstrictor" external carotid nerve. The cervical preganglionic trunk was then prepared for recording, and the effect of depressor region stimulation was tested on the centrally-evoked preganglionic potentials. Typical records illustrated in Figure 6 show the same pattern of reciprocal effects of depressor region stimulation as that observed while monitoring postganglionic sympathetic discharges. The short- and long-latency preganglionic potentials were evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied to the same medullary pressor site from

Figure 6. Effects of depressor region stimulation on potentials evoked in preganglionic cervical sympathetic nerve by trains of pulses applied to medullary pressor sites

Records in A and B are from two different cats. A: summed long-latency response (16 trials) evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied once each sec. A1 - control response. A2 - response during depressor region stimulation (10v, 0.5 msec, 100 Hz). A3 - response after depressor region stimulation. B: summed short-latency response (32 trials) evoked by 10 msec trains of 3 pulses (7v, 0.5 msec, 300 Hz) applied 4 times each sec. B1 - control response. B2 - response during depressor region stimulation (10v, 0.5 msec, 50 Hz). B3 - response after depressor region stimulation. Horizontal calibration is 50 msec. Vertical calibration is 106 μV.

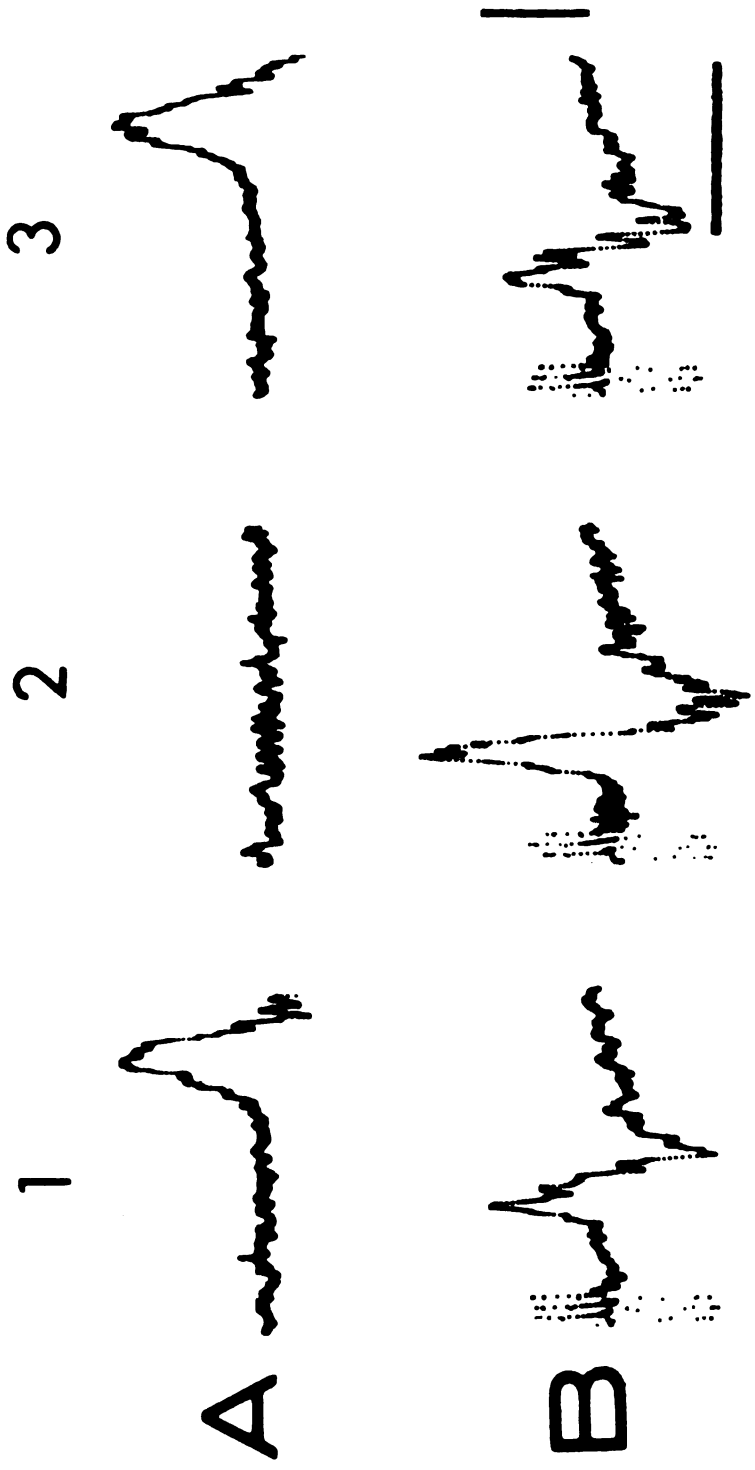


Figure 6

which the postganglionic responses had previously been elicited. The latencies of the potentials recorded from the preganglionic nerve were 15-20 msec shorter than those of the postganglionic responses. The results of these experiments indicate that the reciprocal actions of depressor region stimulation on the short- and long-latency sympathetic nerve responses occurred within the central nervous system.

D. The Effects of Depressor Region Stimulation on Postganglionic Sympathetic Nerve Responses Evoked from the Spinal Cord

To determine if the reciprocal effects of depressor region stimulation are mediated at a spinal or at a supraspinal locus, the effects of depressor region stimulation were tested on postganglionic potentials evoked from descending spinal components of each of the vasopressor systems. Both vasopressor systems have been previously located within the spinal cord (Gebber et al., 1973).

The spinal cord was stimulated at the level of the fourth cervical vertebra in 10 cats. Ten msec trains of 3 pulses (3-10v, 0.5 msec, 300 Hz) were applied to pressor sites in the dorsolateral white column of the midcervical spinal cord. Although the short train of stimuli applied to pressor sites in the spinal cord failed to change blood pressure, stimulation at a frequency of 50 Hz for 15 sec raised mean arterial pressure in excess of 40 mmHg. In

confirmation of the report by Gebber et al. (1973), postganglionic sympathetic nerve potentials evoked from the midcervical spinal cord could be classified into two groups. The first group contained potentials with onset latencies of 34-37 msec, which were inhibited during the pressor action of norepinephrine (1-2 μg/kg i.v.). Potentials of the second group had onset latencies of 28-42 msec and were not inhibited during pressor effects of norepinephrine.

The effects of depressor region stimulation on the two groups of postganglionic potentials evoked from spinal pressor sites are illustrated in Figure 7 and summarized in Table 1B. Those potentials which were inhibited by baroreceptor reflex activation also were blocked during depressor region stimulation (Fig. 7A). Those potentials evoked with supramaximal intensities of stimulation (>8v) which were not inhibited by baroreceptor reflex activation also were not inhibited by depressor region stimulation. However, when evoked with submaximal intensity of stimulation (<8v), these responses were always enhanced during depressor region stimulation (Fig. 7B) and the pressor action of norepinephrine. Stimulation of 34 depressor sites tested either enhanced or did not change the postganglionic responses in this group. Transection of the spinal cord at the level of the first cervical vertebra failed to affect significantly either type of sympathetic nerve response evoked from the midcervical region of the

Figure 7. Effects of depressor region stimulation and C-1 transection on sympathetic nerve responses evoked from dorsolateral white column of midcervical spinal cord

Records in A and B are from two different cats. Ten msec trains of 3 pulses (10v, 0.5 msec, 300 Hz in A; and 8v, 0.5 msec, 300 Hz in B) were applied to the spinal cord once each sec in A and 4 times each sec in B. Each record in A is sum of 16 trials. Records in B are sum of 32 trials. A1, B1: control responses. A2, B2: responses during stimulation (10v, 0.5 msec, 100 Hz) of a site in medullary depressor region. A3, B3: responses after depressor region stimulation. A4, B4: responses 20 min after transection of spinal cord at level of 1st cervical vertebra. Horizontal calibration is 50 msec. Vertical calibration is 106 μV.

spinal cord (Fig. 7, Row 4). Thus, both groups of potentials were evoked from descending spinal tracts. Therefore, the reciprocal effects of depressor region stimulation were mediated at a spinal locus.

E. Drug Effects on the Enhancement of the Short-Latency Sympathetic Nerve Response Evoked from the Medulla

The reciprocal effects of depressor region stimulation on the short- and long-latency potentials evoked from the medulla could not be dissociated and had the same threshold of activation (see Fig. 4). This observation suggests that activation of one system in the medial medulla produced the reciprocal effects. It is conceivable, therefore, that spinal connections between the two vasopressor pathways exist, so that the level of nervous activity in the long-latency pathway controls impulse transmission in the short-latency pressor pathway. That is, enhancement of the short-latency potential may have resulted as the consequence of the removal of an existing inhibition exerted by the long-latency pressor pathway on the short-latency pressor pathway.

Such an interaction between the two vasopressor pathways might be similar to the regulation of the discharge pattern of α motoneurons by the Renshaw system (recurrent collateral inhibition; Eccles et al., 1954a; Renshaw, 1941). If so, collateral fibers may arise from the preganglionic neurons to excite interneurons which, in turn, would

inhibit transmission in the short-latency pathway. Thus, the effect of depressor region stimulation on the short-latency response was tested in the presence of drugs which are known to block Renshaw mediated postsynaptic inhibition.

Enhancement of the short-latency response to that level, produced by depressor region stimulation in the control situation, would be expected, following the administration of drugs which release the inhibition on the short-latency pathway. Blockade of the inhibitory interneuron would also eliminate the enhancement of the short-latency potential during depressor region stimulation.

1. Effects of Strychnine on the Enhancement of the Short-Latency Potential Evoked from the Medulla

Strychnine (0.1-1.0 mg/kg i.v.) did not alter the enhancement of the short-latency postganglionic response evoked from a medullary pressor site at submaximal intensity of stimulation (<8v) during depressor region stimulation (Fig. 8-II). Furthermore, the amplitude of the control submaximal short-latency response evoked by 10 msec trains of 3 pulses was not changed following the administration of strychnine. The results of three experiments are summarized in Table 2. These doses of strychnine are known to block Renshaw inhibition of impulse generation by α motoneurons (Eccles et al., 1954a).

From these negative results, one may suspect that a Renshaw-type mechanism is not responsible for the spinal

Figure 8. Lack of drug effects on the enhancement of the short-latency response during depressor region stimulation

I: Negative effect of dihydro-β-erythroidine (DHβE) and mecamylamine (MECA) on the enhancement of the short-latency response during depressor region stimulation (10v, 0.5 msec, 50 Hz). CONT: predrug effect. DHβE: response following administration of DHβE (2 mg/kg i.v.). MECA: response following administration of MECA (10 mg/kg i.v.). CONT 1, DHβE 1 - summed control preganglionic responses (32 trials) evoked by 10 msec trains of 3 pulses (4v, 0.5 msec, 300 Hz) applied 2 times each sec to same medullary pressor site. CONT 2, DHβE 2 responses during depressor region stimulation. CONT 3, DHβE 3 responses after depressor region stimulation. MECA 1-MECA 3 same sequence as described for CONT 1-CONT 3 except response evoked at stimulus intensity of 6v.

II: Negative effect of strychnine on the enhancement of short-latency response during depressor region stimulation. CONT - predrug effect. STRY - effect following administration of strychnine (1 mg/kg i.v.). CONT 1, STRY 1 summed control postganglionic responses (32 trials) evoked by 10 msec trains of 3 pulses (7v, 0.5 msec, 300 Hz) applied 4 times each sec to same medullary pressor site. CONT 2, STRY 2 responses during depressor region stimulation. CONT 3, STRY 3 responses after depressor region stimulation.

Records in I and II are from two different cats. Horizontal calibration is 50 msec. Vertical calibrations are 53 μV in I and 106 μV in II.

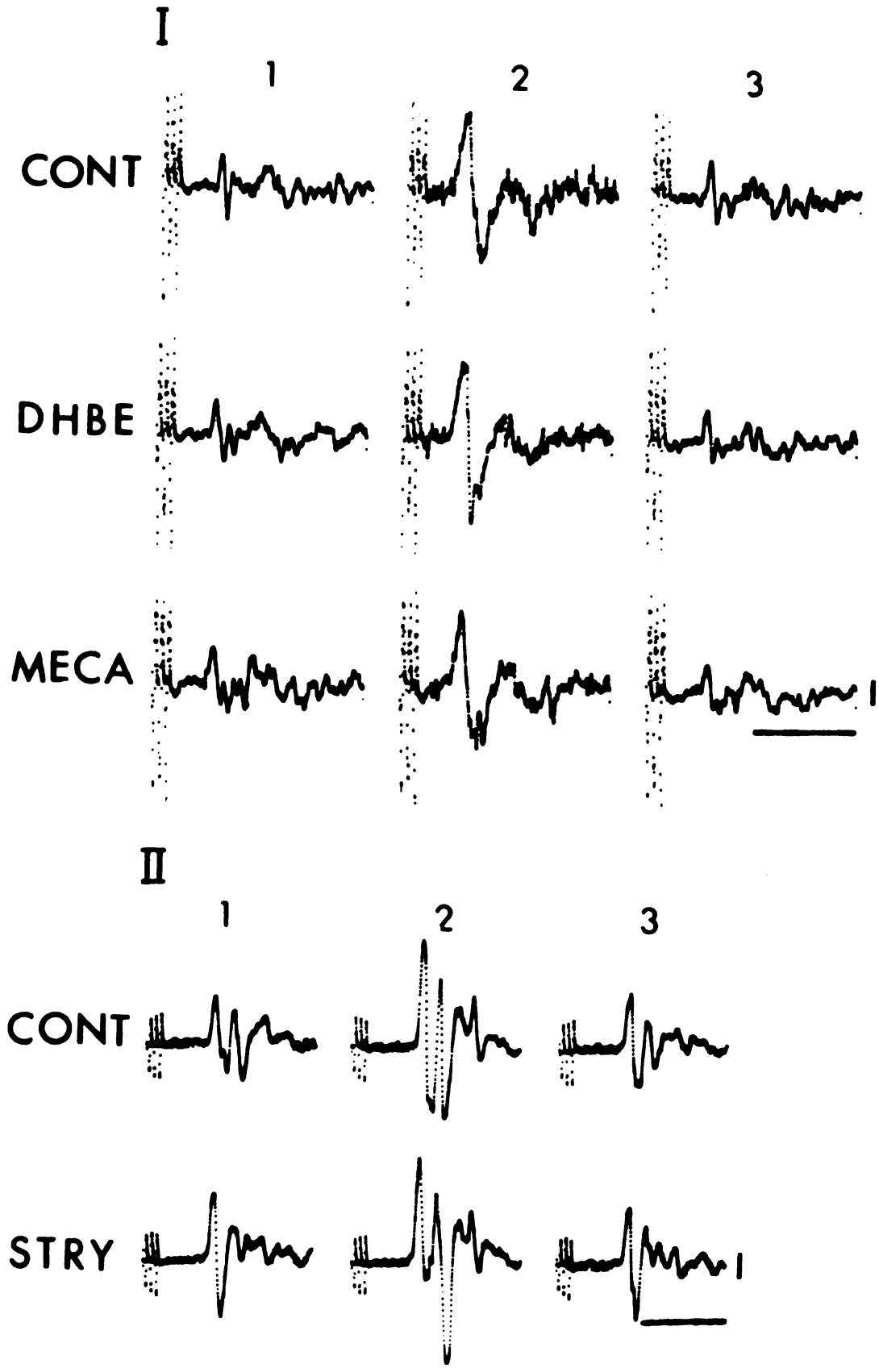


Figure 8

Table 2 - Lack of effect of dihydro-β-erythroidine (DHβE), mecamylamine (MECA) and strychnine (STRY) on the enhancement of the short-latency response evoked from the medulla during depressor region stimulation

	% Change from Control Amplitude
Treatment	Enhancement of Short-Latency Responses
Control	184 ± 17 (4)
DHβE	186 ± 15
Control	178 ± 23 (3)
MECA	183 ± 17
Control	193 ± 16 (3)
STRY	182 ± 10

Values for % change are means ± SE with N given in parenthesis.

interaction between the two pressor systems. However, it is still possible that a Renshaw-type system might be involved in the facilitation of the short-latency postganglionic response if the inhibitory transmitter was different. Yet, the preganglionic sympathetic neuron is cholinergic in nature. Therefore, acetylcholine would serve as the transmitter from the axon collaterals arising from the preganglionic nerve to the inhibitory interneuron. Dihydro-β-erythroidine (DHβE) is known to block activation of the Renshaw cells by cholinergic recurrent collaterals of α motoneurons (Eccles et al., 1954a). Mecamylamine (MECA), which has a central action (Freis, 1959), is more effective in blocking cholinergic transmission at the sympathetic ganglion than at the neuromuscular junction (Stone, 1956). Therefore, both DHβE and MECA were tested on the enhancement of the short-latency response evoked from the medulla.

2. Effects of Dihydro-β-erythroidine and Mecamylamine on the Enhancement of the Short-Latency Potential Evoked from the Medulla

Figure 8-I illustrates that DHβE (0.1-2.0 mg/kg i.v.) and MECA (0.5-10.0 mg/kg i.v.) failed to affect the ability of depressor region stimulation (10v, 0.5 msec, 50 Hz) to enhance the short-latency preganglionic response evoked from the medulla at submaximal intensities of stimulation (<8v). The medullary site from which the short-latency potential was evoked was first defined while

monitoring postganglionic sympathetic discharges on the external carotid nerve. Then, the recording electrode was moved to the preganglionic trunk. Following the administration of MECA, blood pressure was reduced (10-40 mmHg). The fall in blood pressure was associated with the expected increase in the level of spontaneously-occurring preganglionic sympathetic discharges. Concomitant with these changes, the amplitude of the control submaximal short-latency potential was reduced. The intensity of stimulation was increased to return the short-latency response to its pre-drug level. The degree of enhancement during depressor region stimulation was virtually the same (Fig. 8-I-MECA). If MECA had been effective in blocking the spinal interaction, then an increase in the amplitude of the control submaximal short-latency response would have been expected, as opposed to the observed decrease. Only transient and smaller changes in blood pressure and spontaneous activity were observed with DHβE. The results of five experiments are summarized in Table 2. The doses of DHβE used in the present experiments effectively reduced the IPSP evoked by ventral root stimulation in α motoneurons (Eccles et al., 1954a). In addition, the doses of MECA employed in this study have been shown to produce effective ganglionic blockade (Stone et al., 1956). Thus, drugs which are known to block cholinergic transmission at autonomic and somatic junctions were without effect on the

spinal interaction between the two sympathetic vasopressor pathways.

F. Sympathoinhibition of Non-Baroreceptor Origin Evoked by Medial Medullary Stimulation

1. Inhibition of the Short-Latency Response Evoked from the Medulla

Enhancement of the short-latency postganglionic responses was usually observed during depressor region stimulation. However, on six occasions, inhibition of the short-latency potential was observed. In four instances, small movements (0.5-1 mm) of the electrode in the depressor region changed the effect of stimulation from enhancement to inhibition. An example of such a reversal is illustrated in Figure 9-I. In the two remaining instances, small movements (<1 mm) of the stimulating electrode in R.v. of the pressor region had little effect on the onset of the short-latency response. Yet, stimulation of the same depressor site in the medial medulla at the same parameters of stimulation (10v, 0.5 msec, 50 Hz) enhanced the short-latency postganglionic potential evoked from one pressor site, and blocked the response elicited from the other (Fig. 9-II). The inhibitory effects of depressor region stimulation on the short-latency responses evoked from the medulla are summarized in Table 3.

It should be stressed that on those six occasions when depressor region stimulation inhibited the short-latency

Figure 9. Reversal of effect of depressor region stimulation on short-latency postganglionic potentials evoked from medullary pressor sites

I: Reversal produced by moving electrode in depressor region by 1 mm (A-electrode position 1; B-electrode position 2). A1, B1 - summed control responses (16 trials) evoked by 10 msec trains of 3 pulses (7v, 0.5 msec, 300 Hz) applied 4 times each sec to same medullary pressor site. A2, B2 - responses during stimulation of 2 different medullary depressor sites (10v, 0.5 msec, 50 Hz). A3, B3 - responses after depressor region stimulation.

II: Reversal produced by moving electrode in pressor region of R.v. by 0.5 mm (A-electrode position 1; B-electrode position 2). A1, B1 - summed control responses (32 trials) evoked by 10 msec trains of 4 pulses (8v, 0.5 msec, 400 Hz) applied 4 times each sec to 2 different medullary pressor sites. A2, B2 - responses during stimulation of same medullary depressor site (10v, 0.5 msec, 50 Hz). A3, B3 - responses after depressor region stimulation.

Records in I and II are from two different cats. Horizontal calibrations are 50 msec. Vertical calibrations are 106 μV in I, 27 μV in II-A, and 53 μV in II-B.

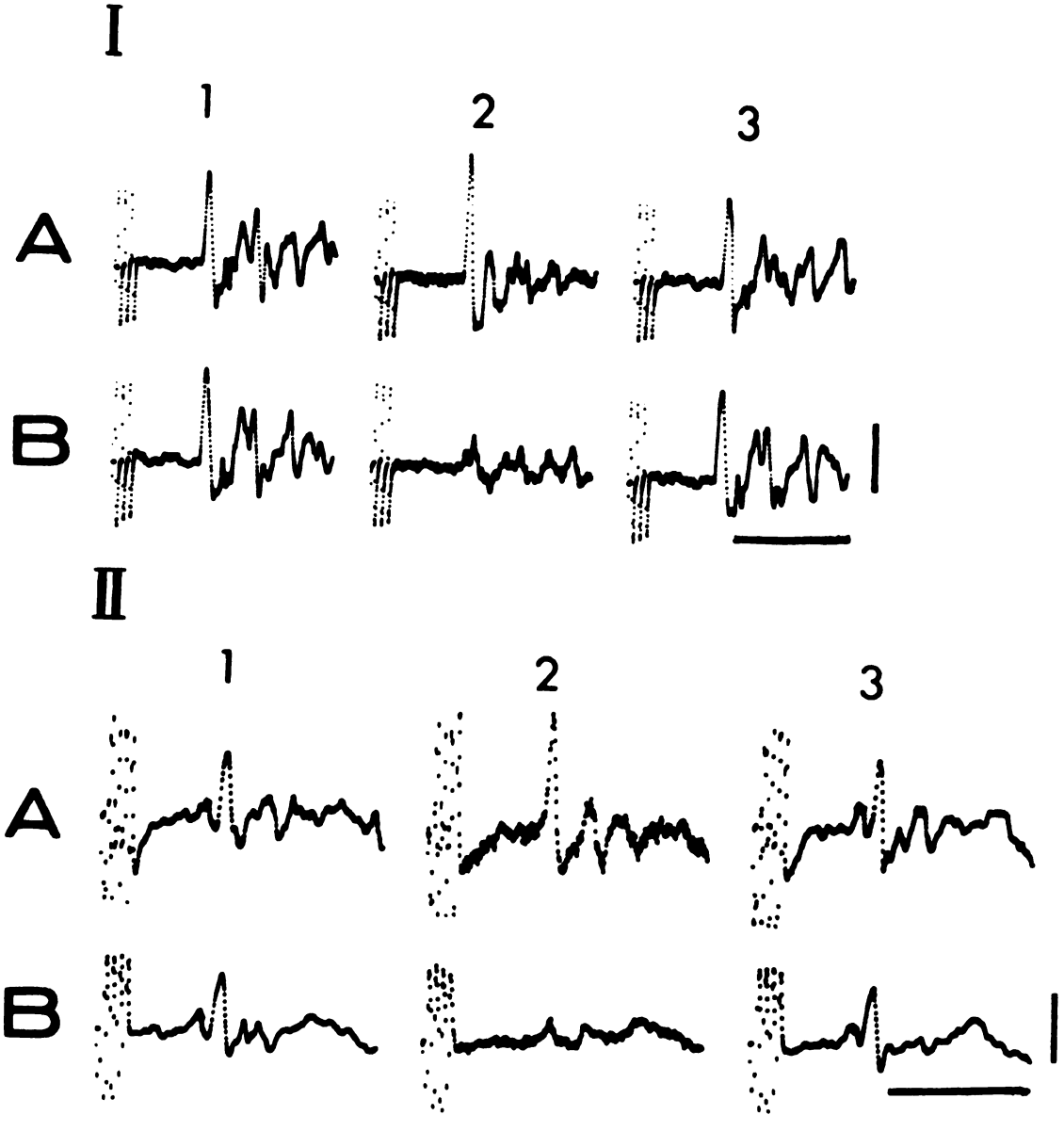


Figure 9

Table 3 - Inhibitory effects of depressor region stimulation on postganglionic sympathetic potentials evoked from the medulla

Potential Type, % Change from Control Amplitude	
Short-latency (<50 msec)	Long-latency (>50 msec)
-68 ± 8 (6)	-78 ± 12 (3)

Values for % change are means ± SE with N given in parenthesis.

potential, the same response was enhanced or unchanged during the pressor action of norepinephrine. Thus, baroreceptor reflex activation and depressor region stimulation did not always produce the same effect on the short-latency response evoked from the medulla.

2. Postganglionic Sympathetic Responses Evoked from the Midbrain

Depressor region stimulation never inhibited the sympathetic nerve responses evoked from the descending spinal tracts of the short-latency pathway. Since the short-latency postganglionic potentials evoked from the medulla were inhibited by depressor region stimulation on six occasions, it is conceivable that, in the majority of cases, the short-latency responses were evoked from medullary sites distal to the site of non-baroreceptor induced inhibition. Thus, if the short-latency potential was evoked from higher levels of the neuraxis, perhaps this non-baroreceptor inhibitory effect of depressor region stimulation would become more apparent. Both vasopressor pathways have been previously located at midbrain levels (Gebber et al., 1973). Therefore, the effects of depressor region stimulation were tested on postganglionic potentials evoked from midbrain components of the two vasopressor systems.

In eight cats, activation of 71 sites in the medial medulla was tested upon the postganglionic discharges

evoked from the midbrain. Postganglionic sympathetic nerve responses were evoked by 10 msec trains of 3 pulses (3-15v, 0.5 msec, 300 Hz) applied to pressor sites distributed diffusely throughout the central gray and reticular formation of the midbrain. A train of 3 pulses applied to pressor sites in the midbrain failed to change blood pressure, although stimulation at a frequency of 50 Hz for 15 sec raised mean arterial pressure in excess of 40 mmHg. As previously reported by Gebber et al. (1973), the sympathetic nerve responses evoked from the midbrain took the form of a multiple discharge with early (onset latency <50 msec) and late (onset latency >50 msec) components. The early and late components monitor the simultaneous activation of the short-and long-latency pressor pathways, respectively.

a. Comparison of the Effects of Baroreceptor Reflex Activation and Depressor Region Stimulation on the Early Postganglionic Response Evoked from the Midbrain

The early component of the postganglionic discharge evoked from the midbrain was blocked by stimulation of over 50% of the sites tested in the medial medulla. However, the early component was never inhibited during the pressor action of norepinephrine (1-2 µg/kg i.v.). A representative experiment is illustrated in Figure 10. Figure 10A shows an early response which was blocked by stimulation of a site in the medullary depressor region.

Figure 10. Comparison of effect of depressor region stimulation and norepinephrine on early component of postganglionic discharge evoked from a midbrain pressor site

A1, B1: control summed responses (32 trials) evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied three times each sec to midbrain. A2: response during depressor region stimulation (10v, 0.5 msec, 50 Hz). B2: response during inhibition of spontaneously-occurring postganglionic discharges accompanying pressor action of norepinephrine (1 µg/kg, i.v.). A3, B3: responses after depressor region stimulation and norepinephrine, respectively. Horizontal calibration is 25 msec. Vertical calibration is 106 µV.

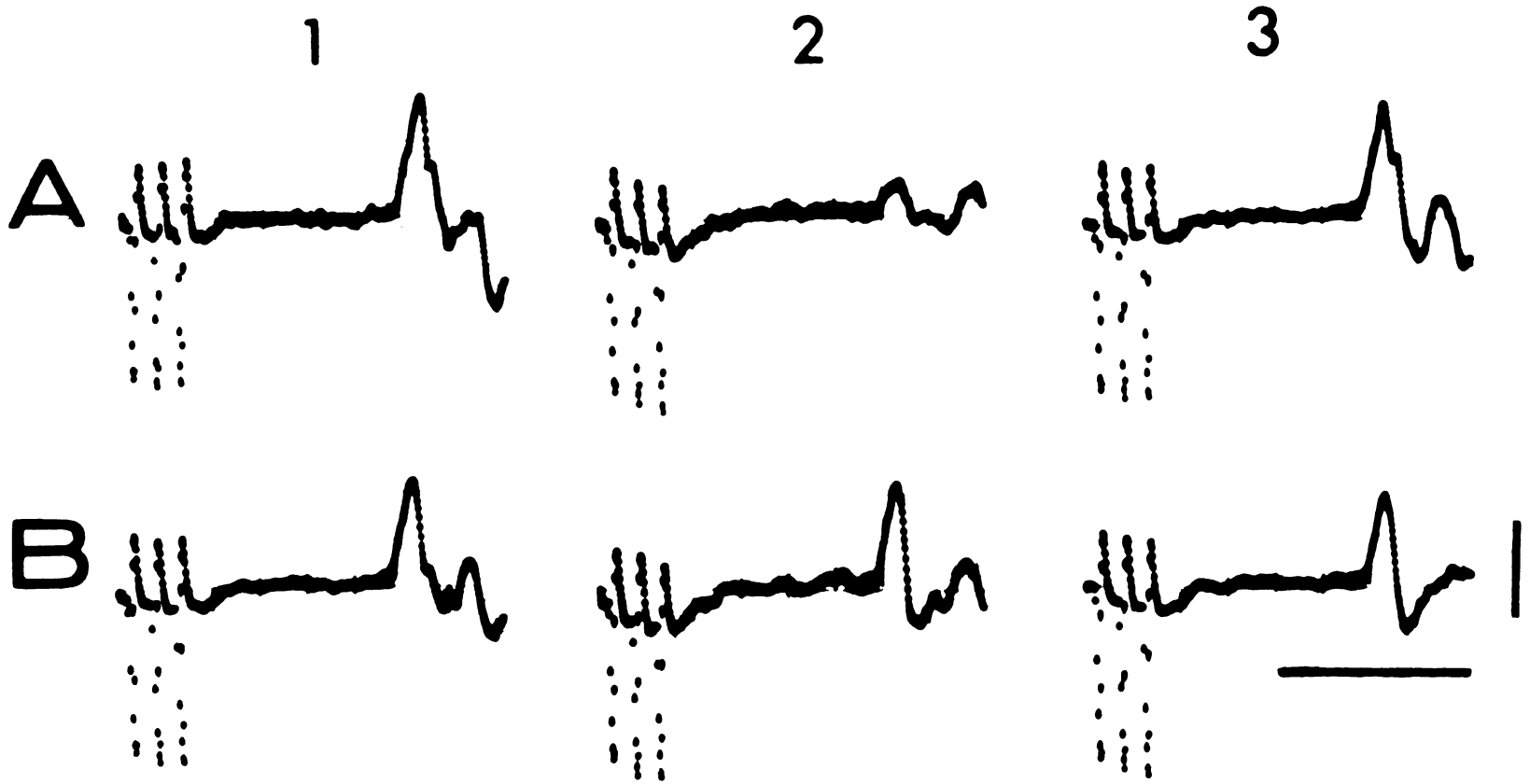


Figure 10

The same response was unchanged during the inhibition of spontaneously-occurring postganglionic discharges associated with the rise in blood pressure produced by norepinephrine (Fig. 10B). The late components of the postganglionic discharge elicited from the midbrain were always reduced or abolished during the rise in blood pressure produced by norepinephrine.

b. Differential Actions of Depressor Region Stimulation on the Early and Late Components of the Discharge Evoked from the Midbrain

When the effect of depressor region stimulation was tested on both components of the multiple discharge evoked from a midbrain pressor site, three patterns of results were observed. These are illustrated in Figure 11. The first pattern (Fig. 11A) consisted of: 1) enhancement or no change in the early components, and 2) inhibition of the late components of the multiple discharge elicited from the midbrain. Stimulation of 25 medial medullary sites which produced these effects also lowered blood pressure and inhibited spontaneously-occurring postganglionic discharges.

The second pattern consisted of inhibition of both the early and late components of the multiple discharge evoked from the midbrain (Fig. 11B). These effects were elicited from 36 medial medullary sites which, when activated, also lowered blood pressure and inhibited spontaneously-occurring postganglionic discharges.

Figure 11. Effects of stimulation of three different medial medullary sites (A-C) on postganglionic nerve response evoked from the same midbrain pressor site in a cat

A-C: top tracing is blood pressure (mmHg). Second tracing is time base (1 sec/division). Third tracing is spontaneously-occurring postganglionic discharges. Bottom tracings depict: (1) control responses evoked from midbrain; (2) responses during stimulation (10v, 0.5 msec, 50 Hz) of medial medullary sites; and (3) responses after medial medullary stimulation. A1-3 are summed potentials (32 trials) evoked with 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied 4 times each sec. B1-3 and C1-3 are summed potentials (32 trials) evoked with same parameters of stimulation applied twice each sec. Horizontal calibration is 100 msec. Vertical calibrations are 20 μV for records of spontaneously-occurring activity, 213 μV for A1-3 and B1-3, and 106 μV for C1-3.

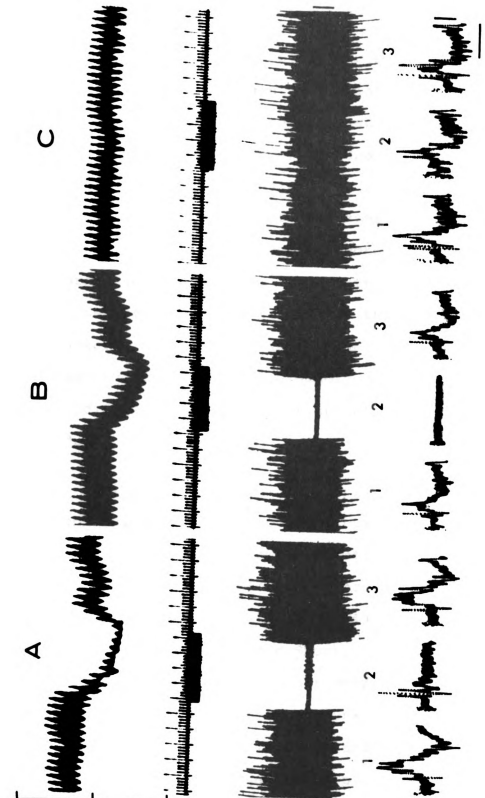


Figure 11

The third pattern (Fig. 11C) consisted of: 1) inhibition of the early components, and 2) no consistent change of the late components of the multiple postganglionic discharge evoked from the midbrain. Stimulation of ten sites within the medial medulla which produced these effects had little or no effect on blood pressure and spontaneously-occurring postganglionic discharges.

A summary of the effects of depressor region stimulation on the postganglionic discharge evoked from the midbrain is presented in Table 4. The distribution of sites within the depressor region, from which the early and late components were inhibited, is shown in Figure 12. It is evident that the two sympathoinhibitory effects were elicited from diffuse and widely-overlapping areas of the medial medulla.

G. Silent Period

The depression of spontaneously-occurring sympathetic nervous discharge, which follows excitation of supraspinal origin, has been commonly referred to as the silent period (Koizumi and Brooks, 1972; Sell et al., 1958), or post-excitatory depression (Pitts and Bronk, 1942). An example of this phenomenon (10 superimposed records) recorded on the splanchnic preganglionic nerve trunk is illustrated in Figure 13A. The splanchnic discharge and following silent period were evoked by a 10 msec train of 3 supramaximal pulses (10v, 0.5 msec, 300 Hz) applied once every 4 sec

Figure 12. Distribution of medullary sites which, when stimulated, affected postganglionic sympathetic nerve discharges evoked from the midbrain pressor region

A: represents a frontal section about 2 mm rostral to the obex. Abbreviations are those used in Fig. 3. B: distribution of 46 sites from which inhibition of the early components of the midbrain response was produced. C: distribution of 61 sites from which inhibition of the late components of the midbrain response was produced.

Table 4 - Effects of depressor region stimulation on post-ganglionic sympathetic nerve responses evoked from midbrain

Response Pattern	Potential Type, % Change from Control Amplitude	
	Early component (<50 msec)	Late component (>50 msec)
I	+88 ± 12 (25)	-65 ± 6 (25)
II	-81 ± 4 (36)	-75 ± 5 (36)
III	-57 ± 8 (10)	- 1 ± 4 (10)

Values for % change are means ± SE with N given in parenthesis.

to a pressor site in the dorsolateral medullary reticular formation. The onset latency of the preganglionic splanchnic nerve discharge evoked from the medulla ranged between 45-80 msec, which agrees with previous reports (Gootman and Cohen, 1971; Nathan, 1972). In the 30 cats used in this study, the duration of the silent period evoked by central stimulation ranged between 800-2000 msec.

1. Does True Neural Inhibition Account for the Silent Period?

   a. Dissociation of the Splanchnic Nerve Discharge and the Silent Period

Figure 13 is representative of four experiments in which it was possible to dissociate the splanchnic nerve discharge and silent period evoked by stimulation of the medullary pressor region. The splanchnic nerve discharge and silent period elicited by a conditioning train of 3 pulses (10v, 0.5 msec, 300 Hz) applied to a pressor site in the dorsolateral medulla is shown in Panel A. Panel B illustrates the discharge and silent period evoked by a single test shock (8v, 0.5 msec, 0.25 Hz) applied to the same medullary site. The test shock was then applied during the silent period produced by the conditioning train (Panel C). Although the test shock failed to elicit a splanchnic discharge, the silent period, produced by a combination of conditioning and test stimuli, was considerably longer than that produced by the conditioning train

Figure 13. Dissociation of sympathetic discharge and silent period evoked by stimulation of dorsolateral medulla

A: 10 superimposed traces of splanchnic discharge and silent period elicited by a conditioning 10 msec train of 3 pulses (10v, 0.5 msec, 300 Hz) applied to medullary pressor site at beginning of record. Insert shows discharge (sum of 16 trials) on expanded time base. B: 10 superimposed traces of discharge and silent period evoked by a test shock (10v, 0.5 msec) applied to the same medullary site. Time of application of shock is designated by white dot. Insert shows discharge (sum of 16 trials) on expanded time base. C: 10 superimposed traces showing prolongation of silent period when the test shock was applied 460 msec following the conditioning stimuli. Insert shows that test discharge was blocked. Horizontal and vertical calibrations for superimposed traces are 200 msec and 10 μV, respectively. Horizontal calibration is 25 msec for insert traces. Vertical calibration is 67 μV for insert A and 33 μV for inserts B and C.

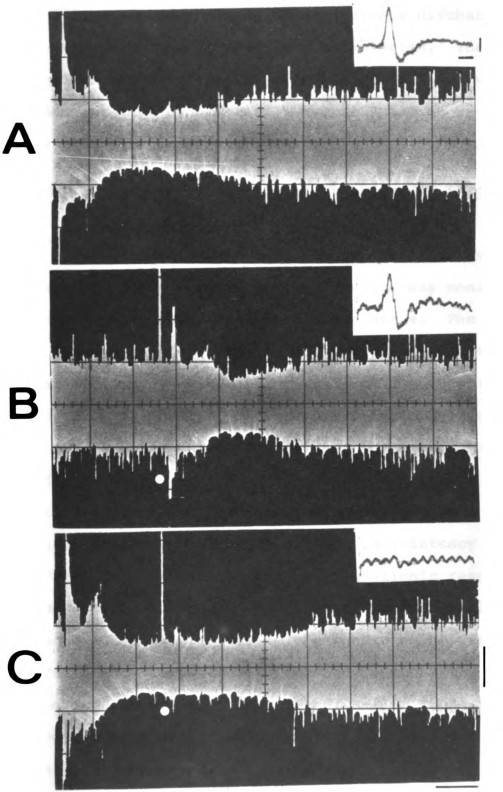


Figure 13

alone. This would suggest that independent neural mechanisms are involved in the sympathetic nervous discharge and silent period produced by medullary stimulation. These results confirm those of Koizumi et al.(1968) and Iwamura et al. (1969a) obtained by afferent nerve stimulation in demonstrating true neural inhibition.

b. Selective Inhibition of Transmission in the Long-Latency Pressor Pathway

Figure 14 illustrates the typical results of three experiments in which the silent period was monitored from the external carotid postganglionic nerve. The long-latency postganglionic discharge and following silent period, elicited by a conditioning train of 3 pulses (10v, 0.5 msec, 300 Hz) applied to a pressor site in the dorsolateral medulla, are shown in Panel A. The test 10 msec train of 3 pulses (7v, 0.5 msec, 300 Hz) was applied to a medullary site in R.v. The test stimulus evoked a postganglionic response with both short-and long-latency components (Panel B-1). When the test postganglionic response was summed during the peak depression of spontaneously-occurring discharges produced by the conditioning stimulus, the short-latency response was enhanced, whereas the long-latency discharge was inhibited (Panel B-2). Thus, the silent period acts selectively to block transmission in only the long-latency pressor pathway.

Figure 14. Effects of the silent period on postganglionic discharges evoked in external carotid nerve.

A: 10 superimposed traces of postganglionic discharge and silent period elicited by a conditioning 10 msec train of 3 pulses (10v, 0.5 msec, 300 Hz) applied to medullary pressor site at beginning of record. Insert shows discharge (sum of 16 trials) on expanded time base. B: summed postganglionic response (16 trials) evoked by test 10 msec trains of 3 pulses (7v, 0.5 msec, 300 Hz) applied to a different medullary pressor site. B1 - control response; note that the short- and long-latency responses were evoked simultaneously from this site. B2 - summed response when the test stimulus was applied 250 msec following the conditioning stimuli. B3 - test response alone. Horizontal calibrations are 100 msec in A and 50 msec in B. Vertical calibrations are 20 μV for A and 106 μV for insert and B.

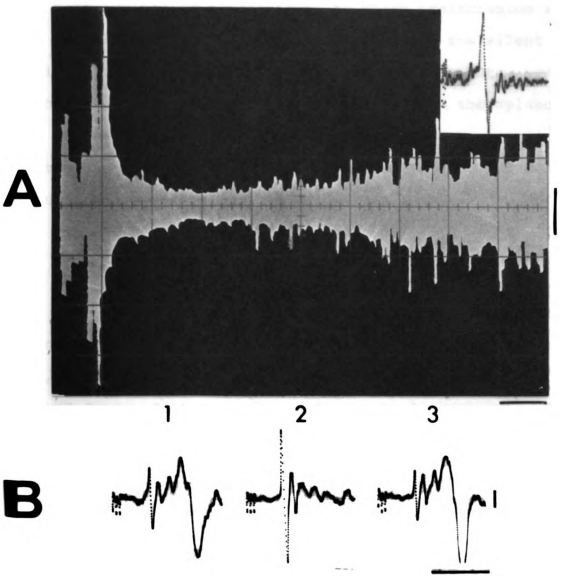


Figure 14

2. Site of Sympathoinhibition Responsible for the Silent Period

The purpose of these experiments was to determine the site (spinal or supraspinal) at which transmission in the long-latency pathway is inhibited during the silent period. To simplify the interpretation of the data, sympathetic nervous discharge was monitored from the splanchnic preganglionic trunk since only the long-latency pressor system has been identified in this nerve (Taylor and Gebber, unpublished observation).

a. Comparison of the Silent Period Evoked from the Medulla and Spinal Cord

The temporal characteristics for depression of spontaneously-occurring preganglionic discharges are similar, regardless of whether the preceding excitation was evoked from medullary or descending spinal components of the long latency pressor pathway. Figure 15 is typical of the results observed in four experiments. Trains of 3 pulses of supramaximal intensity (10v, 0.5 msec, 300 Hz) were applied to pressor sites in the medulla (Fig. 15B), and in the dorsolateral white column of the midcervical spinal cord (Fig. 15C). The silent period evoked in either case lasted for more than 1 sec. Two observations indicated that the splanchnic nerve discharge evoked from the dorsolateral white column of the midcervical spinal cord monitored the activation of descending pathways. First, the onset

Figure 15. Comparison of the temporal characteristic of silent period evoked by stimulation of pressor sites in the medulla and spinal cord

A: 10 superimposed traces of spontaneously-occurring preganglionic sympathetic activity recorded on the splanchnic nerve. B: 10 superimposed traces of splanchnic discharge and silent period evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied to medullary pressor site at beginning of record. C: 10 superimposed traces of splanchnic discharge and silent period evoked by 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied to descending spinal tracts. Horizontal calibration is 100 msec. Vertical calibration is 10 μV.

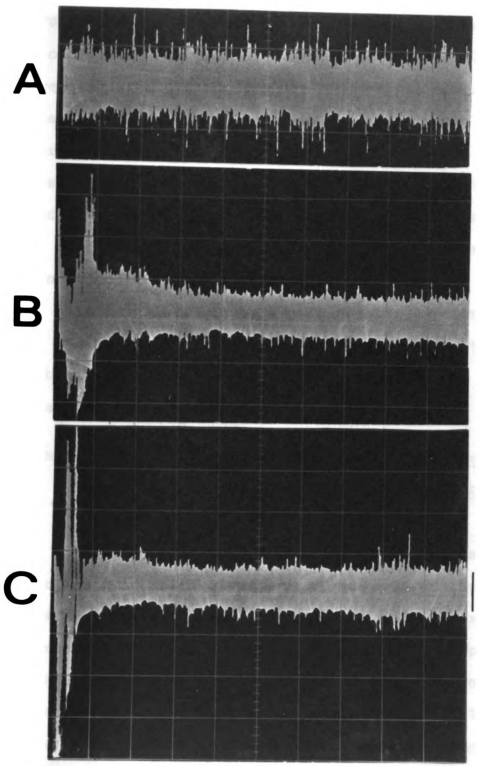


Figure 15

latency (25-36 msec) of the splanchnic discharge evoked by spinal stimulation was shorter than for the response elicited from the medulla. This is in agreement with previous reports of Gootman and Cohen (1971). Second, the splanchnic nerve discharge evoked from the midcervical spinal cord was not eliminated by transection of the spinal cord at the first cervical vertebra.

These experiments suggested that the silent period was generated at a spinal locus. However, it is also possible that the electrode in the cervical spinal cord activated afferents ascending to the medulla, which could have initiated the silent period. Thus, a more precise experimental approach had to be developed to test the hypothesis that the inhibition responsible for the silent period was exerted at a spinal level.

b. Relationship Between the Silent Period and the Depression of the Test Splanchnic Nerve Discharge

Figure 16 illustrates the typical results of six experiments in which a comparison was made of the temporal characteristics of the silent period and the excitability recovery curve of a test splanchnic nerve response. The conditioning and test stimuli were 10 msec trains of 3 supramaximal pulses (10-15v, 0.5 msec, 300 Hz) applied once every 4 sec to the same pressor site in the dorsolateral medullary reticular formation. As can be noted in

Figure 16. Comparison of silent period and excitability-recovery curve of a splanchnic nerve response

The conditioning and test stimuli were 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) applied once every 4 sec to the same pressor site in the dorsolateral medulla. A: 10 superimposed traces of splanchnic discharge and silent period evoked by conditioning stimuli applied at beginning of record. B: excitability-recovery curve of test splanchnic discharge. Cond.-Test Int. is the interval (msec) between the onset latencies of the sympathetic discharges evoked by the conditioning and test stimuli. This interval also provides the time base for A. C: top trace (T) is control test response. Lower traces are test responses at specified cond.-test int. (msec). Each trace is the sum of 16 trials. Horizontal calibration is 25 msec for records in C. Vertical calibrations are 10 μV in A and 67 μV in C.

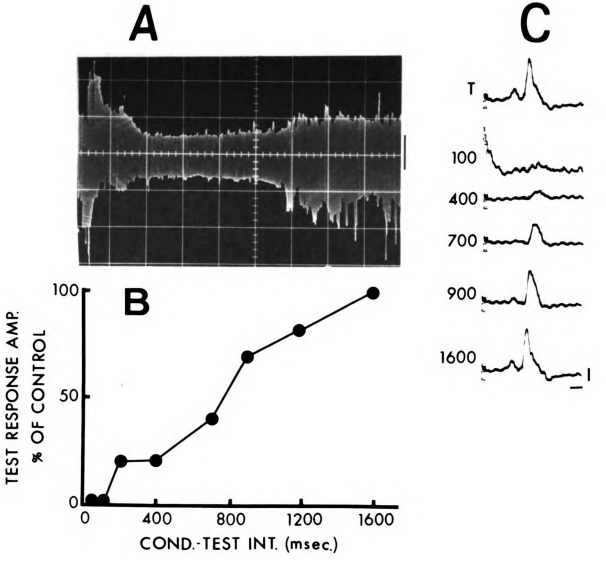

A
B
C
100
50
0
TEST RESPONSE AMP.
% OF CONTROL
0
400
800
1200
1600
COND.-TEST INT. (msec.)
T
100
400
700
900
1600

Figure 16

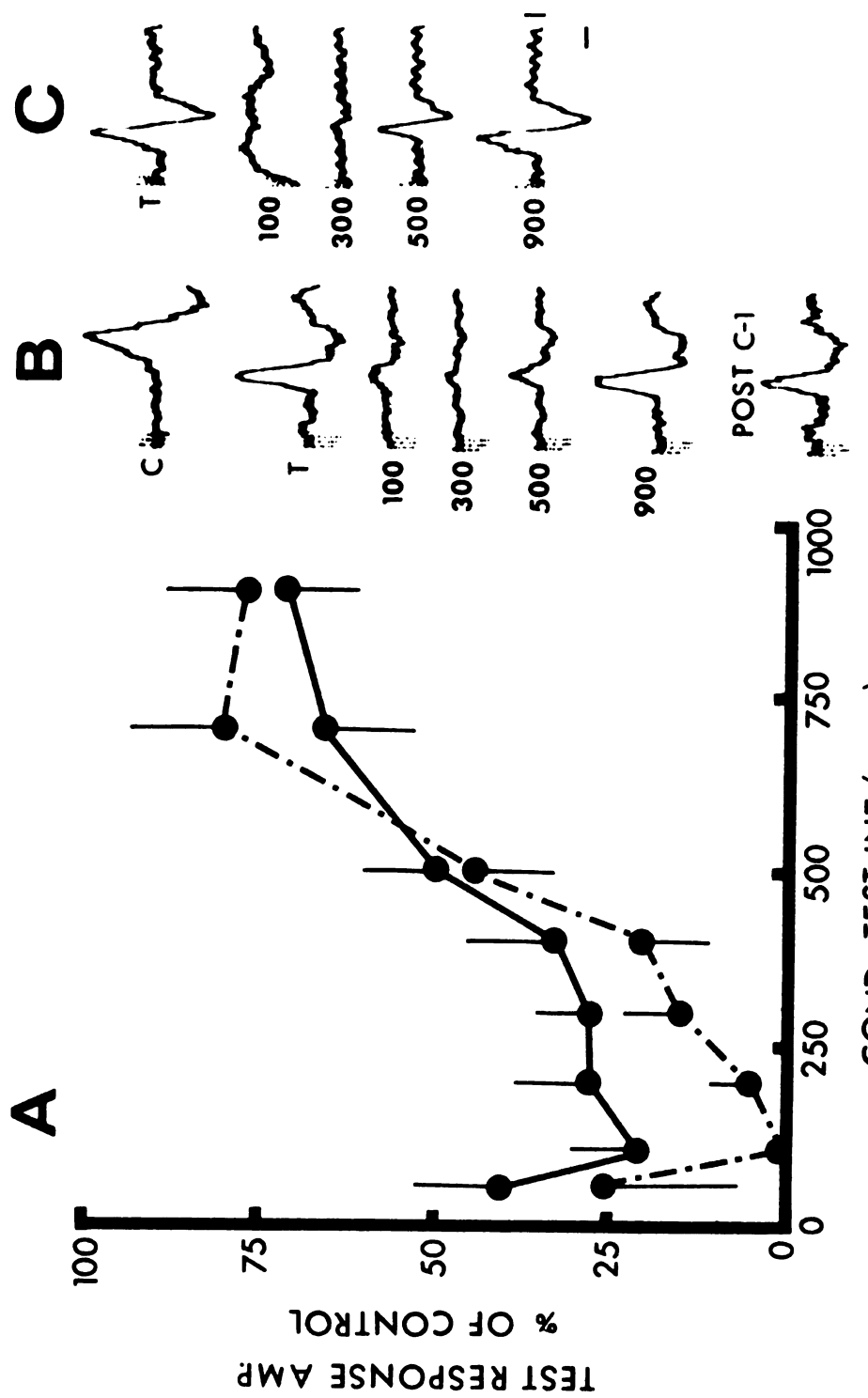
Figure 16A-B, the time course of depression of the test splanchnic nerve response closely followed that of the silent period produced by the conditioning stimulus. Experiments in which paired shocks were applied to somatic afferent nerves have revealed similar results (Coote and Perez-Gonzalez, 1970; Iwamura et al., 1969a; Koizumi et al., 1968; Sato et al., 1967). Thus, the excitability recovery curve of a test splanchnic nerve discharge can be used as a monitor of the true silent period which follows excitation evoked from the medulla or other levels of the neuraxis. With this in mind, a series of experiments was designed to more carefully examine the possibility that the silent period is generated at a spinal level.

c. Sympathoinhibition Representing the Silent Period is Generated at a Spinal Locus

The time course of depression of a test splanchnic nerve discharge evoked by a 10 msec train of 3 pulses (10-15v, 0.5 msec, 300 Hz) applied to a pressor site in the midcervical spinal cord was compared with that of the test response evoked by stimulation of a medullary pressor site. The conditioning train of 3 pulses was applied to the medullary pressor site in each case. The excitability recovery curves depicted in Figure 17 summarize the results obtained in eight experiments. The time course of depression of the splanchnic nerve discharge evoked from the spinal cord was essentially the same as that for the response elicited by

Figure 17. Comparison of excitability-recovery curve of test splanchnic nerve discharge evoked by stimulation of midcervical spinal cord with that of the test discharge elicited by stimulation of the medullary pressor region

The test stimuli were 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz). The conditioning 10 msec train of 3 pulses (10v, 0.5 msec, 300 Hz) was applied to the medulla in each case. A: excitability-recovery curves of test responses produced by stimulation of spinal cord (solid line) and medulla (dot-dash line) in 8 cats. Values are mean ± SE. Records from one cat are shown in B and C. Each trace is sum of 16 trials. B: time course of depression of test response evoked by stimulation of spinal cord. Top trace (C) is response elicited by conditioning stimuli applied to medulla. Second trace (T) is control response elicited by test stimuli applied to spinal cord. Lower traces show test response at specified cond.-test int. (msec). The bottom trace shows the test response 10 min after C-1 transection of spinal cord. C: time course of depression of test response (T) evoked from medulla. Conditioning and test trains were applied through the same electrode. Horizontal calibration is 12.5 msec for traces in B and 25 msec for traces in C. Vertical calibration is 33 μV.


A
B
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TEST RESPONSE AMP
% OF CONTROL
100
75
50
25
0
0
250
500
750
1000
COND.-TEST INT. (msec.)
C
T
100
300
500
900
POST C-1

Figure 17

stimulation of the medulla. The splanchnic nerve discharge evoked from the dorsolateral white column of the midcervical spinal cord was elicited from descending pathways. This was based on the following observations: first, the onset latency (25-36 msec) of the splanchnic discharge evoked by spinal stimulation was shorter than for the response elicited from the medulla (Fig. 17B). Second, the splanchnic nerve discharge evoked from the midcervical spinal cord was not eliminated by transection of the spinal cord at the first cervical vertebra (Fig. 17B). Table 5 shows that the maximum depression of the test splanchnic nerve response produced by the conditioning train was significantly greater than the reduction of the test response following C-1 transection. Transection of the spinal cord at the first cervical vertebra reduced spontaneously-occurring discharges of the splanchnic nerve to the level observed during the peak of the silent period. This suggests that the sympathoinhibition responsible for the silent period occurs at a spinal synapse.

It has been suggested that the medial medulla is involved in the sympathetic silent period (Iwamura et al., 1969a; Koizumi et al.,1968; Koizumi and Brooks, 1972; Sato, 1972b). Stimulation of the medial medullary depressor region is known to interrupt sympathetic nervous activity at a spinal level (Coote et al., 1969; Gootman and Cohen, 1971; Kirchner et al., 1971). Further, the data illustrated in Figures 4 and 7 support this hypothesis. Stimulation of

Table 5 - Maximum changes of test splanchnic nerve discharge evoked from the midcervical spinal cord

Conditioning Procedure	% Reduction in Amplitude of Test Response
Conditioning medullary stimuli	88 ± 4 (8)*
Conditioning spinal stimuli before C-1 transection	69 ± 8 (6)*
C-1 transection	42 ± 6 (8)

Values for % reduction are mean ± SE with N given in parenthesis.

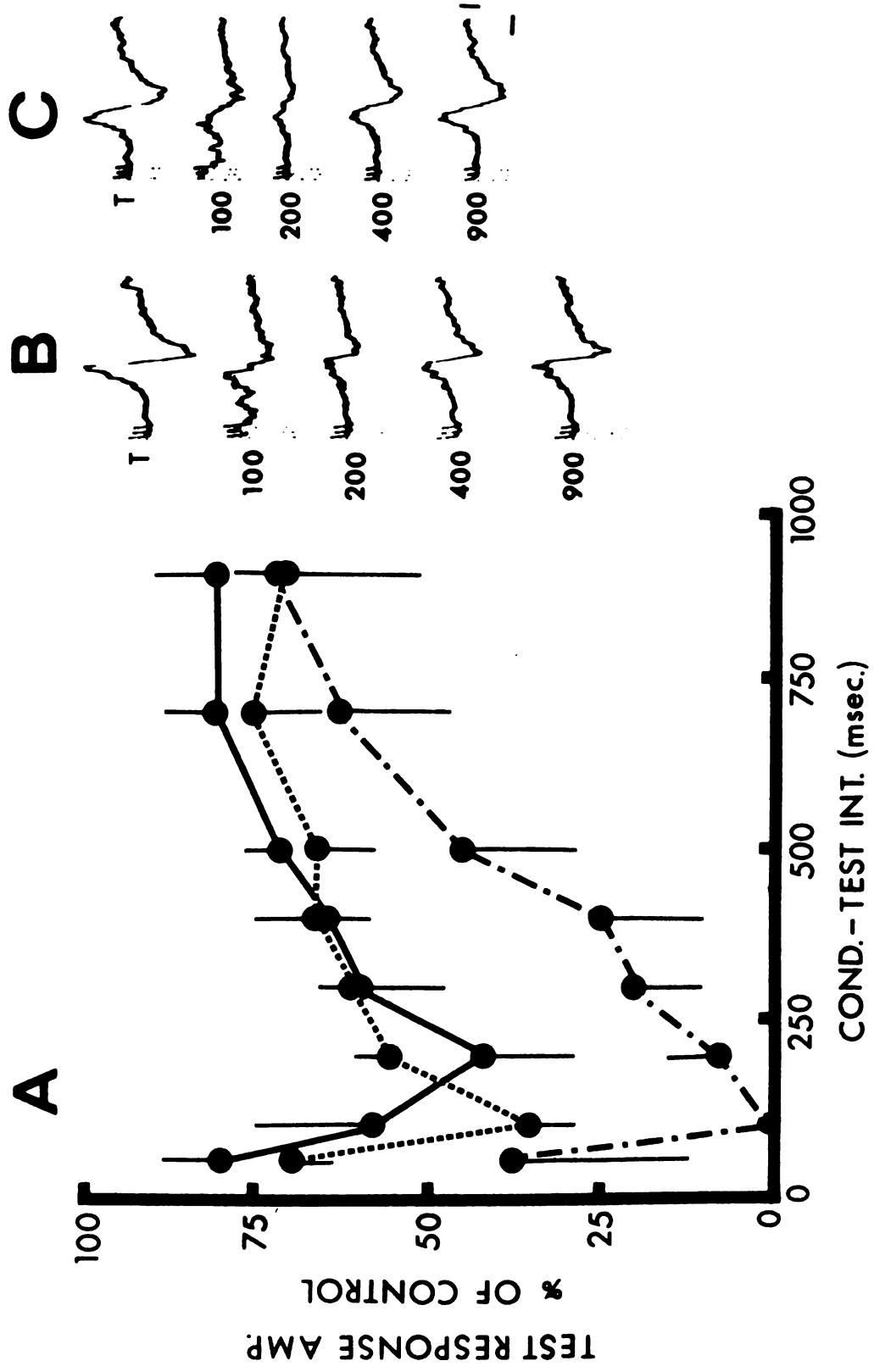
*Significantly different from the effect produced by C-1 transection.

the medial medullary depressor region activates an inhibitory system which controls transmission in the long latency sympathetic pressor pathway at a spinal locus (Fig. 7). Short trains of 3 pulses applied to a site in the medial medulla which activate this spinal sympathoinhibitory system produced a long-lasting depression of spontaneously-occurring sympathetic activity without a preceding excitation (Fig. 4). Thus, the following experiments were performed to determine the extent to which the medial medulla played a role in the generation of the silent period.

Figure 18 summarizes the results of six experiments in which the time course of depression of a test splanchnic nerve response evoked by stimulation of descending spinal pathways was compared before and after C-1 transection. The conditioning and test stimuli (10 msec trains of 3 pulses) were applied to the same dorsolateral site in the midcervical spinal cord. Transection of the spinal cord at the first cervical vertebra failed to alter the degree or time course of depression of the test splanchnic nerve discharge (Fig. 18A). C-1 transection reduced the amplitude of the test response (Fig. 18B-C), but not to the extent of the maximum depression produced by the conditioning train before transection of the spinal cord (Table 5). The excitability recovery curve of the splanchnic nerve discharge elicited in the same cats, when the conditioning and test trains were applied to the medullary pressor

Figure 18. Excitability-recovery curves of test splanchnic nerve discharges evoked by stimulation of the midcervical spinal cord before and after C-1 transection

The conditioning and test 10 msec trains of 3 pulses (10v, 0.5 msec, 300 Hz) were applied to the same spinal site (6 experiments). A: excitability-recovery curves of test responses before (solid line) and 10-20 min after (dotted line) C-1 transection. Values are mean ± SE. The excitability-recovery curve (dot-dash line) of test responses elicited in the same cats when the conditioning and test stimuli (10 msec trains of 3 pulses) were applied to the medulla before C-1 transection is also shown. Records from a representative experiment are illustrated in B and C. Each trace is sum of 16 trials. B: time course of depression of control response (T) evoked by test stimuli applied to midcervical spinal cord before C-1 transection. Lower traces show test response at specified cond.-test int. (msec). C: time course of depression of response (T) elicited from the same spinal site after C-1 transection. Horizontal calibration is 12.5 msec. Vertical calibration is 33 μV.


A
B
C
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100
200
400
900
TEST RESPONSE AMP.
% OF CONTROL
100
75
50
25
0
0
250
500
750
1000
COND.-TEST INT. (msec.)

Figure 18

region before C-1 transection, is also shown in Figure 18A. Note that the time course of depression of the test response evoked from the spinal cord corresponded with that of the splanchnic nerve discharge elicited by stimulation of the medulla. However, the degree of depression of the test response evoked from the medulla was more pronounced during the early portions of the excitability recovery curve.

Figure 19 shows the distribution of sites within the medulla and midcervical spinal cord from which the sympathetic responses and following silent period were evoked in splanchnic preganglionic nerve. The AP levels of medullary stimulation were sufficiently close so that the data could be plotted on one frontal section, slightly rostral to the obex. The majority of the sites from which preganglionic responses were evoked were located in the periventricular gray and the underlying dorsolateral reticular formation (Fig. 19B). Most of the responses evoked from the midcervical spinal cord were elicited from sites in the dorsolateral white column (Fig. 19C). This is in accord with the localization of descending spinal pressor tracts reported by others (Gebber et al., 1973; Illert and Gabriel, 1970; Illert and Seller, 1969; Johnson et al., 1952; Kell and Hoff, 1952; Kerr and Alexander, 1964).

Figure 19. Distribution of medullary and spinal sites from which responses were elicited in ipsilateral splanchnic nerve

A and B: a frontal section about 2 mm rostral to the obex. Abbreviations are those used in Fig. 3. C: a frontal section of the spinal cord at C-4.

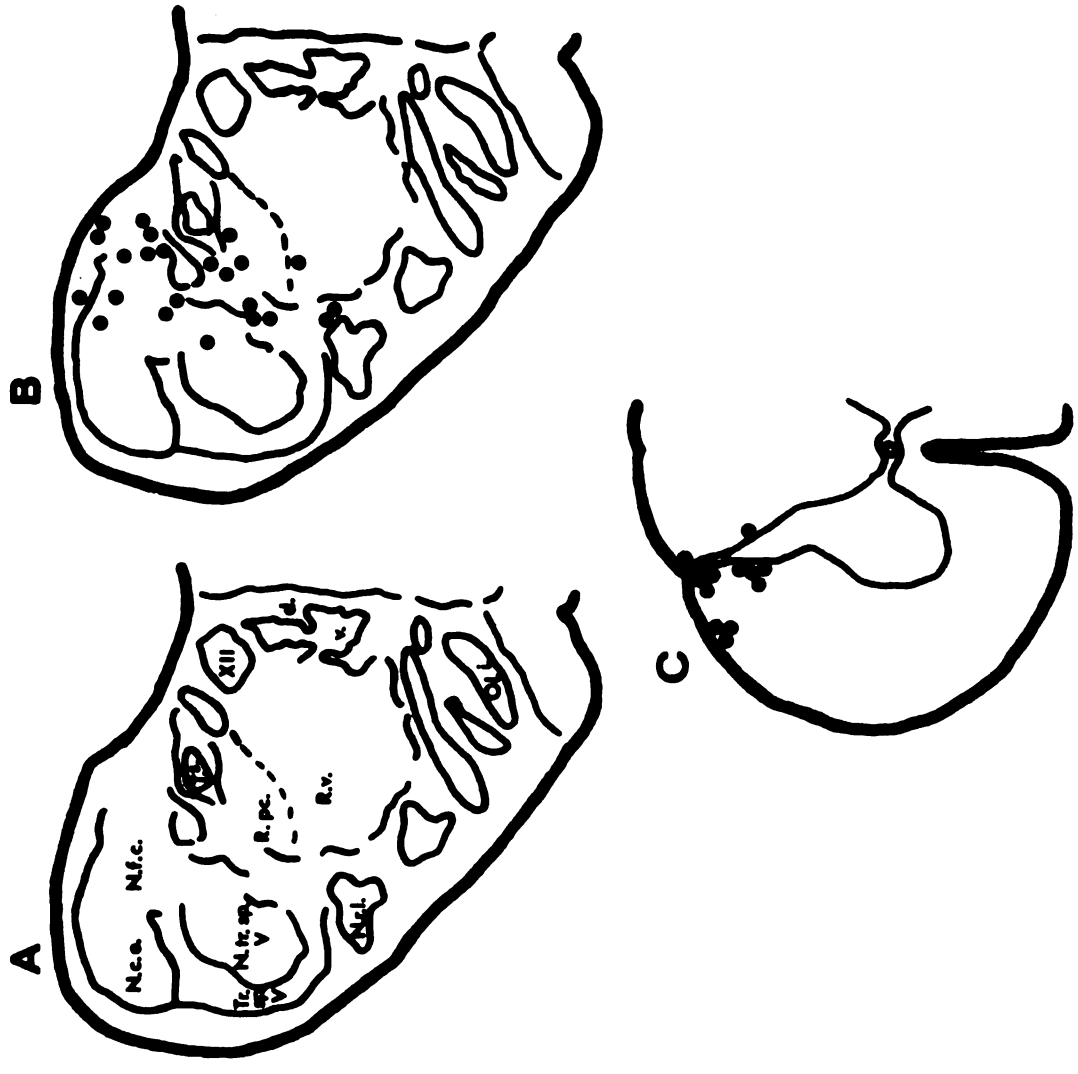

Figure 19

DISCUSSION

Sympathetic vasopressor outflow from the hypothalamus is distributed over two distinct systems of parallel pathways. Postganglionic potentials, characteristic of the two vasopressor circuits, have been evoked from various levels of the neuraxis (Gebber et al., 1973). For the purpose of this discussion and with reference to this previous study (Gebber et al., 1973), postganglionic potentials evoked from one vasopressor system include: 1) the late components of the midbrain multiple discharge; 2) long-latency (>50 msec) medullary responses; 3) responses elicited from the spinal cord, all of which were inhibited during the pressor action of norepinephrine. This system will be referred to as the "long-latency" pathway. Postganglionic potentials evoked from the other vasopressor system include: 1) the early components of the midbrain responses; 2) short-latency (<50 msec) medullary responses; and 3) responses elicited from spinal sites, all of which were insensitive to the pressor action of norepinephrine. This vasopressor system will be referred to as the "short-latency" pathway.

The results presented in this dissertation have demonstrated that transmission in the two vasopressor pathways

is controlled centrally by different sympathoinhibitory systems. Two distinct inhibitory systems were activated by stimulation of the medial medullary depressor region. Each system functions to control transmission in a different vasopressor pathway and at a different level of the neuraxis. One inhibitory system mimics the effects of baroreceptor reflex activation, while the other does not.

The results have also demonstrated the existence of a third inhibitory system which is activated by spinal components of the long-latency vasopressor pathway. The function of this inhibitory system is to control impulse transmission in the short-latency vasopressor pathway. Depressor region stimulation, which blocks transmission in the long-latency pathway, leads indirectly to an inhibition of activity in this control system (i.e., disinhibition), thus removing the regulatory function of the long-latency pathway on the short-latency pathway. Thus, the two vasopressor systems interact reciprocally at the spinal level.

Finally, the data presented allows some speculation with regard to the nature and origin of the neural inhibition responsible for the silent period which follows sympathetic nervous excitation of supraspinal origin.

It was possible to distinguish between two of the four sympathoinhibitory mechanisms studied because of the differential effect of depressor region stimulation on the postganglionic potentials evoked from the short- and long-latency vasopressor pathways. For example, stimulation of

some medullary depressor sites blocked the postganglionic responses elicited by stimulation of the midbrain components of both the short- and long-latency vasopressor pathways. Electrical activation of other depressor sites inhibited transmission only in one or the other of the two vasopressor pathways activated electrically from the midbrain. This suggested that transmission in each of the vasopressor pathways was controlled by a different sympathoinhibitory system.

A. Inhibitory System (I_B "baroreceptor-like") Which Controls Transmission in the Long-Latency Pressor Pathway

Activation of sites within the medial medulla which reduced blood pressure and spontaneously-occurring discharges always inhibited sympathetic nerve responses evoked from spinal, medullary, and midbrain components of the long-latency pressor pathway. In contrast, sympathetic responses evoked from the short-latency pathway were generally enhanced or unchanged during the depressor effect of medial medullary stimulation. At times, however, the short-latency potential evoked from medullary or midbrain pressor sites was blocked by stimulation of a medial medullary site which lowered blood pressure. This resulted from the simultaneous activation of two distinct inhibitory systems in the medial medulla, as will be discussed below.

This inhibitory system, which will be referred to as I_B (baroreceptor-like), functions to control transmission

in the long-latency pressor pathway at a spinal site. This was indicated by the fact that postganglionic potentials evoked from descending spinal tracts of the long-latency pressor pathway were inhibited by stimulation of all depressor sites tested in the medial medulla. Blockade of impulse propagation in the long-latency pressor pathway, produced by depressor region stimulation, occurred at a spinal site central to the preganglionic neuron. This was evident since Taylor and Gebber (1973) have demonstrated that both pressor pathways converge onto the same preganglionic neuron; and the data demonstrated that postganglionic responses evoked from descending spinal components of the short-latency pathway were not blocked by depressor region stimulation.

The inhibitory effect of depressor region stimulation on the long-latency pathway mimicked that produced by baroreceptor reflex activation. Either baroreceptor reflex activation or stimulation of medial depressor sites produced the following: 1) reduced spontaneously-occurring postganglionic discharges; 2) blocked the long-latency potential evoked from all three levels of the neuraxis, and 3) did not block transmission in the short-latency pathway. Gebber et al. (1973) have previously demonstrated that reflex inhibition induced by the pressor action of norepinephrine acted, in part, at a spinal level to control transmission in the long-latency pathway. It is not surprising that baroreceptor reflex-like effects were observed upon

stimulation of the depressor region, in view of reports which have demonstrated that the carotid sinus nerve makes primary and secondary connections with neurons within the medial medulla (Biscoe and Sampson, 1970a; Crill and Reis, 1968; Homma et al.,1970; Humphrey, 1967; Miura and Reis, 1969, 1972). Thus, electrical stimulation of the depressor region most likely activated the central projections of the baroreceptor afferents to produce the baroreceptor reflex-like effect at a spinal level.

However, other investigators have suggested that baroreceptor reflex inhibition of sympathetic nervous discharge occurs primarily at a brain stem locus. The most direct evidence for this has been presented by Koizumi et al. (1971). They found that reflex discharges of spinal origin, recorded from sympathetic white rami, were not inhibited during carotid sinus distension. In contrast, spontaneously-occurring activity and reflex discharges evoked over supra-spinal pathways were inhibited. On the basis of these observations, it was concluded that baroreceptor reflex inhibition of sympathetic nervous discharge occurred mainly at the medullary level. The results presented here and previously by Gebber et al. (1973) do not refute a medullary site of action of the baroreceptor reflexes, but suggest that the conclusions of Koizumi et al. (1971) should be regarded with some caution. Other possibilities may be raised to explain the results of Koizumi et al. (1971): 1) the spinal reflex discharge was mediated over the

interneuronal components of the short-latency vasopressor pathway, which is not under the inhibitory influence of the baroreceptor reflex (Gebber et al.,1973); 2) the baroreceptor-induced inhibition may occur at a spinal site central to the interneuronal components of the pathway mediating the spinal reflex discharge; 3) the spinal reflex discharge may be mediated over a different group of preganglionic neurons from those mediating the long-and short-latency response evoked from the brain stem.

The parallel reductions observed in the amplitudes of spontaneously-occurring activity and the long-latency potentials during depressor region stimulation raises the possibility that both types of sympathetic nerve discharges were mediated over the same central pathway. That is, it appears likely that the long-latency pathway would be responsible, at least under the existing experimental conditions, for basal or spontaneously-occurring discharges in the external carotid nerve. This contention is further supported by the following observations made on the external carotid nerve which indicate that the short-latency pathway is not spontaneously active in the anesthetized cat. First, stimulation of medial medullary sites that failed to inhibit postganglionic potentials evoked from brain stem and spinal levels of the short-latency pathway markedly reduced or eliminated spontaneously-occurring activity on the external carotid nerve. Second, stimulation of some medial medullary sites inhibited the early components of the postganglionic

response evoked from the midbrain without altering spontaneously-occurring discharges.

B. Inhibitory System (I_{NB} "non-baroreceptor-like") Which Controls Transmission in the Short-Latency Pressor Pathway

High frequency stimulation of certain sites within the medial medulla activated a second inhibitory system (I_{NB}) which functions to selectively block transmission in the short-latency vasopressor pathway. This conclusion was based on the results from experiments in which the effects of medial medullary stimulation were tested on the early and late components of the multiple postganglionic discharge evoked, respectively, from the midbrain components of the short-latency and long-latency vasopressor pathways. First, stimulation of some medial medullary sites inhibited the early component and had virtually no effect on the late component of the multiple discharge evoked from the midbrain. Second, electrical activation of these same sites in the medial medulla had little effect on blood pressure or spontaneously-occurring postganglionic discharges. Third, transmission in the long-latency pathway was inhibited only when stimulation of sites in the medial medulla produced a depressor response. The majority of the sites activated in the medial medulla blocked both components of the midbrain multiple discharge, which suggested that two inhibitory systems (I_B and I_{NB}) were activated simultaneously. This is not surprising since the two

sympathoinhibitory systems were evoked from widely overlapping sites distributed throughout the medial medulla (Fig. 12).

The second sympathoinhibitory system (I_{NB}) blocks transmission in the short-latency vasopressor pathway at a supraspinal site. This was indicated by the contrasting effects of medial medullary stimulation on the sympathetic nerve responses evoked from the midbrain and cervical spinal components of the short-latency pressor pathway. More than 50% of the sites tested in the medullary depressor region inhibited the early components of the multiple discharge elicited from the midbrain. In contrast, medial medullary stimulation never reduced the sympathetic nerve responses evoked from descending spinal tracts of the short-latency pathway. Thus, inhibition of transmission presumably occurred at a site between the midbrain and cervical spinal components of the short-latency vasopressor pathway.

The effects of medial medullary stimulation on the short-latency responses evoked from the medulla occasionally were reversed from enhancement to blockade by small movements of the electrode in the pressor region. This observation suggests that inhibition of transmission in the short-latency pathway may occur in the lower brain stem. The fact that the short-latency response evoked from the medulla was only inhibited in six instances would suggest that the short-latency potentials were being evoked caudal to the site of inhibition in the majority of the experiments.

Unlike the first sympathoinhibitory system which controls transmission in the long-latency pathway, the inhibitory effect of the second sympathoinhibitory system on the short-latency nerve response was not mimicked by baroreceptor-induced inhibition. Stimulation of certain medial medullary depressor sites inhibited the early components of the midbrain response and the short-latency postganglionic potential evoked from the medulla. These same early postganglionic discharges were not inhibited during baroreceptor reflex activation. In agreement with the previous reports by Gebber et al. (1973), transmission in the short-latency pressor pathway was not inhibited by baroreceptor reflex activation. Thus, blockade by depressor region stimulation of transmission in the short-latency pathway resulted from activation of a sympathoinhibitory system of non-baroreceptor origin. This supports the view that the inhibitory function of the depressor region does not merely involve mediation of the baroreceptor reflex (Alexander, 1946; Bard, 1960; Coote et al., 1969; Johansson, 1962; Lofving, 1961a). Nevertheless, the peripheral and/or central inputs which coordinate the activity of this second inhibitory system remain to be defined.

C. Enhancement of Transmission in the Short-Latency Pressor Pathway: A Disinhibitory Phenomenon?

The following observations suggest that another control system may be acting to regulate transmission in the

short-latency pressor pathway. First, stimulation of many sites in the medullary depressor region, as well as baroreceptor reflex activation, enhanced the amplitude of postganglionic potentials evoked from the brain stem and spinal components of the short-latency pathway at a time when basal discharges and the long-latency postganglionic response were reduced. Second, the amplitude of the short-latency potential was enhanced when evoked during the peak depression of spontaneously-occurring discharge (silent period), which follows sympathetic excitation. Third, the short-latency potential evoked from the medulla was reduced when basal discharges in the long-latency pathway were reflexly increased following the administration of mecamylamine, which lowered blood pressure. Fourth, intensity response curves revealed the proportionality of the inverse relationship between the degree of enhancement of the short-latency responses elicited from the medulla, and inhibition of spontaneously-occurring discharges and the long-latency postganglionic potentials. The reciprocal effects noted on the short- and long-latency responses could have represented independent actions of the depressor region on the two vasopressor systems. This explanation is unlikely, however, in view of the proportionality of the intensity-related reciprocal actions of depressor region stimulation. Alternatively, the possibility may be raised that transmission in the short-latency pathway is influenced by the level of spontaneously-occurring discharges in the long-latency

pathway. Thus, an interaction between the two pressor pathways most likely exists.

Enhancement of the short-latency potential was observed when it was evoked from descending spinal tracts of the short-latency pathway, and when recordings were made from the preganglionic cervical sympathetic trunk. Thus, it can be concluded that the interaction between the two vasopressor systems occurred at a spinal locus.

Several possible mechanisms may be entertained in describing the spinal interaction between the two vasopressor pathways. First, the facilitation of transmission in the short-latency pathway may be the result of deocclusion, since both pressor pathways converge upon the same preganglionic neuron (Taylor and Gebber, 1973). However, Taylor and Gebber observed that the probability of discharge of a relatively fixed latency unit response was increased during depressor region stimulation, independent of whether or not the preganglionic neuron exhibited basal discharges. According to these investigators, the relatively fixed unit response monitored the activation of the short-latency pressor pathway. This rules out deocclusion at the level of the preganglionic neuron as the mechanism responsible for the facilitatory effect. However, the phenomenon of deocclusion might occur at an interneuronal site. This could only be investigated by single unit recording from such interneurons.

Alternatively the possibility may be raised that the facilitatory effect on transmission in the short-latency pathway involves the removal of an existing inhibition (i.e., disinhibition). Normally, basal discharges in the long-latency pathway would activate a spinal inhibitory interneuron which controls impulse transmission in the short-latency pathway. Enhancement of the short-latency response would result indirectly as the consequence of selective inhibition of impulse transmission in the long-latency pathway, thereby eliminating the inhibitory effect of the spinal interneuron. This occurred during baroreceptor reflex activation, depressor region stimulation, which activated the I_B system, and the silent period, which follows sympathetic excitation.

The spinal inhibitory interneuron which controls transmission in the short-latency pathway may be activated by collateral fibers which arise from the preganglionic neurons of the long-latency pathway. This would be similar to the recurrent collateral pathway which activates the Renshaw cell to control the discharge pattern of α motoneurons. However, the following observations indicate that a recurrent collateral pathway is not involved in this system: 1) dihydro-β-erythroidine, mecamylamine and strychnine, which are known to block Renshaw-mediated inhibition of motoneurons (Eccles et al., 1954a), failed to alter the enhancement of the short-latency response during depressor region stimulation. 2) Taylor and Gebber (1973)

have recently reported that both vasopressor pathways converge on the same preganglionic neuron. 3) Rethelyi (1972) failed to observe axonal collateral branches of preganglionic sympathetic neurons, and Fernandez DeMolina et al. (1965a) could not demonstrate the existence of a preganglionic sympathetic recurrent inhibitory system in electrophysiological experiments.

A more likely system leading to the activation of the spinal inhibitory interneuron involved in the interaction between the two vasopressor pathways is depicted in Figure 20. Axon collateral fibers arising from neural components of the long-latency pathway (LL spinal) would activate spinal inhibitory interneurons (I), which converge on spinal neural components of the short-latency pathway (SL spinal) to block transmission in the short-latency system. Inhibition of impulse transmission in the long-latency pressor pathway at a site central to the collateral axonal pathway would deactivate the inhibitory interneuron (i.e., disinhibition), and thereby facilitate transmission in the short-latency pressor pathway.

The relationships between the other two sympathoinhibitory systems identified by medial medullary depressor region stimulation and the two excitatory vasopressor pathways are also depicted in Figure 20. Inhibitory system I_B (baroreceptor-like), activated by depressor region stimulation, acts at a spinal site to block transmission in the long-latency pressor pathway. As stated above, the site

Figure 20. Diagram of the relationship between the three sympathoinhibitory systems identified by medial medullary stimulation and the two excitatory vasopressor pathways.

Open circles represent excitatory synapse. Closed circles represent inhibitory synapse. B: baroreceptor afferent input. I_B: inhibitory system of baroreceptor origin which controls transmission in the long-latency pathway at a spinal locus. I_{NB}: inhibitory system of non-baroreceptor origin which controls transmission in the short-latency pathway at a medullary locus. I: spinal inhibitory interneuron representing the interaction between the two vasopressor pathways. LL hypo, LL med, LL spinal: hypothalamic, medullary and spinal components of the long-latency vasopressor pathway. SL hypo, SL med, SL spinal: hypothalamic, medullary and spinal components of the short-latency vasopressor pathway. Pre: cervical sympathetic preganglionic neuron. Details are described in the text.

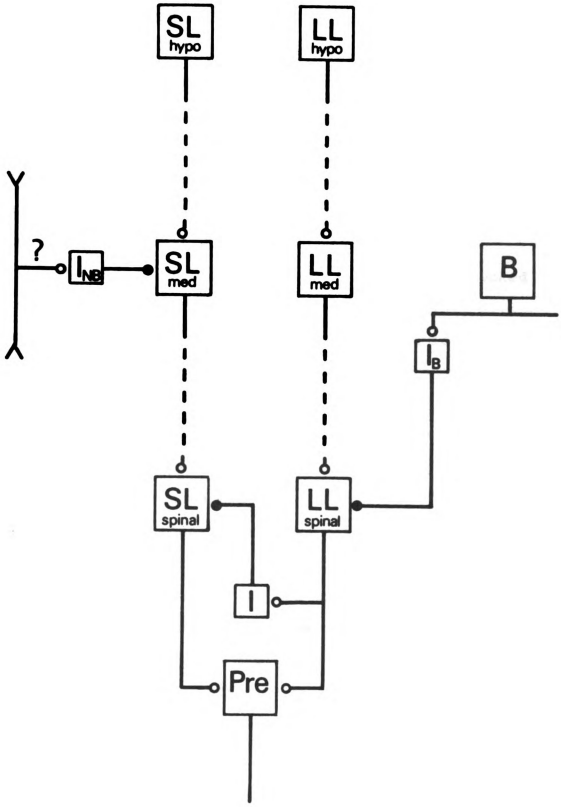

Figure 20

of this inhibition is central to the collateral axonal pathway responsible for the spinal interaction and, therefore, is central to the preganglionic neuron (Pre). Since the inhibitory actions of this system mimicked those produced by baroreceptor reflex activation, it is proposed that depressor region stimulation resulted in the activation of central neural elements of the baroreceptor reflex arc. Therefore, this system (I_B) must have peripheral input from the baroreceptor afferent (B).

The second sympathoinhibitory system (I_{NB}) evoked by medial medullary stimulation acts at a medullary site to selectively block transmission in the short-latency pathway (SL med). Activation of this inhibitory system was not mimicked by baroreceptor reflex activation. Therefore, this inhibition was of non-baroreceptor origin. The central and peripheral inputs to this second control system require further investigation.

The significance of the two vasopressor pathways and the spinal interaction in the normal unanesthetized cat is a matter of speculation at this time. Since these two excitatory pathways are both vasoconstrictor in function, they may play a role in the tonic and phasic control of blood pressure. As stated above, the long-latency pathway appears to be responsible for the generation of basal sympathetic nervous discharge. Sympathetic nervous activity in the short-latency pathway is not associated with spontaneous activity in the anesthetized or unanesthetized state.

Presumably, it is activated in the unanesthetized animal by specific behavioral cues. When this occurs, transmission is further enhanced in the short-latency pathway by baroreceptor induced inhibition of nervous impulses in the long-latency pressor pathway via the spinal interaction. In this way, baroreceptor reflex activation acts as a positive, rather than negative, feedback system specifically for the short-latency pathway.

This mechanism may play a role in the redistribution of blood flow during emotional stress. The central nervous system has the ability to impose local or regional control on blood flow (Lofving, 1961a; Peiss, 1965). Lofving (1961a) has demonstrated that stimulation of different sites in the pressor area of the subcallosal area produced differential effects on muscle and intestinal blood flow. Stimulation of some pressor sites had little effect on muscle blood flow resistance and increased intestinal blood flow resistance over 50%. Stimulation of other pressor sites produced opposite effects on blood flow to the two vascular beds. Further, sympathetic vasoconstrictor control to certain cutaneous vascular beds is relatively insensitive to baroreceptor reflex inhibition (Lofving, 1961b; Folkow and Neil, 1971). Thus, selective activation of the short-latency pathway may explain the blush versus blanch response in humans under different emotional stresses. However, to date, the two pressor systems, which

are related differently to the baroreceptor reflex arc, have only been identified in the cat (Gebber et al., 1973).

D. Silent Period

The significance of the sympathetic silent period has been recently reviewed by Koizumi and Brooks (1972). They concluded that the silent period is responsible for the diverse vasomotor responses produced by stimulation of somatic afferents at varying intensities and frequencies. At low rates of stimulation of myelinated afferents, the silent periods tend to fuse in such a manner that there is a diminution of total sympathetic discharge. This augmentation of the silent period is represented as a depressor response, indicating peripheral vasodilatation. Pressor responses are observed at stimulus parameters which predominantly increase sympathetic nervous discharges above the basal level. Thus, the inhibition represented by the silent period modulates the discharge pattern of sympathetic neurons.

True neural inhibition is a major factor involved in the genesis of the silent period. This was indicated by the fact that it was possible to set the interval between the conditioning and test stimuli applied to a medullary pressor site so that the test discharge, but not the attendant silent period, was blocked. Thus, the splanchnic nerve discharge could be dissociated from the silent period. Koizumi et al. (1968) and Iwamura et al. (1969a) came to similar conclusions in experiments in which the condition and test stimuli were applied to somatic afferent nerves.

The present study further demonstrated that the silent period represents a selective inhibitory control on transmission in the long-latency pressor pathway. The amplitude of the short-latency response was enhanced, whereas the long-latency potential was blocked when evoked during the silent period elicited by a conditioning stimulus applied to the medullary pressor region. Since both short-and long-latency pressor systems converge on the same preganglionic neuron (Taylor and Gebber, 1973), it is clear that, in contrast to the conclusions of Polosa (1967), post-firing depression of the excitability of the preganglionic neurons (positive after-potential) plays little if any role in the genesis of the silent period.

Questions relating to the mechanism and site of initiation of the silent period, which followed sympathetic nervous excitation, were approached primarily by comparing the excitability-recovery curves of splanchnic nerve discharges elicited by stimulation of various sites in the central nervous system. This approach was justified, since the time course of depression of a test splanchnic nerve discharge following a conditioning response corresponded to that of the silent period produced by the conditioning stimulus. This was demonstrated when the conditioning and test stimuli were applied to the medulla or spinal cord. Similar results have been reported when conditioning and test stimuli were applied to somatic afferent nerves (Coote and Perez-Gonzalez, 1970; Iwamura et al., 1969a; Koizumi

et al., 1968; Sato and Schmidt, 1971; Schmidt and Schonfuss, 1970). With this method, the phenomenon of the silent period could be studied in situations in which spontaneously-occurring sympathetic nervous activity was absent, such as following C-1 transection.

Using the excitability-recovery curves, two observations indicated that the inhibition of spontaneously-occurring discharge (the silent period) occurred at a spinal synapse. First, the test splanchnic nerve discharge evoked by stimulation of descending tracts in the midcervical spinal cord was depressed following conditioning stimuli applied to the medullary pressor region. The time course of depression of the test response was the same as that observed for the test splanchnic nerve discharge evoked by medullary stimulation. Second, the test splanchnic nerve discharge evoked by stimulation of descending midcervical spinal tracts was depressed following conditioning stimuli applied to the same spinal site. The time course of depression of the test response, either before or after C-1 transection, was the same as that observed when the conditioning and test stimuli were applied to the medulla.

It could be questioned whether depression of the splanchnic nerve discharge elicited from the spinal cord by conditioning stimuli applied to the medullary pressor region provides sufficient evidence to conclude that the silent period was initiated in the spinal cord. The depression of the test response evoked by spinal

stimulation may have resulted from the removal of the facilitatory subliminal fringe produced by spontaneously-occurring sympathetic impulses on the transmission of electrically-evoked activity at spinal synapses. If this were the case, the subliminal fringe produced by spontaneously-occurring discharges would be important in the initiation of sympathetic discharge in the preganglionic neuron. Furthermore, it would be difficult to decide whether the inhibition of spontaneously-occurring discharges (silent period) had occurred at a synapse in the brain or spinal cord. However, the maximum depression of the splanchnic nerve discharge evoked from the spinal cord by conditioning stimuli applied to the medulla was significantly greater than the reduction of the test response produced by C-1 transection. Transection of the spinal cord reduced spontaneously-occurring discharges of the splanchnic nerve to the level observed during the peak of the silent period. It should also be stressed that the test splanchnic nerve discharge was depressed to the same extent before and after C-1 transection in experiments in which the conditioning and test stimuli were applied to the mid-cervical spinal cord.

The existence of a supplementary brain stem site, at which inhibition responsible for the silent period may be exerted, should be considered since the conditioning spinal stimuli were not as effective in depressing the test splanchnic nerve discharge elicited from the spinal cord,

as were conditioning medullary stimuli in depressing the test response evoked from the medulla (Fig. 18). However, this possibility is unlikely since conditioning medullary stimuli were almost equally effective in depressing the test splanchnic nerve responses evoked by stimulation of descending spinal pressor tracts and the medullary pressor region (Fig. 17). Thus, there is little question that the inhibition responsible for the silent period occurred at a spinal synapse. The experimental approach in which conditioning and test stimuli were applied to brain stem and spinal pressor sites resolved the controversy (Sato and Schmidt, 1973) regarding the site at which sympathoinhibition (silent period) occurs.

The spinal inhibition responsible for the silent period does not interrupt transmission in the long-latency pressor pathway at the level of the preganglionic neuron. This was indicated since: 1) the short-latency response evoked from the medulla was enhanced, whereas the long-latency response was inhibited when evoked during the silent period which followed excitation of the long-latency pressor pathway at the medullary level; and 2) impulses from both pressor systems activate the same preganglionic neuron (Taylor and Gebber, 1973).

A number of possibilities arise concerning the pathway taken from the medullary pressor region by impulses which generate the silent period in the spinal cord. First, separate inhibitory and excitatory pathways may project

directly from the dorsolateral pressor region to the spinal cord. This seems improbable, however, since the splanchnic nerve discharge evoked from the medulla or spinal cord was always followed by a silent period. If separate pathways did mediate the sympathetic discharge and silent period, it would be highly coincidental if they were anatomically adjacent at each and every site stimulated in the medulla and spinal cord. In addition, sympathoinhibitory systems comprise the medial regions of the medulla and descending inhibitory tracts are distributed in the ventrolateral areas of the spinal cord (Illert and Gabriel, 1972; Illert and Seller, 1969). In this study, histological verification showed that the sites of stimulation were localized in the periventricular gray and underlying dorsolateral reticular formation of the medulla and the dorsolateral white column of the spinal cord (Fig. 19). Thus, it is highly unlikely that separate sympatho-excitatory and inhibitory pathways were activated simultaneously.

A more likely possibility for the mediation of the silent period involves a feed-forward inhibitory pathway. Spinal collateral branches of excitatory reticulospinal fibers of the long-latency pathway descending from the medulla would excite inhibitory interneurons which block transmission in spinal neural elements of the excitatory long-latency pathway. Inhibition initiated in this manner would be similar to that of α motoneurons induced by inhibitory interneurons excited by Ia afferent fibers from

skeletal muscle (Eccles et al., 1954b, 1956, 1960). This scheme is particularly attractive since it would explain why the splanchnic nerve discharge elicited by stimulation of the medulla or spinal cord was always followed by a silent period. Likewise, a feed-forward inhibitory pathway would explain how the silent period could appear in those instances when the test discharge was blocked following the application of conditioning stimuli to the medulla. Inhibition of excitatory transmission would occur at a site downstream from where the collateral branches of reticulospinal neurons excited the inhibitory interneurons.

The time course of depression of a test splanchnic nerve response evoked from the spinal cord was virtually the same when conditioning stimuli were applied to either brain or spinal sites (Figs. 17, 18). The fact that C-1 transection failed to significantly alter the degree or time course of depression of the test splanchnic nerve response when conditioning and test stimuli were applied to the same spinal site indicates that the functional integrity of higher brain stem structures is not required for the initiation of a silent period. However, conditioning medullary stimuli were more effective than conditioning spinal stimuli with regard to the degree of depression of the test response evoked from the spinal cord (Figs. 17, 18). This would suggest that the silent period evoked from the medullary pressor region involves more than the simple spinal feed-forward inhibitory system described

above. In view of this observation, the question may be raised as to whether the participation of sympathoinhibitory systems of the medial medulla may explain how conditioning medullary stimuli were more effective than conditioning spinal stimuli in depressing the test response evoked from the spinal cord. In regard to this, Iwamura et al. (1969a) reported that lesions placed in the ventral portions of the cat medullary depressor region attenuated the sympathetic nervous silent period elicited by stimulation of a somatic afferent nerve. This occurred without a significant change in the sympathetic nervous discharge of supraspinal origin which preceded the silent period.

Sympathoinhibitory systems of the medial medulla may play a subordinate role in the generation of the silent period evoked from the medulla through a second feed-forward inhibitory system which connects the medullary pressor and depressor regions. Axon collaterals which interconnect reticulospinal neurons have been identified in the medulla (Brodal, 1957). Yet, when conditioning stimuli were applied to the medulla, the time course and degree of depression was virtually the same for the test responses evoked from either medulla or spinal cord. This observation suggests that the inhibition which was exerted by sympathoinhibitory systems of the medial medulla in the generation of the silent period evoked from the medulla occurred at a spinal synapse, perhaps by the activation of the same inhibitory interneuron linked to the spinal

feed-forward system described above. Related to this, inhibition of sympathetic nervous discharge produced by reflex or electrical activation of the depressor region of the medial medulla is mediated at least in part at a spinal locus (Coote et al.,1969; Gebber et al.,1973; Gootman and Cohen, 1971; Kirchner et al.,1971; Sato, 1972b).

In fact, the reticulospinal inhibitory pathway of the I_B (baroreceptor-like) system described above may be mediating the supraspinal component of the silent period evoked by medullary stimulation. This was indicated by the following observations: 1) the I_B system functions to inhibit transmission in the long-latency pressor pathway at a spinal site; 2) short trains of 3 pulses applied to sites in the depressor region, from which the I_B system was activated, produced a long-lasting depression of spontaneously-occurring discharges without a preceding excitation.

It appears that the silent period results from an interaction between sympathetic excitatory and inhibitory neurons. Activation of excitatory neural elements leads to the activation of a sympathetic inhibitory system which, in turn, functions to control the discharge pattern of the excitatory pathway. It is proposed that the interaction between the excitatory and inhibitory neural elements occurs through two feed-forward inhibitory systems which result in the silent period being generated at a spinal level. The summary diagram illustrated in Figure 21 represents the

Figure 21. Diagram of the proposed two feed-forward inhibitory pathways involved in the generation of the sympathetic silent period

Open circles represent excitatory synapse. Closed circles represent inhibitory synapse. B: baroreceptor afferent input. I_B MDR: inhibitory system of baroreceptor origin within the medullary depressor region. LL MPR: long-latency vasopressor system within the medullary pressor region. SI_e: spinal excitatory interneuron of the long-latency pressor pathway. SI_i: spinal inhibitory interneuron responsible for the silent period. PRE: cervical sympathetic preganglionic neuron. Details are described in the text.

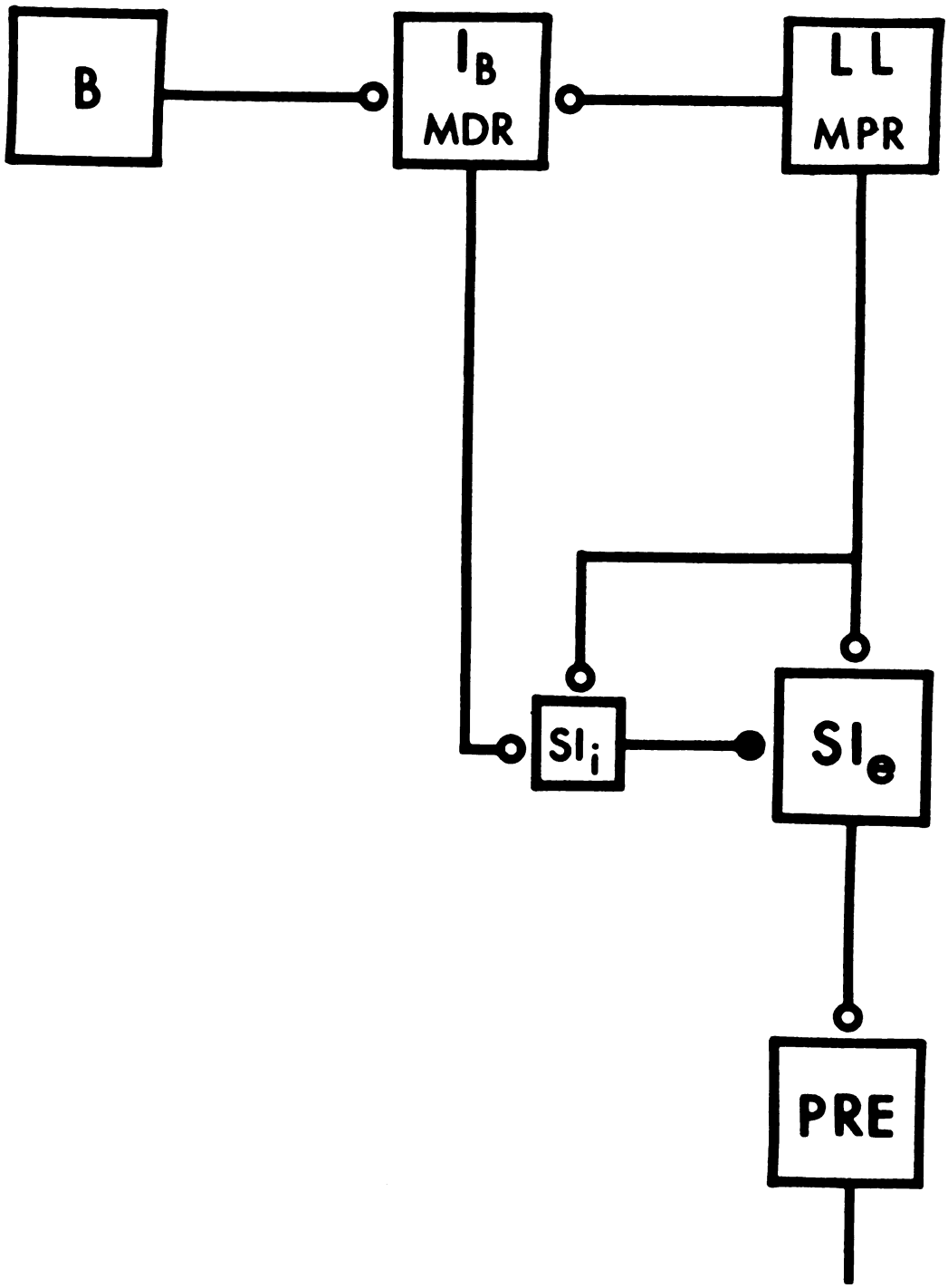

Figure 21

proposed model of the two feed-forward inhibitory systems which may be involved in the generation of the sympathetic silent period. Sympathetic nervous impulses descending the long-latency pressor pathway activate spinal inhibitory interneurons via axon collateral fibers arising from spinal neural elements of the excitatory pathway. The interneuron, in turn, acts in a forward direction to block transmission caudal to the site from which the collateral fiber arose.

The second feed-forward system (Fig. 21) involves the subordinate role played by the sympathoinhibitory systems of the medial medulla. Axon collateral fibers arise from medullary neural elements of the long-latency pathway which converge on descending spinal inhibitory neurons in the medial medulla. The inhibitory reticulospinal pathway may excite the same inhibitory interneuron synapsed upon by the spinal feed-forward system, or may act directly on spinal components of the excitatory system to block transmission.

SUMMARY

This investigation demonstrated that four distinct sympathoinhibitory systems function to control central sympathetic vasoconstrictor outflow in the cat. Three sympathoinhibitory systems were identified by stimulation of the medial medullary depressor region. The fourth inhibitory system studied was the sympathetic silent period or depression of spontaneously-occurring discharge following sympathetic excitation.

Sympathetic vasopressor outflow from the brain to the external carotid nerve is distributed over two systems of excitatory pathways (Gebber et al., 1973). These two systems were composed of the long-latency pressor pathway which was under the inhibitory influence of the baroreceptor reflex arc and the short-latency pressor pathway which was not under the reflex control of the baroreceptor afferents. Sympathoinhibitory systems of the medial medullary depressor region which influence vasoconstrictor function were identified, using this model which describes the central organization of the sympathetic vasopressor system.

Activation of sites in the medial medulla which reduced blood pressure and spontaneously-occurring postganglionic discharges recorded on the external carotid nerve always

inhibited long-latency potentials evoked from midbrain, medullary and descending spinal components of the long-latency pressor pathway. In contrast, sympathetic nerve responses evoked from descending spinal components of the short-latency pressor pathway were not inhibited by depressor region stimulation. These effects of depressor region stimulation were mimicked by baroreceptor reflex activation. These observations suggested that activation of a sympathoinhibitory system of baroreceptor reflex origin in the medial medulla selectively blocked transmission in the long-latency pathway at a spinal locus.

Stimulation of many sites in the depressor region inhibited the short-latency responses evoked only from midbrain or medullary components of the short-latency pressor pathway. In contrast, baroreceptor reflex activation failed to reduce these same short-latency responses. This indicates that a second sympathoinhibitory system of non-baroreceptor origin controls transmission in the short-latency pressor pathway at a supraspinal locus.

Baroreceptor reflex activation and depressor region stimulation produced reciprocal effects on the amplitude of the pre- or postganglionic nerve responses evoked from brain stem or descending spinal components of the two vasopressor pathways. Sympathetic nerve responses evoked from the long-latency pathway were blocked, whereas those evoked at submaximal intensity from the short-latency vasopressor pathway were enhanced. Intensity-response curves revealed

that the degrees to which the short-and long-latency potentials changed were related similarly to the intensity of depressor region stimulation. It was suggested that the facilitatory effect on transmission in the short-latency pressor pathway was the result of the removal of an existing spinal inhibition (i.e.,disinhibition) which was modulated by the basal sympathetic discharge in the long-latency pressor pathway. Thus, a spinal interaction between the two vasopressor pathways was identified as the third control system.

The silent period, or depression of spontaneously-occurring sympathetic discharge following splanchnic nervous excitation produced by the stimulation of brain stem or spinal cord, was the fourth control system studied in this investigation. Experiments supported the view that true neural inhibition is a major factor involved in the genesis of the silent period. This was evident, since it was possible to set the interval between conditioning and test stimuli so that the test splanchnic discharge, but not the attendant silent period, was blocked. Thus, the silent period could be dissociated from the test discharge.

Excitability-recovery curves of the test splanchnic nerve response evoked by stimulation of medullary and spinal pressor sites were compared to determine the site at which the inhibition mediating the silent period occurred. This approach was justified since the time course of depression of a test splanchnic nerve discharge, following a

conditioning response, corresponded to that of the silent period produced by the conditioning stimulus. The time course of depression of the test splanchnic nerve response evoked from descending tracts in the midcervical spinal cord was virtually the same, independent of whether conditioning stimuli were applied to either brain or spinal sites. C-1 transection failed to alter the degree or time course of depression of the test splanchnic nerve response when conditioning and test stimuli were applied to the same spinal site. It was suggested that the inhibition mediating the silent period occurred at a spinal synapse through a feed-forward pathway which did not require the functional integrity of higher brain stem structures. However, additional experiments suggested that a subordinate role was played by sympathoinhibitory systems of the medial medulla in the generation of the silent period produced by stimulation of medullary pressor sites. The subordinate effect of the medial medulla was also exerted at a spinal locus through a second feed-forward pathway.

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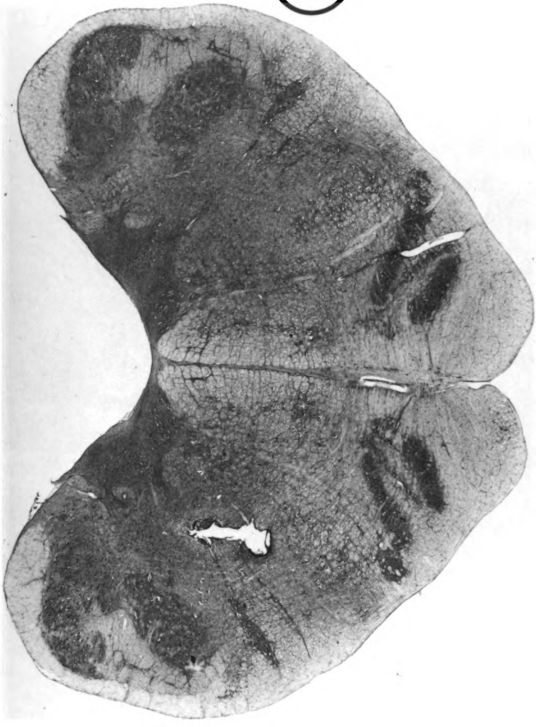
APPENDICES

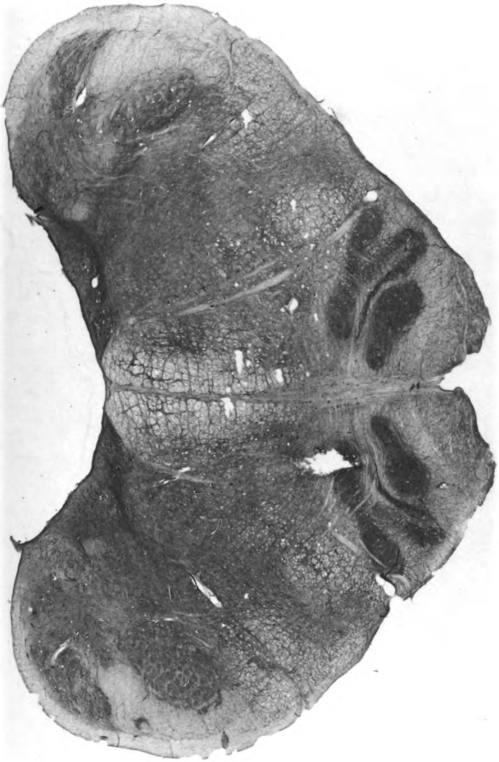
Appendix A. Photomicrograph showing location of concentric electrode in lateral pressor region of the medulla

The lesion in the lateral area of nucleus reticularis ventralis was made with direct current of 2.0 mA for 10 sec. The nucleus was approached from the ventral surface of the brain. This section corresponds to a level at approximately P12.



Appendix B. Photomicrograph showing location of concentric electrode in medial depressor region of the medulla.

The lesion, near the inferior olive, was made with direct current of 2.0 mA for 10 sec. The site was approached from the ventral surface of the brain. This section corresponds to a level at approximately P10.



Appendix C. Photomicrograph showing location of concentric electrode in dorsolateral region of the midcervical spinal cord.

The lesion was made with direct current of 2.0 mA for 10 sec. The site was approached from the dorsal surface.