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Ascending Spinal Pathways for the Somatosympathetic Reflex

Jin Mo Chung
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ASCENDING SPINAL PATHWAYS FOR THE
SOMATOSYMPATHETIC REFLEX

by

Jin Mo Chung

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

June
1977
Dedicated to my parents,

Mom and Dad
ACKNOWLEDGEMENTS

I would like to take this opportunity to express my sincere appreciation to my advisor, Dr. Robert D. Wurster, for his guidance and direction throughout the graduate study. With his exceptional teaching skill, he guided me in developing as an independent and creative researcher. He had to demonstrate much patience and effort with me because of my language barrier and lack of background.

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personnel, including the secretarial staff. Mr. David L. Gudewicz was always ready to help me in electronics. Mr. George E. Barratt prepared some of the figures. Mr. David E. Euler and G. Steven Geis never hesitated helping me with English and their discussion helped to develop this work.
VITA

Jin Mo Chung was born on April 1, 1945, in Seoul, Korea. In 1948, he moved to Kang-Kyung, South Choong-Chung Province, where he spent most of his young life. He attended Sogang University, Seoul, Korea, from 1963 to 1967, and graduated with a Bachelor of Science Degree in Physics. He joined the Korean Air Force and served as a Communications Officer from 1967 to 1971. He returned to Sogang University and worked as an assistant in the Department of Physics from 1971 to 1972.

In September, 1972, Jin Mo Chung came to the United States and entered the Department of Physiology, Loyola University, Maywood, Illinois, where he studied under the direction of Dr. Robert D. Wurster. He was supported by a Basic Science Fellowship from 1972 to 1976 and Arthur J. Schmitt Doctoral Fellowship from 1976 to 1977.

In December, 1972, he married Kyungsoon Chang who is a graduate student, Department of Anatomy, Loyola University, Maywood, Illinois.

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CHAPTER I

INTRODUCTION

The somatosympathetic reflex is a reflex change in sympathetic nerve activity during afferent nerve stimulation. The term has been used loosely and some visceral as well as somatic afferent fibers are included in afferent nerve stimulation.

Stimulation of afferent fibers can elicit pressor or depressor responses depending upon the intensity (55, 69) and frequency (32) of stimulation. Historically, two major theories have been proposed to explain these different blood pressure responses. Hunt (38) suggested that stimulation of some afferent nerve fibers gave rise to pressor responses while stimulation of others resulted in depressor responses. Thus, blood pressure responses were determined by the number and type of afferent fibers activated. On the other hand, Ranson and associates (70, 71, 72, 74) proposed that these different blood pressure responses were not due to activation of two different fiber types. They suggested that different intensities or frequencies of stimulation of the same afferent fiber types selectively activate a distinct ascending spinal pressor or depressor
pathway due to the characteristics of each pathway. The former theory has been tested and is generally supported by recording blood pressure and sympathetic nerve activity (43, 50, 82). However, the concept of dual ascending spinal pathways has received little attention despite the fact that the above two theories are not necessarily mutually exclusive. That is, different afferent nerve fibers may excite separate ascending spinal pathways.

Schmidt and Weller (85) elicited a short latency somatosympathetic reflex when afferent A fibers in a peripheral nerve were stimulated. In addition, a long latency reflex was present when they stimulated both A and C fiber groups. They concluded that the activation of afferent A fibers elicited the short latency reflex (sympathetic A reflex) while C fiber activation was responsible for the long latency reflex (sympathetic C reflex). Koizumi et al. (51) also reported a similar result of sympathetic A and C reflexes. They further concluded that the sympathetic A reflex is responsible for the depressor response, because the brief excitation was followed by a prolonged inhibition of spontaneous sympathetic activity (silent period). On the other hand, activation of the sympathetic C reflex produced a pressor response because there was no silent period after the excitation.

The purposes of this study were (1) to test the concept of dual ascending spinal pathways for blood pressure
changes during afferent nerve stimulation; (2) to investigate these possible pathways using neurophysiological techniques; (3) to localize the ascending spinal pathways for the somatosympathetic A and C reflexes; (4) to test the possibility of combining the concepts of Hunt's afferent nerve specificity and Ranson's spinal pathway specificity to form a new theory for blood pressure determination during afferent nerve stimulation.
CHAPTER II

LITERATURE REVIEW

A. History of the Somatosympathetic Reflex

Somatosympathetic reflexes were initially observed by recording cardiovascular responses by the Ludwig school (reviewed in 43, 58 and 82). They observed blood pressure changes during electrical stimulations of limb nerves. Based on observations after a series of brain lesions, they concluded that the blood pressure changes were due to a reflex coursing up the spinal cord to the medulla oblongata and back to the sympathetic neurons.

Hunt (38) observed that depressor responses were obtained when weak stimuli were applied to the central end of a mixed nerve, while pressor effects occurred at higher stimulus intensities. On the basis of these findings he suggested that the peripheral nerves contain two types of afferents, depressor and pressor fibers, which differed in their excitability by electrical stimuli.

Hunt's findings were confirmed by Martin and Lacey (55) using more quantitative stimuli. After stimulating many different nerves, they concluded that a reflex drop of blood pressure occurred with stimulus intensities which
were approximately threshold for a spinal reflex from skeletal muscle. The threshold for the pressor reflex was at least 20 times the threshold intensity for reflex blood pressure drop.

Hunt's concept of pressor and depressor afferent fibers was criticized by Ranson and co-workers (70, 71, 72, 74) who proposed that the different blood pressure responses were attributed to differences in the organization of the spinal pressor and depressor pathways. Furthermore, they localized the pressor and depressor pathways in the dorsolateral sulcus area and lateral funiculus of the spinal cord, respectively (details in section D3).

Gruber (32) was first to demonstrate that not only intensity but also rate of stimulation affects the reflex blood pressure responses. He found that with the same strength of stimulus depressor and pressor responses were obtainable by varying the rate of stimulation from 1 to 20 Hz. This rate sensitivity did not support Hunt's hypothesis for different rates of stimulation must somehow activate different components in the central nervous system.

Nevertheless, Hunt's postulate of specific pressor and depressor afferents in somatic nerves was widely accepted and gained experimental support from many studies. One notable study was carried out by Gordon (31). Application of cocaine to sciatic nerve fibers selectively blocked the pressor reflex, while depressor reflex was
blocked by asphyxia. He suggested that small diameter, unmyelinated fibers which are more susceptible to the cocaine block, were responsible for the pressor reflex; while larger diameter, myelinated fibers, which are more susceptible to asphyxia, were responsible for the depressor reflex. In addition, increasing the frequency of stimulation without altering the intensity reversed the depressor response to a pressor response. Consequently, he concluded that variations in both afferent fiber types and central nervous system mechanisms determined the cardiovascular responses to afferent nerve stimulation.

As briefly reviewed above, the importance of both afferent nerves and the central nervous system in the somatosympathetic reflex were appreciated. However, up to the 1940's all studies performed were based on cardiovascular effects of the peripheral nerve stimulations. The neurophysiological approach to the study of the somatosympathetic reflex began after Adrian et al. (1, 2) recorded action potentials from sympathetic nerves and Alexander (4) recorded somatically-evoked sympathetic reflexes. In the 1960's rigorous investigations of the somatosympathetic reflexes were started using advanced neurophysiological techniques.

B. Background

1. Spontaneous Activity of Sympathetic Neurons and the Silent Period.
The tonic or spontaneous discharges of the sympathetic nerves supplying blood vessels has been known since the mid 1800's (reviewed in 58). In the 1930's Bronk and associates first recorded single unit spontaneous activity from the cervical sympathetic trunk and inferior cardiac nerves (8) of cats. The rates of discharge at rest ranged from less than one to several Hz but these increased to 10-20 Hz during intense asphyxia (9).

Recently, Kaufman and Koizumi (46) made single unit recordings from the lumbar white rami of cat with vagus and carotid sinus nerves intact. The average discharge frequency of 27 spontaneous units ranged from 0.1-9 Hz and averaged 1.4 Hz. Sato (76) also made single unit recordings from 76 spontaneously active units of lumbar white rami of cats whose vagi and carotid sinus nerves were sectioned. Spontaneous discharges ranged from 0.1 to 4.0 Hz, with a mean of 2.1 ± 1.4 (S. D.) Hz. Jäning and Schmidt (42) recorded from the cervical sympathetic trunk and found an average discharge rate of 1.7 Hz in case of myelinated fibers and 2.9 Hz in unmyelinated fibers.

Not all sympathetic nerve fibers are spontaneously acting. Jäning and Schmidt (42) recorded over 500 units from the cervical sympathetic trunk; each unit was identified by direct stimulation. About 70% of these units were not spontaneously active and did not respond to afferent nerve stimulation, whereas 25% had both properties.
The other 5% had either one or the other property. They further concluded that a sympathetic unit showing reflex discharges usually is spontaneously active and vice versa.

Koizumi et al. (54) recorded and compared the patterns of sympathetic spontaneous discharges in the white rami at T1-2, T10-11, L1-2 spinal level and in the cervical sympathetic nerve. Spontaneous discharges recorded from preganglionic fibers at various levels of sympathetic outflow were similar.

Much of the spontaneous discharge of sympathetic fibers is apparently driven by the medulla oblongata, since this activity is significantly reduced by severance of the spinal cord from the medulla. In 1871 Owssjannikow (reviewed in 5 and 58) described the cardiovascular effects of serial transections of the brainstem in the cat and rabbit. He showed that blood pressure remained relatively stable until a section was made through the rostral medulla which caused a marked fall in blood pressure. Sections in the lower medulla further decreased systemic blood pressure until a level was reached which was characteristic of spinal animals.

However, the importance of the spinal cord in maintaining vasomotor tone in spinal animals was supported by several investigators. Brooks (10) noted that three to seven days following cervical spinal cord transection, animals had a nearly normal blood pressure. Alexander (3)
observed spontaneous inferior cardiac nerve discharges in decentralized, deafferented spinal cord preparations. In a recent study, Polosa (67) reported sympathetic preganglionic spontaneous firing rate of 0.3 Hz in decentralized, deafferented spinal cord preparations. This discharge rate was much lower than that found in intact preparations. This residual discharge indicates that some mechanism other than input drive to preganglionic neurons from the periphery or supraspinal centers is responsible for maintaining activity in a spinal animal. Polosa suggested several possible mechanisms which may account for spontaneous discharges in sympathetic neurons: (1) direct or indirect activation of preganglionic neurons by chemical or physical factors (e.g., respiratory gases, metabolites, pH); (2) spontaneous leakage of excitatory transmitter from presynaptic terminals; or (3) true pacemaker activity of preganglionic neurons. Since in intact preparations, antidromic impulses caused a resetting of the spontaneous discharge pattern, Polosa concluded that the rhythm is endogenous to the sympathetic neuron itself.

The mechanism of the spontaneous activity as well as its exact origin is not known at the present time. However, intracellular recordings from preganglionic neurons may provide the necessary detailed information.

The phenomenon of the silent period in the sympathetic neuron reflex response was first described by Pitts
and Bronk (64). They reported that the spontaneous discharge of single cervical sympathetic fibers was suppressed for nearly one second following excitation by repetitive stimulation of the hypothalamus. This phenomenon was called postexcitatory depression by these authors.

Sato et al. (79) used conditioning-testing stimuli to the sciatic or radial nerves and recorded from lumbar sympathetic trunks, in order to study the excitability and recovery properties of sympathetic neurons. They found that nearly one second is required for complete recovery of these neurons following excitation and no positive test response could be elicited for the first 500-800 msec. Similar phenomena have been observed in various other pre- and postganglionic sympathetic nerves (17, 40, 53, 81, 84). The silent period is commonly defined as the period of depression which follows excitation of sympathetic neurons.

It is not known what processes are responsible for producing such a long lasting depression. Koizumi et al. (53) reported that a test shock occurring during the silent period of a conditioning shock produced no excitation but it did prolong the silent period. They suggested that somewhat independent mechanisms were involved in excitation and inhibition of the sympathetic efferent discharge. Koizumi and Sato (52) presented similar evidence. They recognized the single unit activity from sympathetic postganglionic fibers supplying skeletal muscle during stimulation
afferent nerves. Under favorable stimulation conditions, they could elicit pure depression (without preceding excitation) of spontaneous activity from about 30% of units tested. This again suggested that the silent period can be generated independently from excitation. Furthermore, Iwamura et al. (40) were able to abolish selectively the silent period or the reflex excitation by placing lesions in the medulla. They recorded from the renal nerve while stimulating either the radial or sciatic nerve. Medullary lesions near the dorsal surface selectively abolished or greatly depressed the reflex excitation, whereas lesions in the ventral medullary reticular formation selectively abolished or greatly depressed the silent period.

On the other hand, Polosa (66) proposed that the preganglionic neuron itself may be responsible for the generation of the silent period. By recording extracellularly from preganglionic cell bodies in the spinal cord, he found that repetitive antidromic excitation of spontaneously active units could produce a subsequent, long lasting inactivity or silent period. A similar silent period could be produced by antidromic excitation of units in the actually isolated and deafferented spinal cord. He concluded that the silent period of sympathetic preganglionic neurons is generated in the spinal cord and is due to accumulation of post-activation depression by the cell bodies of the affected units.
The origin and mechanism of the silent period is not clear at the present time as in the case of spontaneous activity. Future studies have to be directed in these directions.

2. Sympathetic Preganglionic Neuron

In the late 1800's Gaskell (28, 29) suggested that sympathetic preganglionic cell bodies were located in the lateral horn of the spinal cord. He pointed out that the position and constitution of the lateral horn differed in different parts of the spinal cord. He noted the cells which in all probability gave rise to the motor nerves of the vascular and glandular systems were small cells lying in groups in the intermediolateral cell column. Utilizing the Golgi stain, Bok (7) and Poljak (65) conducted extensive studies on the neurons in the intermediolateral cell column which were assumed to be sympathetic preganglionic neurons.

Cummings (18) and Petras and Cummings (63) observed identified preganglionic neurons in the dog and monkey spinal cord. Following transection of preganglionic axons, chromatolytic cells were located in the intermediolateral cell column, lateral funiculus, central autonomic area and intercalated nuclei.

Recently, Chung et al. (13) and Schramm et al. (86) injected horseradish peroxidase into sympathetic ganglia and adrenal gland, respectively. They traced the cells
bodies which contain retrogradely transported peroxidase in the spinal cord and confirmed the general locations of the preganglionic neurons. According to Chung et al. (13), 82% of the preganglionic cells in the upper thoracic cord were located in the intermediolateral cell column while 12% were located in the lateral funiculus. The remaining 6% were found in the intercalated nucleus and central autonomic area.

Not all fibers in the cervical sympathetic trunk are of sympathetic preganglionic origin. Foley and DuBois (24) and Foley (22, 23) carried out detailed histological investigations on the cervical sympathetic trunk of the cat. Although there was considerable animal-to-animal variability, about 3000 (37%) of approximately 8000 fibers were unmyelinated. Judging from the results of various types of degeneration experiments, the myelinated fibers of the cervical sympathetic trunk are about 90% preganglionic, 1% postganglionic, and 9% aberrant vagal fibers. The corresponding percentages for the unmyelinated fiber group are 51%, 32%, and 17%, respectively. Ranson and Billingsley (73) and Foley and DuBois (24) claimed that there are no dorsal root afferents in the cervical sympathetic trunk.

The physiological evidence for the presence of both myelinated and unmyelinated fibers in the cervical sympathetic trunk was presented by Eccles (20). He recorded action potentials from the superior cervical ganglion while
stimulating the cervical sympathetic trunk and noted the appearance of four different peaks which had different activation thresholds. He suggested that the first three peaks were action potentials from myelinated fibers and the last peak was from unmyelinated fibers. Jänig and Schmidt (42) recorded single unit activity from the cervical sympathetic trunk. Based on the measurements of conduction velocity, they concluded that 27.5% of the total number of units recorded were unmyelinated fibers.

The spinal origin of the preganglionic fibers in the cervical sympathetic trunk was studied by Herring (34). He observed neurons which showed chromatolytic reaction in the spinal cord after section of the cervical sympathetic trunk of the cat. He found that the cells showing chromatolytic changes were in the ipsilateral lateral horn of the spinal cord. Cell bodies were located in C8 to T6 spinal cord segments and were most numerous in the T2-T3 segments. By applying horseradish peroxidase to the superior cervical ganglion, Chung (14) observed a similar spinal distribution of cervical preganglionic sympathetic cell bodies.

C. Afferent Nerves Affecting Somatosympathetic Reflex

Johansson (43) made an extensive study of the cardiovascular responses to somatic afferent nerve stimulation. He recorded arterial blood pressure and regional blood flows to the kidney, skeletal muscle, intestine and skin. He
demonstrated a marked depressor response and vasodilatation in all vascular beds during activation of myelinated muscle afferent fibers. This was particularly evident in cats with high spontaneous vasoconstrictor tone. Though this response was most noticeable at low stimulus frequencies, it was also noted at higher frequencies (100-500 Hz). When the intensity of stimulation was increased to activate unmyelinated C fibers, high frequency stimulation resulted in a pressor reflex, while low frequency stimulation produced a depressor response. Stimulation of myelinated cutaneous fibers caused a decrease in blood pressure at low stimulus frequencies, but an increase in pressure was elicited by high intensity stimulation of unmyelinated cutaneous afferent fibers.

Since blood pressure responses depended on the type of afferent fiber being stimulated, studies were undertaken to relate sympathetic discharges to specific afferent nerve volleys. Schmidt and Schönfuss (84) and Sato and Schmidt (80), by using a computer to average transients, recorded mass discharge responses to stimulation of afferent fibers in cutaneous and muscle nerves. They reported that stimulation of low threshold cutaneous afferent fibers (A-beta) was most effective in eliciting sympathetic reflexes. If cutaneous A-delta afferent fibers were also stimulated there was a further increase in reflex size. Activation of A-alpha afferents from muscle never produced sympathetic
reflexes, but A-beta and -delta afferents were quite effective.

Schmidt and Weller (85) provided a detailed description of reflex discharges in the cervical and lumbar sympathetic trunk following activation of unmyelinated afferent fibers. At a slow repetition rate, single shock stimulation of both A and C afferent fibers elicited reflex discharges that were related to the A volley only (sympathetic A reflex). However, if pairs or short trains of pulses were given (pulse frequency 10-30 Hz), an additional late reflex component appeared. This late reflex disappeared if the stimulus strength was lowered beyond the threshold of the unmyelinated fibers. The A reflex was abolished following anodal block of myelinated fibers but the late reflex still persisted. Therefore, the late reflex was elicited by activation of unmyelinated C fibers and was called the sympathetic C reflex by these authors. The C reflex had a latency of about 250-500 msec from the beginning of the stimulus and a duration of 250-600 msec. The most effective stimuli for eliciting the C reflex were pairs of short trains of pulses. This suggested that unmyelinated fibers require temporal summation of afferent volleys to evoke a sympathetic discharge. Furthermore, 10-20 stimuli were necessary for the maximal reflex responses (recruitment phenomenon). Schmidt and Weller concluded that much of the latency of the C reflexes was due to the low conduction
velocity of the unmyelinated afferent fibers in the peripheral nerve.

Koizumi and co-workers (51) also demonstrated the C reflex in lumbar white rami after anodal current block of the myelinated afferent fibers. In addition, they correlated sympathetic nerve activity with the blood pressure responses. Low frequency stimulation of both cutaneous A and C fibers or A fibers alone resulted in a depressor response. During this stimulation, sympathetic nerve activity showed a prolonged silent period following reflex excitation. Low frequency stimulation of unmyelinated fibers alone induced a pressor response and simultaneous sympathetic nerve recordings showed no silent period. They concluded that myelinated fibers have synaptic input to both excitatory and inhibitory reflex centers, while unmyelinated fibers influence only excitatory areas.

Jänig et al. (41) and Koizumi and Sato (52) analyzed the reflex influence of somatic afferent C fibers on single postganglionic units in cutaneous and muscle nerves. Only a minority of the cutaneous units were excited by C fiber volleys in either cutaneous or muscle afferent nerves. More often the C fiber volleys increased and prolonged the inhibition induced by the concomitant stimulation of the myelinated afferent fibers. They suggested that those units which were activated by C fiber volleys subserve different functions (vasodilators, pilomotor, etc.) from those which
were inhibited by these volleys. In contrast, C fiber volleys in cutaneous nerves resulted mainly in excitatory responses in the majority of muscle sympathetic units. Koizumi and Sato (52) observed that the C fiber reflex excitation was not followed by a silent period whereas the A fiber reflex excitation was followed by a period of marked depression. This again seems to indicate that myelinated fibers have synaptic input to both excitatory and inhibitory reflex centers, while unmyelinated fibers influence only excitatory areas.

D. Central Pathways for the Somatosympathetic Reflex

1. Descending Spinal Pathways

One of the earlier descriptions of the location of the efferent spinal pathway mediating the somatosympathetic reflexes was presented by Ranson and Billingsley (71). Following dorsal hemisection in the upper thoracic cord, the vasomotor response to brachial nerve stimulation was not affected. They indirectly concluded that the efferent limb of this reflex was in the lateral or ventral funiculus of the spinal cord.

Chen et al. (12) stimulated both sides of the medulla and observed sympathetic responses in various organs including the cardiovascular system. After sympathetic responses were elicited, the ventrolateral funiculus of the spinal cord was destroyed on one side. The ipsilateral or contralateral side of the medulla was then stimulated but no
sympathetic responses were observed on the side of the spinal lesion. They concluded that the sympathetic tract arises either from the ventromedial part of the vestibular nuclei or from dorsolateral cells in the reticular formation and descends uncrossed in the ventrolateral funiculus.

Wang and Ranson (90) noted that lesions in either the anterolateral funiculus or just dorsal to the dentate ligament at the Cl to C2 segments significantly reduced blood pressure elevations during hypothalamic stimulation.

Kerr and Alexander (48) examined the sympathetic descending pathway by directly stimulating the surface of the thoracic spinal cord between the dorsolateral sulcus and a point midway between the dentate ligament and ventrolateral sulcus. They noted the greatest concentration of vasopressor fibers were located two to three millimeters ventrolateral to the dorsolateral sulcus.

Illert and Gabriel (39) localized descending excitatory pathways in the dorsolateral funiculus and inhibitory pathways in the ventrolateral funiculus. Both blood pressure and sympathetic activity in the renal nerve increased when the dorsolateral funiculus was stimulated, but decreased when the ventrolateral pathway was stimulated.

Gebber and co-workers (30) recorded blood pressure and evoked discharges in the postganglionic external carotid nerve during stimulation of the dorsolateral funiculus in anesthetized cats. The majority of the effective stimulation
sites for the vasomotor outflow were near the surface of the cord ventral to the dorsolateral sulcus.

Foreman and Wurster (25) did an extensive electrophysiological and functional study of the descending spinal sympathoexcitatory pathway in anesthetized cats. They demonstrated that maximal pressor responses and maximal T2 preganglionic nerve evoked discharges were elicited by stimulation of the surface of the dorsolateral funiculus, 1.5-2 mm ventrolateral to the dorsolateral sulcus. Discrete lesions on the surface of the dorsolateral funiculus decreased the blood pressure to the level of a spinal animal, indicating that the majority of fibers responsible for maintaining vasomotor tone are located in this area. Conduction velocity in this uncrossed pathway was determined to be about 6 m/sec. In a later study (26) these investigators showed that the responses to afferent nerve stimulation are dependent upon an intact dorsolateral funiculus, indicating that this pathway is the descending limb of the somatosympathetic reflex.

Recently, Szulczyk (87) recorded sympathetic evoked responses from the renal nerve while stimulating the hypoglossal nerve. Evoked activity was abolished by T1 dorsolateral funiculus lesion placed ipsilateral to both the stimulating and recording nerves. Similar to Foreman and Wurster (26), Szulczyk suggested that the sympathoexcitatory pathway in the dorsolateral funiculus is the descending
spinal pathway for the somatosympathetic reflex.

2. Central Relay Areas

Sato et al. (83) recorded mass reflex discharges from the lumbar sympathetic trunk while stimulating the sciatic nerve. They observed two reflex discharges with latencies of 25-50 and 80-120 msec. They termed these responses the early and late reflex potentials, respectively. The late potential was seen consistently and the early potential was observed in about 60% of all experiments. After serial transection of the brain stem, starting at the upper midbrain level down to the lowest level of the pons, the late as well as the early reflex could be still elicited. However, after spinal transection at C1 or T8, only the early reflex was preserved. They concluded that the early response was transmitted via spinal pathways, the late one via the medulla oblongata.

Sato and Schmidt (81) recorded reflex discharges in thoracic and lumbar white rami elicited by single shock stimulation of intercostal, spinal and hind limb nerves. The early (spinal) reflex component had its largest amplitude with afferent volleys entering the spinal cord at the same or adjacent spinal segments to that of the white ramus being recorded. The size of the late (supraspinal) component was independent of the segmental level of the afferent input. They concluded that somatic afferent volleys have a twofold action on sympathetic responses: a more generalized
action via the supraspinal sympathetic reflex centers and a more circumscribed action on the preganglionic neurons at the segmental level.

The spinal and supraspinal components of the reflex activity in the cervical sympathetic trunk were studied by Kirchner et al. (49). In the cervical sympathetic trunk, sciatic or radial nerve stimulation evoked a reflex discharge which also consisted of fast and slow components. However, these two reflex components were found to be mostly attributed to the myelinated and unmyelinated fibers in the cervical sympathetic trunk. The reflexes in spinal cats showed discharge patterns similar to those in CNS-intact cats, although the amplitudes of the reflexes were much smaller in spinal cats. They concluded that in CNS-intact cats, the cervical sympathetic reflex discharges, evoked by a sciatic or radial nerve stimulation, utilized both spinal and supraspinal pathways which have similar latencies.

Sato (75) observed a very late reflex discharge (300-350 msec) in addition to the early (spinal) and late (medullary) reflexes while recording mass discharges from lumbar white rami during somatic afferent nerve stimulation. The very late reflex was only observed 8-10 hours after the initial dose of alpha-chloralose (50 mg/kg, i.p.). It disappeared completely after an additional injection of alpha-chloralose (5-10 mg/kg, i.v.), although the early and late reflexes persisted. After surgical transection of the brain
stem at the supra- or mid-pontine level, the very late reflex discharge disappeared completely while the other two reflexes remained unabated. He concluded that the very late reflex discharge has a suprapontine reflex pathway and is very sensitive to the level of anesthesia.

Because the sympathetic C reflex was found and characterized later, the central relay areas of the somatosympathetic reflex described above had dealt with only the sympathetic A reflex. After the sympathetic C reflex was characterized, Sato (77) studied the pathways for the C reflex by recording sympathetic activity from the lumbar white rami while stimulating afferent C fibers of various hindlimb and lumbar spinal nerves. The sympathetic C reflex evoked in CNS-intact cats remained after decerebration, whereas it disappeared completely after spinal transection. However, in the chronic spinal cats, 8-12 weeks after T8 spinal transection, this reflex reappeared. The sympathetic C reflex evoked by stimulation of spinal nerves adjacent to the segmental level of the recorded white ramus persisted after acute spinal transection. This apparent spinally mediated C reflex did not require temporal summation to elicit a maximum reflex response. He concluded that the sympathetic C reflex evoked by stimulation of hindlimb nerves had a central pathway through the medulla oblongata when the CNS neuraxis was intact. However, a spinal reflex pathway can also exist under special conditions such as in
very long term chronic spinal animals. In addition to the supraspinal pathway, a spinal pathway also exists for the sympathetic C reflex in CNS-intact cats. This spinally mediated sympathetic C reflex could only be elicited by stimulation of spinal nerves entering the spinal cord at the same or adjacent segments to the sympathetic outflow.

3. Ascending Spinal Pathway

Ranson and co-workers (72, 74) proposed that pressor responses to peripheral nerve stimulation are mediated by distinct ascending pathways localized bilaterally in the dorsolateral sulcus area of the spinal cord. After cats had recovered from various lesions in the lower thoracic or upper lumbar spinal cord, the authors recorded and compared systemic blood pressure changes during stimulation of the brachial and sciatic nerves. Depressor or only small pressor responses were observed with sciatic nerve stimulation after bilateral lesions in the dorsolateral sulcus area. However, similar stimulation of the brachial nerve (above the lesion) resulted in much larger pressor responses. They concluded that the afferent pressor impulses travel up the spinal cord in the dorsolateral sulcus area on both sides of the cord with the ipsilateral side being more important.

Ranson and Billingsley (71) used similar techniques to localize the ascending spinal depressor pathway. The depressor responses during weak stimulation of the sciatic nerve was abolished by placing bilateral lesions in the
lateral funiculus at the L1 segment while the brachial nerve stimulation elicited depressor responses. They concluded that the afferent spinal pathway for depressor reflexes lies in the lateral funiculi bilaterally in the same position as the spinothalamic tract, but this pathway was not the spinothalamic tract itself.

Johansson (43), however, failed to confirm Ranson's results. He stimulated the superficial peroneal nerve and could not abolish the pressor response following a lesion of dorsal half of the C3 spinal cord. He suggested that afferent spinal pressor pathways are a rather diffuse system. Furthermore, Johansson was able to abolish the depressor response during hamstring nerve stimulation by placing a lesion at C1 which included both the bilateral ventral and ventrolateral funiculi. Stimulation of the central cut end of the vagus nerve still elicited a depressor response after the spinal lesion. He concluded that the ascending spinal depressor pathways are located in the ventral or ventrolateral funiculus bilaterally.

An attempt to localize the ascending spinal pathway for the somatosympathetic reflex was made by Coote and Downman (15) using electrophysiological techniques. They recorded reflex activity from the renal nerve evoked by stimulation of the intercostal nerves while making C4 spinal lesions. The late reflex (supraspinal) in the renal nerve elicited by stimulation of the tenth intercostal
nerve was abolished by a lesion which included both dorsal horns and the dorsal part of the lateral funiculi. The renal nerve responses to stimulations of the second cervical nerve was still present. A lesion at the C4 level which was confined to the dorsal columns had no effect on the renal nerve reflexes elicited by either the C2 spinal nerve or the tenth intercostal nerve. They concluded that the ascending spinal pathways for the somatosympathetic reflexes are in both dorsal horns and the dorsal portions of the lateral funiculi.

E. Functional Significance of the Somatosympathetic Reflex

The physiological significance of the somatosympathetic reflex is poorly understood. Two major themes have been proposed as the physiological role of the somatosympathetic reflex: cardiovascular adjustments during exercise and autonomic defense mechanisms to noxious stimuli.

Wildenthal et al. (91) studied the effects of arterial infusions of small amounts of KCl (0.3-1.0 mmole) into the vascularly isolated but innervated hind leg of the dog. Arterial pressure, heart rate and ventilatory volume increased significantly during potassium infusions. Neither vagotomy nor beta adrenergic blockade attenuated the blood pressure rise whereas cutting the femoral and sciatic nerves abolished all changes. Since potassium is released from
muscles during exercise, they suggested that potassium can induce cardiovascular reflexes from the leg during muscular exercise.

Coote and co-workers (16) elicited tetanic contraction of the hind limb muscle by stimulating the ventral roots L6-S1 in anesthetized or decerebrated cats. This tetanic muscle contraction caused a rise of arterial blood pressure. When muscle contraction had been abolished by gallamine, or when dorsal roots L6-S1 had been sectioned, ventral root stimulation no longer caused a pressor response. They concluded that the response was a reflex, initiated in the contracting muscle. Furthermore, they suggested that the stimulus arising in the contracting muscle was probably chemical because occluding the arterial inflow to the hind limb increased the magnitude and rate of rise of the pressor response to contraction.

McCloskey and Mitchell (56) conducted similar experiments and confirmed the results of Coote et al. McCloskey and Mitchell further studied the effects of chemical infusions into the artery. Injection of small volumes of 5% NaCl or isotonic KCl into the arterial blood supplying the hind limb musculature gave rise to a cardiovascular response similar to those evoked by contraction. Like the response to contraction, this response was abolished by dorsal root section. Selective anodal current block of large myelinated fibers from the contracting muscle did not abolish
the evoked cardiovascular response. However, the response was abolished by selective blockade of unmyelinated and small myelinated fibers by local anesthesia of the nerves. They concluded that the reflex cardiovascular responses were mediated by small myelinated and unmyelinated fibers originating from exercising muscle.

Hnik et al. (35) recorded sensory afferent nerve activity from simulated exercising muscle in both rats and cats. A brief tetanic contraction of the gastrocnemius muscle increased transiently the overall sensory activity in the muscle nerve and in the dorsal root filaments. This increased activity was due to the increased activity in both proprioceptive and myelinated nonproprioceptive nerve fibers. Most afferent fibers did not respond to either increased perfusion pressure (up to 250 mmHg) or increased arterial blood CO₂ content by about 20%. Intraarterial infusion of KCl at concentrations found in the venous effluent from contracting muscles (10 mEq K⁺ per liter), enhanced the activity of both proprioceptive and myelinated nonproprioceptive fibers. They concluded that muscle afferents may be activated by nonproprioceptive stimuli associated with metabolic changes of the muscle. The increased concentration of potassium is important to enhance most of activity of both proprioceptive and nonproprioceptive nerve endings. The activation of nonproprioceptive fibers might be involved in mediating cardiovascular and respiratory responses.
during muscle exercise.

Ranson and co-workers (71, 74) failed to correlate spinal pain pathways to the ascending vasomotor pathways. They made a series of spinal lesions (lateral hemisection, posterior hemisection, section of the posterior funiculus, bilateral dorsolateral sulcus area lesions) and waited several weeks; this had no noticeable effects on the conduction of pain. They concluded that the pain pathways in the cat spinal cord are bilateral and are not identical to the ascending pressor and depressor pathways which they localized in the dorsolateral sulcus area and lateral funiculus, respectively.

In order to find adequate stimulation for blood pressure responses, Johansson (43) applied various physiological stimuli to the cat hind limb while recording blood pressure. He concluded that pinching or applying pressure to the muscle was found to be the most effective method for inducing the depressor responses whereas pressor responses were mainly obtained by nociceptive cutaneous stimuli.

Horeyseck and Jänig (36, 37) also studied natural stimuli which elicited somatosympathetic reflexes. They recorded single unit activity from sympathetic postganglionic nerves supplying skin and muscle while applying various forms of mechanical non-noxious and noxious stimuli to the skin. Non-noxious stimuli (air jets over hair,
constant force to the foot pads, and sinusoidal stimulation of pacinian corpuscles in fat pads) decreased activity to muscle postganglionic fibers and increased activity to skin postganglionic fibers. These data suggested that non-noxious stimuli to the skin activated A-beta and -delta afferent fibers which resulted in a redistribution of blood flow from the skin to the muscle. Noxious stimuli (mechanical damage or thermal stress) caused the opposite response, i.e., attenuation of skin sympathetic discharges and activation of muscle sympathetic discharges. They suggested that these reflexes were initiated by small myelinated and unmyelinated fibers.

Guzman, Braun and Lim (33) carried out extensive studies on the effects of intraarterial injection of various chemicals (KCl, acetylcholine, histamine, serotonin, bradykinin) in anesthetized dogs and cats. Intraarterial injection of these substances into organs in various areas throughout the body evoked vocalization, hypertension and hyperpnea. The responses mimicked the symptoms of pain provoked by applying mechanical, thermal and electrical stimuli to the body surface. They concluded that these substances induce pain from somatic and visceral sites following intraarterial injection. Furthermore, increased unmyelinated afferent nerve activity following intraarterial injection of these algesic substances was demonstrated by Ménse and co-workers (21, 27, 60).
Juan and Lembeck (44) observed the effects of intraarterial injection of algesic substances (bradykinin, substance P, acetylcholine, histamine, serotonin, KCl) into the isolated perfused rabbit ear connected to the body by only its nerve. Following injection of the algesic substances, afferent nerve activity of the auricular nerve in the isolated ear was increased and systemic blood pressure of the rabbit decreased reflexly. They concluded that the afferent impulses generated from pain receptors (free nerve endings) located in the paravascular tissue were responsible for the reflex fall in systemic blood pressure. In the later study (45) they found both procaine and tetrodotoxin reduced the effects of intraarterial injection of algesic substances.

The available literatures concerning the functional significance of the somatosympathetic reflex have been briefly reviewed. Meaningful conclusions about the physiological role of the somatosympathetic reflex will require further investigation.
CHAPTER III

MATERIALS AND METHODS

A. Animal Preparation

Experiments were performed on adult cats (2-4.5 kg) of either sex. Blood pressure responses during cervical spinal cord stimulation were studied under sodium pentobarbital (35 mg/kg, ip) anesthesia. For the remaining experiments, the femoral vein of the cat was cannulated under chloroform preanesthesia. Then, alpha chloralose (60 mg/kg, dissolved in carbowax 200) was injected slowly (10 sec) into the femoral vein with the animal started to move his hind limb as the chloroform anesthesia was discontinued. The level of anesthesia was adjusted to be just deep enough to inhibit the corneal reflex and muscle tone. After tracheotomy, animals were paralyzed with gallamine triethiodide (4 mg/kg at hourly interval injection) and maintained on a positive pressure respirator (EnSCO RU-4M) with room air. Respiration rate and depth were adjusted to approximate spontaneous breathing patterns. Laminectomies were performed at the appropriate level for each study and animals were placed in an apparatus to stabilize the vertebral
A mineral oil pool was made around the exposed spinal cord and the dura mater was resected with fine scissors under a dissecting microscope. Lactated Ringer solution (600 mg% NaCl, 310 mg% Sodium lactate, 30 mg% KCl, 20 mg% CaCl₂) was infused slowly (1 ml/min) to compensate for blood loss. Rectal temperature was maintained at 37±1°C by a heating pad throughout the experiment.

B. Stimulation Procedure

Appropriate peripheral nerves were cut and the central ends were placed on a pair of bipolar stainless steel electrodes for afferent nerve stimulation. Electrodes were made from insect pins (size 00) and insulated with Insl-X (Insl-X Products Corp.) except at the tips. Small mineral oil pools were made around the exposed nerves to prevent drying. A coaxial electrode (David Kopf, 0.2 mm OD) was placed on the surface of the dorsal root entry zone for afferent nerve stimulation of the cervical spinal cord. Rectangular electrical stimuli were applied by means of squarewave stimulators (Grass S44, S48) and a stimulus isolation unit (Grass SIU5). Stimulation parameters were monitored with a differential oscilloscope (Tektronix 561A with a 3A3 differential amplifier).

C. Recording Procedure

1. Blood Pressure Recording

Blood pressure was recorded from the cannulated femoral artery with a pressure transducer (Statham P23Db)
and a Harvard recording apparatus. The catheter was made from polyethylene tubing (ID 0.045", OD 0.062") fitted over an 18 gauge needle. The arterial cannula was inserted into the femoral artery to the level of the lower aorta. Mean blood pressure was taken as diastolic pressure plus one-third of the pulse pressure.

2. Sympathetic Nerve Recording

Sympathetic nerve activity was recorded from the preganglionic fibers of the cervical sympathetic trunk. The cervical vagus and sympathetic trunk was cut just caudal to the superior cervical ganglion and freed from surrounding tissue. A lateral incision on the neck was made and the skin flaps were tied to the side bars of the mounting apparatus to form a mineral oil pool. The caudal end of the cut nerve was pulled into the pool and laid over a small dental mirror. The sympathetic nerve was separated from the vagus and cardiac depressor nerves with the aid of a dissecting microscope and a pair of fine forceps. Positive identification of sympathetic trunk was made by the presence of spontaneous activity and reflex evoked activity following peripheral nerve stimulation. Usually, the vagus and cardiac depressor nerves showed highly synchronous activity with respiration and heart rate, respectively. Once the sympathetic preganglionic nerve was identified and separated, the nerve was desheathed with fine scissors. The desheathed sympathetic nerve was
divided carefully with two pairs of fine forceps until several spontaneously active units were found. Sympathetic unit activity was recorded from the nerve by using a pair of stainless steel bipolar electrodes (5 mm apart) and amplified by an A-C amplifier (Grass P-9AC) with half amplitude frequencies set at 10 Hz and 2 KHz. This amplified signal was then fed into a window discriminator (57). The window discriminator sends a standard output pulse whenever the peak amplitude of the nerve impulses is between the lower and upper window settings. The window discriminator output signal and time histogram of the window output were displayed on the Harvard recording apparatus and stored on a tape recorder (Precision Instrument PI-6200) for later analysis. The sympathetic unit activity and window discriminator output signals were monitored simultaneously on an oscilloscope (Tektronix DL3). Figure 1 shows the arrangement of the stimulating and recording devices.

D. Lesion and Histology

Spinal cord lesions were made with fine scissors under the dissecting microscope. The lesions were gradually enlarged until a satisfactory change in the response could be observed. At the end of the experiment, the portion of the spinal cord containing lesions was immersed in 10% formalin fixative solution. Subsequently, 20 µm serial sections were cut and stained with hematoxylin and
FIGURE 1

DIAGRAM OF INSTRUMENTS
FIGURE 1

LEGEND

A schematic diagram showing the instruments used for stimulating and recording nerve activity. SIU: Stimulus isolation unit.
eosin. A Leitz microscope with an Orthoplan drawing attachment was used to reproduce the area of the lesion. A drawing of the limits of the lesion was constructed by overlapping serial spinal cord sections.

E. **Data Analysis**

Data are expressed as mean ± Standard error of the mean. Statistical analysis was performed with the Student unpaired-t test (19). P values of less than 0.05 were considered to indicate statistical significance.

F. **Computer Analysis**

A Digital PDP-12 computer was used to analyze the data in one experimental series. Two channels of tape recorded analog information were simultaneously played back to the computer for the generation of a poststimulus time histogram (PSTH). The first channel received the stimulus signal which directed the computer to start a new PSTH sweep through its 100 bins. Bin size was variable from 1 to 4095 msec therefore giving a wide range in PSTH times from 100 msec to 409.5 sec. Delay logic was added to the trigger channel so that triggers received within one second of an accepted trigger would be rejected. Thus, stimulus trains of any frequency less than one second in duration could be used as a PSTH trigger without early resetting of the sweep. This delay, however, increased the minimum PSTH time from 100 msec to 1 sec.

The second channel received the response signal.
Whenever a channel 2 trigger was accepted, a count of one was added to the corresponding bin number of elapsed time from the accepted channel 1 or stimulus trigger. Response events occurring beyond the 100th bin did not contribute to the PSTH but were counted. Therefore, the operator could successively increase the bin width until no response spikes were lost if so desired.

The number of sweeps read in to build the PSTH was selectable. In some cases it was necessary to generate a single PSTH from different tape-recorded data. This was readily accomplished by selecting an add to PSTH mode in the computer program.

The completed PSTH was plotted on the cathode ray tube as the bin contents and as a function of bin number (or time). Full scale calibrations were selectable as either 50 or 100 counts on the Y-axis and 50 or 100 bins on the X-axis. The scaled plot could then be transferred to a hard-copy plot as generated on a CALCOMP digital plotter. Appropriate digital values could also be printed out on the teletype and included the contents of each bin plotted, the number of cycles added together, the number of pulses rejected, bin size in msec, etc.
CHAPTER IV

RESULTS

A. Blood Pressure Study

The initial study of the ascending pathway for the somatosympathetic reflex was performed by observing blood pressure responses to afferent nerve stimulation. The cardiovascular system has been extensively used as an effector organ for the study of the somatosympathetic reflex. Therefore, the findings can be readily compared with earlier investigations.

Bilateral vagus nerves were cut to eliminate vagal induced changes in heart rate and blood pressure. Blood pressure changes during afferent nerve stimulation were observed and compared before and after various lesions of the spinal cord. Data are expressed as mean ± standard error of the maximum change in mean blood pressure elicited by stimulation of afferent nerve fibers.

1. Determination of Stimulus Parameters

In order to determine the appropriate stimulus parameters, the dorsal roots (L7 and S1) of three cats were cut, and one or two rootlets were stimulated peripherally while monitoring antidromic compound action potentials from
the sural nerve. This procedure was used to determine the activation threshold of dorsal root fibers. Activation thresholds \(T_C\) of afferent C fibers (conduction velocity less than 2 m/sec) ranged from 3 to 5 V with a 1 msec stimulus duration. The form of the compound action potential did not change when the stimulus intensity was increased beyond 2 \(T_C\) (two times the C fiber threshold), suggesting that 2 \(T_C\) is a supramaximal stimulus intensity for all fiber groups. Therefore, a stimulus intensity of 10 V with a 1 msec duration, which is at least 2 \(T_C\), was chosen for dorsal root stimulation. This stimulus intensity activated all fiber groups which affect blood pressure, including C fibers.

The frequency of the stimulus was varied depending on the experimental conditions. The stimuli were applied for 20 sec because usually about 15 sec was required for blood pressure responses to be stabilized.

2. Localization of Ascending Pressor Pathways.
   a. Cervical Spinal Cord Study

   Laminectomies were performed at the C2 to C7 segments of the spinal cord. A David Kopf coaxial electrode was placed on the surface of the C6 dorsolateral sulcus (DLS) for afferent nerve stimulation because of the short dorsal roots in this area.

   Prior to any stimulation, a lesion was made in the ipsilateral dorsolateral funiculus (DLF) caudal to the
stimulating site. This procedure prevented direct activation of the descending sympathetic pathway, since the descending sympathetic pathway was localized in the DLF (25, 30, 39). To verify this lesion, the DLF was stimulated (50 Hz, 10 V, 1 msec) caudal and rostral to the lesion. DLF stimulation rostral to the lesion did not produce an increase in blood pressure whereas stimulation caudal to the lesion resulted in a marked increase in blood pressure (25).

Figure 2A shows an example of blood pressure responses during the stimulation of the cervical spinal cord. A pressor response of 50 mmHg was observed when the surface of the DLS at C6 was stimulated at a frequency of 50 Hz. This pressor response was abolished by bilateral lesions in the portion of the spinal cord surrounding the DLS (DLS area) placed more than three segments rostral to the stimulating site. Stimulation of the DLS rostral to the lesion elicited a pressor response of 50 mmHg. Figure 2B summarizes the data from seven cats. Pressor responses to control stimulation (32 ± 6 mmHg) were significantly different (P<0.002) from the responses to identical stimulation following bilateral lesion of the DLS area (-2 ± 2 mmHg). DLS stimulation rostral to the lesion elicited pressor responses 28 ± 6 mmHg) which were significantly different (P<0.005) from stimulation caudal to the lesion but not significantly different (P<0.5) from control.
FIGURE 2

LOCALIZATION OF PRESSOR PATHWAYS

A

BP (mmHg)  
Control  Caudal  Rostral

200
100

30 sec

B

BP Change (mmHg)

Control  Stim.
Caudal to DLS  Rostral to DLS
FIGURE 2

LEGEND

Functional localization of ascending pressor pathways. A: blood pressure tracings during stimulation (50 Hz, 10 V, 1 msec) of cervical dorsolateral sulcus (DLS). Pressor response (Control) to C6 DLS stimulation was abolished by placing bilateral lesions in C3 DLS area (Caudal). Stimulation of C2 DLS (Rostral) still elicited a pressor response. Solid bars above blood pressure tracings indicate stimulation periods. B: averaged effects on blood pressure from 7 cats. Prestimulus basal blood pressure levels for Control Stim., Caudal to DLS, and Rostral to DLS were 127 ± 9, 119 ± 7, and 120 ± 8 mmHg, respectively. C: averaged blood pressure changes during L7 dorsal root stimulation (50 Hz, 10 V, 1 msec) from 12 cats. Bilateral DLS area lesions were made at L3 - L4. Prestimulus basal blood pressure levels for Control Stim., Caudal to DLS, and Rostral to DLS were 113 ± 6, 106 ± 4, and 107 ± 4 mmHg, respectively. Data are expressed as mean ± SE of the change in mean blood pressure for both B and C. Dots indicate a significant difference from responses to stimulation caudal to lesions in DLS area (solid bars).
b. Lumbar Spinal Cord Study

To avoid involvement of descending sympathetic pathways and the possibility of current spread within the spinal cord, responses to lumbar dorsal root stimulation were investigated. Laminectomies were performed at the L2 and S2 segments of the spinal cord. The central cut end of dorsal roots (one or two rootlets) were placed on the stainless steel bipolar electrode for afferent nerve stimulation. Figure 3 diagrammatically indicates a portion of lumber spinal cord to which the stimulations and lesions were applied in this study. The L7 dorsal roots were stimulated with a frequency of 50 Hz, and bilateral lesions were made in the DLS area between L3 and L4. Figure 2C summarizes these results. Blood pressure responses were similar to those noted with stimulation in the cervical spinal cord. Control stimulation resulted in a $37\pm 4$ mmHg increase in pressure. Following bilateral lesions of the DLS area, identical stimulation elicited a fall in blood pressure of $-4\pm 2$ mmHg. Stimulation of another dorsal root (L3) rostral to the lesions increased blood pressure $31\pm 6$ mmHg. Responses after bilateral lesions were significantly different from responses to control stimulation ($P<0.001$) and to stimulation rostral to the lesions ($P<0.002$).

It can be concluded from the experiments illustrated by Fig. 2 that there are ascending pathways in the DLS
FIGURE 3
DORSAL ROOT STIMULATION OF LUMBAR SPINAL CORD
FIGURE 3

LEGEND

A diagrammatic drawing of the lumbar spinal cord. L7 dorsal roots were stimulated while lesions were placed between L3 and L4. Dotted lines indicate the site of lesions.
region of the cervical and lumbar spinal cord which mediate pressor responses to afferent nerve stimulation.

3. Localization of Ascending Depressor Pathways

After lesions were made in the ascending pressor pathways, depressor responses became obvious when lower stimulus frequencies were applied. Localization of ascending depressor pathways were investigated by recording blood pressure responses during L7 dorsal root stimulation before and after lesions in selected parts of the spinal cord.

Figure 4A shows the pressor response (25 mmHg) to low frequency stimulation (5 Hz) of the L7 dorsal root prior to the lesions (control). Following placement of bilateral lesions in the ascending pressor pathways in the DLS area, identical stimulation elicited a depressor response of -20 mmHg. This depressor response was abolished by additional bilateral lesions in the DLF three segments rostral to the L7 dorsal root. Results from 10 animals are summarized in Fig. 4B. With an intact spinal cord, L7 dorsal root stimulation elicited a pressor response of 30 ± 6 mmHg. This was shifted to a depressor response of -13 ± 2 mmHg after bilateral lesions in the DLS area. The depressor response was abolished (1 ± 2 mmHg) after bilateral lesions in the DLF, and the difference was highly significant (P<0.001).

Data from Fig. 4 indicate the presence of ascending depressor pathways in the DLF which are anatomically
FIGURE 4
LOCALIZATION OF DEPRESSOR PATHWAYS

A

B

BP (mmHg)

200

100

0

Control

DLS

DLS & DLF

30 sec

BP Change (mmHg)

40

20

0

-20

Control

DLS

DLS & DLF
FIGURE 4

LEGEND

Functional localization of ascending depressor pathways. A: blood pressure responses to L7 dorsal root stimulation at low frequency (5 Hz, 10 V, 1 msec). Pressor response to stimulation (Control) was shifted to a depressor response following bilateral lesions of L3 dorsolateral sulcus area (DLS). This depressor response was abolished by additional dorsolateral funiculus lesions (DLS & DLF). Solid bars above blood pressure tracing indicate stimulation periods. B: averaged responses of A from 10 cats. Data are expressed as mean ± SE of change in mean blood pressure, whereas dots indicate a significant difference from response to stimulation after lesions in DLS area (stippled bar). Prestimulus basal blood pressure levels for Control, DLS, and DLS & DLF were 117 ± 6, 107 ± 5, and 108 ± 8 mmHg, respectively.
separate from ascending pressor pathways.

4. Histological Examination of Spinal Cord Lesions

The spinal cord lesions of the DLS area and DLF were examined histologically in each of the five cats. Figure 5 diagrammatically illustrates the spinal cord lesions of the DLS and DLF.

5. Bilateral System

In order to determine whether the ascending pressor pathways are bilateral or unilateral systems, a comparison was made between the effects of unilateral and bilateral DLS lesions in eight cats, as shown in Fig. 6. Responses to L7 dorsal root stimulation (Fig. 6A) with 50 Hz following an unilateral lesion of DLS area (18 ± 3 mmHg) were significantly different (P<0.002) from control stimulus responses (29 ± 3 mmHg). Identical stimulation resulted in a slight fall in blood pressure (-1 ± 1 mmHg) following bilateral lesions of the DLS area. These responses were significantly different (P<0.001) from those elicited following unilateral lesion, suggesting that the ascending pressor pathways are bilateral.

A similar approach was made to learn whether ascending depressor pathways are bilateral or unilateral systems. Pressor responses were eliminated by bilateral lesions in the ascending pressor pathways in the DLS area. Stimulation frequency of 2 Hz was chosen in this study because the maximum depressor responses can be obtained
FIGURE 5

SPINAL CORD LESIONS
FIGURE 5

LEGEND

Schematic drawings of lesion in dorsolateral sulcus area (DLS) and dorsolateral funiculus (DLF) between L3 and L4 spinal cord of five different cats. Different shapes of the spinal cord are due to histological procedures.
FIGURE 6

BILATERAL SYSTEM

- Control Stim.
- Unilat. Lesion
- Bilat. Lesion

B.P. Change (mmHg)
FIGURE 6

LEGEND

Evidence for bilateral organization of ascending pressor and depressor pathways. Data are expressed as mean ± SE of change in mean blood pressure, and dots indicate a significant difference from response to stimulation after unilateral lesion (stippled bar). A: comparison of pressor responses to unilateral and bilateral lesions of dorsolateral sulcus area. L7 dorsal roots were stimulated (50 Hz, 10 V, 1 msec) in 8 cats, whereas lesions were made at L3-L4 spinal cord. Prestimulus basal blood pressure levels for Control Stim., Unilat. Lesion, and Bilat. Lesion were 127 ± 6, 120 ± 6, and 119 ± 6 mmHg, respectively. B: comparison of depressor responses to unilateral and bilateral lesion of ascending depressor pathways. Pressor pathways were cut for control stimulation to eliminate pressor responses. L7 dorsal roots were stimulated (2 Hz, 10 V, 1 msec) in 8 cats, whereas lesions were made at L3-L4 spinal cord. Prestimulus basal blood pressure levels for Control Stim., Unilat. Lesion, and Bilat. Lesion were 108 ± 4, 108 ± 7, and 111 ± 7 mmHg, respectively.
at this frequency (see Fig. 7). Stimulation of the L7 dorsal root elicited a depressor response of $-20 \pm 2$ mmHg. Following a unilateral lesion in the DLF, the depressor response was significantly ($P<0.005$) attenuated to $-11 \pm 3$ mmHg. This depressor response was abolished ($1 \pm 2$ mmHg) by bilateral DLF lesion ($P<0.005$).

It can be concluded from Fig. 6 that ascending pressor and depressor pathways are both bilateral. Furthermore, a small pressor response was observed frequently when the bilateral DLS area lesions were placed less than three segments rostral to the stimulated dorsal root. Moreover, the depressor response was not abolished completely when the bilateral DLF lesions were placed less than three segments rostrally. This may suggest that decussation to the contralateral side is extended three segments rostral to the site of dorsal root entry for both pressor and depressor pathways. The blood pressure response data after unilateral lesion were collected from eight cats, six ipsilateral and two contralateral lesions, because these collective values were not significantly different from the value of six ipsilateral lesions alone.

6. Response of the Pathways to Stimulation at Different Frequencies

Figure 7 summarizes the effects of different stimulus frequencies in 10 cats. When L7 dorsal roots were stimulated at frequencies of 50, 5, 2, and 1 Hz, the blood
FIGURE 7
BLOOD PRESSURE RESPONSES FOR VARIOUS FREQUENCY STIMULATIONS

![Graph showing blood pressure responses for various frequency stimulations. The x-axis represents stimulus frequency (Hz) with values 50, 5, 2, and 1. The y-axis represents blood pressure change (mmHg). The graph compares control stimulation, DLS, and DLS and DLF.](image-url)
FIGURE 7

LEGEND

Blood pressure changes to afferent nerve stimulation at different frequencies. L7 dorsal roots were stimulated at 4 frequencies (50, 5, 2, and 1 Hz) in 10 cats (Control Stim.). Stimulations were repeated after bilateral lesions in dorsolateral sulcus area (DLS) and after both dorsolateral sulcus area and dorsolateral funiculus lesions (DLS and DLF). Data are expressed as mean ± SE of change in mean blood pressure, whereas dots indicate a significant difference from response to stimulation after DLS lesion (stippled bar). Prestimulus basal blood pressure levels for Control Stim., DLS, and DLS and DLF were 117 ± 6, 107 ± 5, and 108 ± 8 mmHg, respectively.
pressure changes were $39 \pm 4$, $30 \pm 6$, $4 \pm 7$, and $-12 \pm 5$ mmHg, respectively. After bilateral lesions in the DLS area, responses changed to $-7 \pm 2$, $-13 \pm 2$, $-16 \pm 3$, and $-14 \pm 2$ mmHg for the same corresponding frequencies. These values were significantly different from the control values for the stimulus frequencies of 50 ($P<0.001$), 5 ($P<0.001$), and 2 ($P<0.05$) Hz but not significantly different for 1 Hz ($P>0.5$). After additional bilateral lesions in the DLF, blood pressure changes were $2 \pm 2$, $1 \pm 2$, $3 \pm 1$, and $4 \pm 2$ mmHg for frequencies of 50, 5, 2, and 1 Hz, respectively. These were significantly different ($P<0.01$) from the responses after DLS area lesions alone at all frequencies tested.

Blood pressure responses to the stimulation of dorsal roots (which contain both pressor and depressor nerve fibers) change more prominently in the high than in the low frequency range when lesions were made in the pressor pathways. This suggests that pressor pathways have high optimal frequencies of activation. On the other hand, depressor pathways have low optimal frequencies of activation as indicated in Fig. 7.

B. Neurophysiological Study

Confirming evidence of the localization of ascending pathways for the somatosympathetic reflex was obtained by neurophysiological study. In addition to the vagotomy, the carotid sinus nerves were sectioned bilaterally to prevent baroreceptor reflex changes in sympathetic nerve
activity. Completeness of carotid sinus denervation was judged by the absence of a pressor response to bilateral carotid artery occlusion.

Figure 8 diagrammatically illustrates the experimental arrangement. A laminectomy was performed at the level of T12 and T13. The common peroneal and superficial radial nerves were cut and stimulated centrally. Sympathetic nerve activity (SNA) was recorded from a total of 28 preparations (each preparation contained several spontaneously active fibers) of the cervical sympathetic trunk in five cats.

An example of nerve activity and window setting of the window discriminator device is shown in Fig. 9. This particular recording contained two spontaneously active sympathetic units within the window setting which can be distinguished by the amplitude of the nerve impulses. The window discriminator sends an output to the oscillograph whenever the peak amplitude of the nerve impulses is between the lower and upper window settings.

This method allowed observation of changes in SNA during stimulation of peripheral nerves. Both superficial radial and common peroneal nerves were stimulated for each preparation, and spinal cord lesions were made at T12. Since the radial nerve enters the spinal cord above the lesions, the response to radial nerve stimulation was used as a control for preparation responsiveness. Since the
FIGURE 8
PREPARATION FOR NEUROPHYSIOLOGICAL STUDY

C. Sym. Trunk
R
S
Radial N.
Lesions
T12
Peroneal N.
FIGURE 8

LEGEND

The arrangement of the recording and stimulating nerves for the neurophysiological study. The unit activity was recorded from the cervical sympathetic trunk while the superficial radial and common peroneal nerves were stimulated. Spinal cord lesions were made at T12 level.
FIGURE 9
NERVE ACTIVITY AND WINDOW DISCRIMINATOR SETTING
FIGURE 9

LEGEND

Actual nerve activity (NA) recording and window discriminator level setting. Whenever the nerve impulse peak amplitude was between lower window (LW) and upper window (UW), window discriminator device produced a window output pulse (WO) which was displayed on oscillograph. This is a picture of a single sweep taken from a storage oscilloscope.
cord lesions were made below the major sympathetic spinal outflow and above the portion of the cord where the peroneal nerve enters, variations in SNA during stimulation of the peroneal nerve before and after spinal cord lesions should be due to interruption of the ascending pathways in the spinal cord.

Figure 10 shows changes in SNA of the cervical sympathetic trunk during stimulation of the superficial radial and common peroneal nerves in the various experimental conditions in three different recording preparations. The lower tracings are the window output displays which indicate sympathetic nerve impulses with peak amplitude within the window setting, and the upper tracings are time histograms of the window output. A stimulus frequency of 10 Hz was used in all experiments. In the control experiment, stimulation of both radial and peroneal nerves increased SNA. A recording was made from another preparation after bilateral DLS area lesions at the level of T12. Stimulation of the radial nerve increased SNA, whereas SNA decreased during the stimulation of the peroneal nerve. After additional bilateral DLF lesions at T12 a recording from another preparation showed that the SNA increased during stimulation of the radial nerve, whereas it did not change during stimulation of the peroneal nerve. Statistical analysis was made from 28 recording preparations as shown in Fig. 11. The data are expressed as percent of
FIGURE 10

CHANGES OF SYMPATHETIC NERVE ACTIVITY
BEFORE AND AFTER SPINAL LESIONS

RADIAL N.  PERONEAL N.

CONTROL

DLS

DLS AND DLF

30 SEC
An example of sympathetic unit activity during peripheral nerve stimulation. Records were taken from three different multifiber preparations (CONTROL, DLS, DLS AND DLF, each). Lower tracings are window output of window discriminator device which indicate nerve impulses with peak amplitude between upper and lower window setting. Upper tracings are frequency histograms of window output. Histogram samples are taken every previous 4 sec interval, and calibrations indicate number of impulses. Black bars under histogram indicate stimulation periods. Stimulus frequencies were 10 Hz. Note that response looks greater in lower panel but actual percent change remains similar. This is due to different levels of spontaneous activity. CONTROL, stimulation of radial and peroneal nerves before making any lesion. DLS, stimulation after bilateral lesions of DLS at T12. DLS AND DLF, stimulation after additional lesion of DLF bilaterally at T12.
FIGURE 11
STATISTICAL ANALYSIS OF SYMPATHETIC NERVE ACTIVITY
BEFORE AND AFTER SPINAL LESIONS

--- Radial N. Stim.
--- Peroneal N. Stim.

% Sym. N. Activity

0 50 100 150 200 250 300

Control (6) DLS (10) DLS&DLF (12)
FIGURE 11

LEGEND

Statistical analysis of sympathetic nerve unit activity during peripheral nerve stimulation (10 Hz). Data are expressed as mean ± SE of percent sympathetic nerve activity during stimulation compared to prestimulus (spontaneous) activity. One triangle indicates no significant difference between two values at level of P > 0.5. Two triangles indicate significant difference at level of P < 0.0001. Number of multifiber preparations recorded in each experiment is shown at bottom.
prestimulus (spontaneous) SNA. These values were obtained by comparing the areas under the time histograms for a given time interval before and during the period of stimulation. Recordings were made from six preparations before any lesion (control). Stimulation of the radial and peroneal nerves increased the SNA to 241 ± 23% and 256 ± 28% of the spontaneous activity, respectively, and these responses were not significantly different from each other (P>0.5). After bilateral DLS area lesions at T12, recordings from ten preparations showed that the SNA increased to 235 ± 27% during radial nerve stimulation, but peroneal nerve stimulation caused a decrease in SNA to 45 ± 7% of spontaneous activity. Additional DLF lesions were placed bilaterally at T12. Recordings from 12 preparations showed little or no change in SNA (96 ± 4%) during stimulation of the peroneal nerve, but SNA increased (239 ± 20%) during radial nerve stimulation. Increases in SNA during stimulations of the radial nerve were consistent (CONTROL, DLS, and DLS AND DLF). Therefore, it can be concluded that the variation of SNA during stimulation of the peroneal nerve under different experimental conditions is due to the interruption of ascending pathways in the spinal cord. These data confirm the localization of ascending pressor and depressor pathways in the bilateral DLS area and DLF, respectively.

Without exception, blood pressure responses agreed
well with SNA throughout the experiment. The marked parallelism of SNA and blood pressure responses under different experimental conditions suggests that the ascending pressor and depressor pathways are a spinal ascending limb of the somatosympathetic reflexes. That is, the ascending pressor pathways are sympathoexcitatory and the ascending depressor pathways are sympathoinhibitory pathways.

C. Ascending Spinal Pathways for Sympathetic A and C Reflexes

The dual nature of the ascending spinal pathway for the somatosympathetic reflex has been established in the preceding section. That is, pressor and depressor responses are mediated by separate ascending pressor and depressor pathways in the spinal cord. On the other hand, it has been known that stimulation of the peripheral afferent A and C fiber groups are responsible for depressor and pressor responses, respectively. Therefore, the possibility of the selective activation of the ascending spinal depressor and pressor pathways by afferent A and C fibers was tested using the characteristics of the sympathetic A and C reflexes.

The animal preparation and experimental procedures were exactly the same as the neurophysiological study except for the stimulus parameters. Sympathetic unit activity was recorded from the cervical sympathetic trunk while stimulating the cut central end of the common peroneal nerve.
The sympathetic unit activity was amplified and fed into the window discriminator device from which output pulses were stored on a tape recorder. Poststimulus time histograms (PSTH) were plotted by a computer. Figure 12 shows an example of single sweep of nerve activity, window discriminator output and plotted PSTH. Usually, 30 successive stimuli were added to form a histogram. Sympathetic A and C reflexes and the following silent periods were compared from the histograms before and after lesions of the ascending sympathetic pathways in the spinal cord to see the relationship between the ascending sympathetic pathways and the A and C reflex pathways.

1. Determination of Stimulus Parameters

The combinations of the stimulus parameters were determined to satisfy the following criteria. First, sympathetic C reflex could be optimally elicited. Second, sympathetic A and C reflexes could be alternatively elicited. Third, the silent period as well as reflexly evoked activity could be observed.

a. Intensity

The sympathetic C reflex is known to be optimally elicited by stimulation of a peripheral nerve with a short pulse train of suprathreshold intensity to the C fibers (51, 52, 85). Figure 13 shows sympathetic A and C reflexes elicited by stimulation of the peroneal nerve with various combinations of stimulus parameters. Stimuli were
FIGURE 12

A POSTSTIMULUS TIME HISTOGRAM
FIGURE 12

LEGEND

An example of a poststimulus time histogram plot. Two pulses were delivered in a train at arrows. This single sweep picture was taken from a storage oscilloscope. The window output was stored on a tape recorder and the poststimulus time histogram was later plotted by computer. Bin size was set at 10 msec and total sweep time was 1 sec. UW, upper window. LW, lower window. NA, nerve activity. WO, window output. HIS, poststimulus time histogram.
FIGURE 13
STIMULUS PARAMETERS FOR SYMPATHETIC A AND C REFLEXES

SINGLE STIM.

TRAIN STIM.

Imp

3T_c

0.5T_c

0 0.6 1.2 Sec.

0 0.6 1.2 Sec.
Sympathetic A and C reflexes elicited by various combinations of stimulus parameters. For the same preparation, single and train stimulations were applied to the peroneal nerve at arrows with intensities which were supra- and subthreshold for C fibers. Stimulus frequencies were 0.3 Hz. All the histograms are formed by 30 successive stimuli. $T_c$, intensity of activation threshold for C fibers. Imp, number of impulses per bin (30 msec).
applied every 3.3 sec. Sympathetic A and C reflexes were elicited simultaneously only by train stimulation with suprathreshold intensity for the C fiber group. Only the A reflex was elicited by any other combinations of stimulus parameters. Therefore, single pulses or trains of pulses with a suprathreshold intensity for the C fibers were used in the present experiment to elicit sympathetic A or simultaneous A and C reflexes, respectively.

Stimulus intensity, number of pulses in the train and pulse frequency were adjusted at the beginning of each experiment to elicit a maximum C reflex; these stimulus parameters were maintained throughout the experiment. At the end of the experiment, the activation threshold of C fibers was determined in five cats by recording compound action potentials from a segment of the peroneal nerve while stimulating at one end. Figure 14 shows an example of this recording. A stimulation which was subthreshold to C fibers elicited an A volley response (Fig. 14A). A late C volley was elicited by high intensity stimulation (Fig. 14B). When a train of two or three pulses were delivered, each stimulation was followed by a C volley response (Fig. 14C and D). Stimulus parameters used in the present experiments had intensities of 3 to 4 Tc, 2 to 3 pulses in a train and 20 to 30 Hz pulse frequency.

b. Stimulus Frequency

Figure 15 shows the sympathetic reflex activity
FIGURE 14

AFFERENT A AND C VOLLEYS
FIGURE 14

LEGEND

Examples of compound action potentials from a segment of the peroneal nerve. Compound action potentials were recorded from one end of a desheathed segment of the peroneal nerve while stimulating the other end. The distance between the stimulating and recording sites was 4 cm. Recordings were made by a pair of bipolar stainless steel electrodes with an AC amplifier with a half amplitude frequency setting of 0.2 Hz and 2 KHz. Single pulse stimuli (1 msec duration) were delivered at arrows. Stimulus intensities were 1V an A and 10 V in B, C and D. Activation thresholds were about 50 mV and 5 V for the A and C volleys, respectively. The A volleys in B, C and D were masked by stimulus artifacts. Calibrations: A, horizontal 5 msec, vertical 100μV; B-D, horizontal 20 msec, vertical 100μV.
FIGURE 15

DETERMINATION OF STIMULUS FREQUENCY

Imp

30

1Hz

0.3Hz

0.2Hz

1.2

2.4 Sec.
FIGURE 15

LEGEND

Sympathetic reflex activity and following silent periods for three different stimulus frequencies. Recordings were made from same recording preparation while three pulses were delivered in train to the peroneal nerve. All the histograms were formed from 30 successive stimuli with bin size set at 30 msec. Imp, number of impulses per bin (30 msec). For 1 Hz stimulation (top figure), no activity could be plotted after 1 sec because computer resets every second.
and the following silent periods for three different stimulus frequencies. As stimulus frequency decreased, the C reflex was attenuated. At the same time, however, the duration of silent period became easier to observe because the successive stimulus came after the nerve activity had recovered to the spontaneous activity level. Therefore, a stimulus frequency of 0.3 Hz was chosen in the present experiment to observe the silent periods as well as a reasonable reflex size.

2. Recruitment of Sympathetic C Reflex

Schmidt and Weller (85) reported a recruitment phenomenon of the sympathetic C reflex. The amplitude and duration of the evoked sympathetic nerve potential progressively increased with repeated stimuli to the peripheral nerve. A similar phenomenon was observed repeatedly in the present experiments. An example is shown in Fig. 16. Twenty-five successive stimuli (two pulses each) were applied at a frequency of 0.3 Hz to the peroneal nerve, while recording sympathetic unit activity. After several minutes, another series of twenty-five stimuli was delivered. This procedure was repeated five times for the same recording preparation. Then a series of PSTH were computed for the first to third, the seventh to ninth, the thirteenth to fifteenth and the nineteenth to twenty-first stimuli from each of the five series of stimulations (Fig. 16). Using this procedure, the dynamic changes
FIGURE 16

RECRUITMENT OF THE SYMPATHETIC C REFLEX

Imp
10-

0

1-3

10-

0

7-9

13-15

19-21

0

0.9

1.8
Sec.

0
Recruitment of the sympathetic C reflex. Five series of 25 stimuli were delivered to the peroneal nerve. Separate poststimulus time histograms were formed after the first to third, the seventh to ninth, the thirteenth to fifteenth and the nineteenth to twenty-first stimuli (three stimuli each from five series of stimulation). Therefore, each histogram consists of 15 stimuli. Note the difference in size of C reflex. Imp, number of impulses per bin (30 msec).
during the initial part of the stimulation period could be observed. As the stimulations were repeated, the size of the C reflex progressively increased. It required about ten stimulations to reach a steady state response. Therefore, all the data in the present study were collected after the twenty-first stimulus, that is, after the responses reached a steady state.

3. Latency of Sympathetic A and C Reflexes

Figure 17 indicates the latency and duration of the sympathetic A and C reflexes. All recordings were made from the cervical sympathetic trunk about 1 cm central (caudal) to the superior cervical ganglion. All stimulations were applied to the peroneal nerve about 2 cm distal to the bifurcation of the common peroneal and tibial nerves. Examples of responses following single and train stimulations are shown in Fig. 17A. Only the A reflex is present for the single stimulation, whereas both A and C reflexes are present when the train stimulation was applied. The averaged response from eight recording preparations is graphically shown in Fig. 17B. The sympathetic A reflex began $73 \pm 5.5$ msec and ended $296 \pm 21$ msec after stimulation. The average latency of the peak or modal response of the A reflex was $158 \pm 32$ msec. The sympathetic C reflex, however, began $435 \pm 21$ msec and ended $889 \pm 23$ msec after the first stimulus in the train. It is apparent that the C reflex is much longer in duration.
FIGURE 17
SYMPATHETIC A AND C REFLEXES

A

SINGLE STIM.

TRAIN STIM.

B

LATENCY

A-reflex

C-reflex
Latency and duration of sympathetic A and C reflexes. A: An example of sympathetic A and C reflexes. Only the A reflex is present for single pulse stimulation and both A and C reflexes are present for train stimulation. The histograms were formed from 30 successive stimuli. B: Average latency and duration of sympathetic A and C reflexes from eight recording preparations. All recordings were made from the cervical sympathetic trunk about 1 cm caudal (central) to the superior cervical ganglion. All stimulations were applied to the peroneal nerve about 2 cm distal to the bifurcation of the common peroneal and tibial nerves. The mean latency of the peak of each reflex is indicated by a single dot. Imp, number of impulses per bin (30 msec).
than the A reflex. The average latency of the peak or modal response of the C reflex was $632 \pm 24$ msec.

4. Conduction Velocity

Conduction velocities of the somatosympathetic reflex in the afferent peripheral nerve and ascending spinal pathway were examined in three cats. Evoked compound action potentials were recorded from the whole nerve preparation of the cervical sympathetic trunk while stimulating the tibial nerve. Train stimulations with a supra-threshold intensity to the C fibers were applied to the two different portions of the tibial nerve which were separated by 15 to 18 cm. The conduction velocity of the afferent component of the sympathetic C reflex was estimated by comparing latencies. It ranged 0.5 to 2.5 m/sec which was in the C fiber conduction velocity range.

On the other hand, the latency difference between sympathetic A and C reflexes is apparently mainly due to the difference of the peripheral afferent conduction time (85). Therefore, sympathetic A and C reflexes probably have similar latency from spinal cord entry to the sympathetic outflow. This view was also supported by the observation that no delayed C reflex could be elicited by the dorsal root stimulation no matter what stimulus parameters were used.

The conduction velocity of the ascending spinal pathway was estimated by stimulating the lumbar and thoracic
segments of the spinal cord. Stimuli were applied through a coaxial electrode placed on the surface of the dorsal root entry zone while recording evoked activity or single unit activity from the cervical sympathetic trunk. Since no delayed C reflex could be elicited, sympathetic A and C reflexes are assumed to have similar (the difference was undetectable by this technique) ascending spinal conduction velocity. The conduction velocity ranged from 5 to 25 m/sec.

5. Ascending Spinal Pathways

The size of the sympathetic A and C reflexes (reflexly elicited number of impulses), the following silent periods and the total nerve activity were compared before and after lesions of the ascending spinal somatosympathetic pathways. Sympathetic nerve activity was recorded from the cervical sympathetic trunk while stimulating the common peroneal nerve. Spinal cord lesions were made at the T12 segment, caudal to the recorded sympathetic spinal outflow. However, these lesions were rostral to the portions of the cord where the peroneal nerve enters. Thus, variations in the A and C reflexes during stimulation of the peroneal nerve before and after spinal cord lesions should be due to the interruption of the ascending pathways in the spinal cord.

For each recording preparation, control spontaneous activity was recorded before and after a series of
stimulations. Statistical analyses were made only from the recording preparations which contained several spontaneously active units.

a. Reflex Size

Figure 18 shows changes in sympathetic A and C reflexes before and after various spinal cord lesions in three different nerve preparations. For prelesion control, the sympathetic A reflex was elicited by single stimulation, and A and C reflexes were elicited by train stimulation of the peroneal nerve. A recording was made from another preparation after the ascending sympathoexcitatory pathway was cut by placing bilateral DLS area lesions at T12 spinal cord level. The sympathetic C reflex was no longer present and only the A reflex was elicited for both single and train stimulations. This remaining A reflex was abolished after bilateral lesions of the ascending spinal sympathoinhibitory pathways in the DLF.

In the present experiment, sympathetic recordings were made from several spontaneously active preganglionic units without knowing the exact number. Consequently, the number of units may vary with each preparation even though the variation is probably small. Recording from a preparation which contains a larger number of units will have a larger reflex than a preparation containing a smaller number of units. Therefore, a method of standardization of the data was necessary to express and compare the reflex size
FIGURE 18

CHANGES IN SYMPATHETIC A AND C REFLEXES BEFORE AND AFTER SPINAL LESIONS

SINGLE STIM. TRAIN STIM.

CONTROL

DLS

DLS & DLF
FIGURE 18

LEGEND

Examples of changes in sympathetic A and C reflexes before and after various spinal cord lesions. Recordings for CONTROL, DLS and DLS & DLF were taken from three different preparations. All the histograms were formed from 30 successive stimuli. Bilateral DLS area and DLF lesions were made at T12 segment of spinal cord. CONTROL, prelesion response. DLS, response after bilateral lesions of DLS area. DLS & DLF, response after additional lesions of bilateral DLF.
of many preparations which contain different numbers of nerve fibers.

A factor, 'Reflex Index (RI)', was defined as the ratio of the frequency of the evoked activity to that of the spontaneous activity, multiplied by the duration of evoked activity (see detailed description in Appendix A). Then, the RI has units of time and indicates the relative size of the reflex independently from the number of recorded nerve fibers.

Figure 19 shows the statistical analysis of the changes of the sympathetic A and C reflexes before and after spinal cord lesions. Data were expressed as mean ± standard error of the RI in sec. The sympathetic A reflex was elicited by single stimulation. Before making any lesion, RI was 1.18 ± 0.14 for control stimulation. The RI was not significantly changed (1.06 ± 0.22) after bilateral DLS area lesions at T12 but the A reflex was abolished by additional lesions in the DLF bilaterally (0.10 ± 0.12). Sympathetic A and C reflexes were elicited by train stimulation. During control stimulation RI values were 1.05 ± 0.13 and 1.86 ± 0.13 for the A and C reflexes, respectively. These two were significantly different (P<0.001). The changes of the A reflex component were similar to single stimulation responses (0.91 ± 0.13 after DLS, -0.03 ± 0.07 after DLS & DLF). However, the sympathetic C reflex was abolished after bilateral lesions
FIGURE 19
STATISTICAL ANALYSIS OF THE SYMPATHETIC A AND C REFLEXES CHANGES (I)
FIGURE 19

LEGEND

Statistical analysis of changes of the sympathetic A and C reflexes before and after spinal cord lesions. Data were expressed as mean ± standard error of the Reflex Index in sec. Number of recorded preparations were indicated at the bottom. Dots indicate a significant difference from responses after DLS lesions, one dot: P<0.05, two dots: P<0.01 and three dots: P<0.001.
in the DLS area (-0.05 ± 0.11) and remained so after additional DLF lesions (-0.17 ± 0.05).

As indicated in Fig. 19 the sympathetic C reflex is almost twice the size of the A reflex. The C reflex is mediated by the ascending sympathoexcitatory pathway in the bilateral DLS area of the spinal cord and the A reflex is mediated by the ascending sympathoinhibitory pathways in the DLF.

It is possible that changes in reflex activity after selective spinal cord lesions, as seen in Fig. 19, may be due to non-specific spinal cord trauma rather than specific pathway cut. To eliminate this possibility, lesions were made in reversed order, i.e. DLF lesions first, then additional lesions in the DLS area. The confirmation of the result by this reversed sequence of lesion also reinforces the conclusion. Figure 20 summarizes these results. The RI of the A reflex for single stimulation was significantly (P<0.05) changed from 1.18 ± 0.14 (control) to 0.70 ± 0.12 after bilateral DLF lesions at T12. The A reflex was further decreased to 0.32 ± 0.04 after additional lesions in the DLS area. The A reflex portion during train stimulation followed a pattern similar to the single stimulation responses (1.05 ± 0.13 for Control, 0.62 ± 0.11 after DLF and 0.28 ± 0.05 after DLF & DLS). The C reflex portion, however, did not change significantly after bilateral lesions of DLF (1.52 ± 0.20).
FIGURE 20

STATISTICAL ANALYSIS OF THE SYMPATHETIC A AND C REFLEXES CHANGES (II)

Single Stim.

Train Stim.

Reflex Index (Sec.)

Control (7)  DLF (6)  DLF&DLS (5)
FIGURE 20

LEGEND

Statistical analysis of changes of the sympathetic A and C reflexes before and after spinal cord lesions. Same as Fig. 19 except sequence of lesions was reversed. Same control values were used with Fig. 19. Dots indicate a significant difference from responses after DLF lesions, one dot: $P<0.05$, two dots: $P<0.01$ and three dots: $P<0.001$. 
Additional lesions of DLS area abolished the C reflex completely to $-0.36 \pm 0.06$.

The results in Fig. 20 indicate again the C reflex is mediated mainly by ascending sympathoexcitatory pathways in the bilateral DLS area. The results for the A reflex are not as clear since it was not abolished completely by bilateral DLF lesions, although decreased significantly. However, the lesion making procedures for the DLS and DLF should be considered and compared. When DLS area lesions were made, the lesions were progressively enlarged until the pressor response for the high frequency (50 Hz) peroneal nerve stimulation was abolished. This was a good functional guide for completeness of DLS area lesions. On the other hand, there was no comparably good functional guide for DLF lesions. Usually, the depressor response mediated by DLF was masked by DLS area pressor response until the pressor pathways were cut. Consequently, DLF lesions without cutting pressor pathways were frequently incomplete. This notion was supported by the observation that a substantial A reflex persisted after lesions of the DLF and DLS area in Fig. 20. In addition to the incompleteness of DLF lesions, there may be some overlapping of the pathways. That is, the pathways for the A reflex may extend to the DLS area as well. This view was supported by the observation of a significant decrease of the A reflex when DLS area lesions were made after the DLF
lesions.

In spite of some difficulties concerning the A reflex in Fig. 20, the general pattern supports the results of Fig. 19. It can be concluded from Fig. 19 and 20 that the sympathetic A and C reflexes are mainly mediated by ascending sympathoinhibitory pathways in the DLF and sympathoexcitatory pathways in the DLS area, respectively.

b. Silent Periods

A silent period followed the sympathetic A reflex when a single stimulus was applied to the peroneal nerve. In addition to the A reflex, the C reflex appeared with train stimulation and was accompanied by a much longer silent period. In the case of train stimulation, a short (usually about 100 msec) silent period was present between the A and C reflexes. This silent period was not counted in the present analysis since the duration between the A and C reflexes depended upon the location of the stimulating electrode on the peripheral nerve rather than central nervous structure (because the A and C reflex delay is mainly due to the peripheral afferent conduction time). Durations of silent periods were measured from computer histograms of 30 successive stimuli. Measurements of silent periods were somewhat arbitrary. The period which has no or very little activity was considered to be the silent period. The silent period was considered to be
started when less than 3 consecutive bins (bin size 30 msec) contained one action potential after the reflex excitation. Although the level of activity was not fully recovered to the spontaneous level, the end of the silent period was taken when one or more action potentials occurred in three consecutive bins (bin size 30 msec) which eventually preceded the return of spontaneous activity. Analyses of silent periods were made from only the preparations which showed fairly high spontaneous activity (2 Hz or more) because it was difficult to judge the termination of silent period in preparations with low spontaneous activity.

Not only the reflex size but also length of the silent periods changed after the ascending sympathoexcitatory or inhibitory pathways were cut. Figure 21 shows the changes of silent periods after various spinal cord lesions. For the prelesion control state, analysis from seven recording preparations showed that the durations of the silent periods were 1101 ± 60 msec and 1537 ± 104 msec for single and train stimulation, respectively. These were significantly different at P<0.01. For single stimulation, the silent period did not change significantly after DLS area lesions (1325 ± 141 msec) but almost disappeared after additional DLF lesions (198 ± 85 msec). The silent period decreased significantly after DLS area lesions (1105 ± 110 msec) for train stimulation and again
STATISTICAL ANALYSIS OF THE SILENT PERIODS

**FIGURE 21**

Silent Periods (Sec)

<table>
<thead>
<tr>
<th></th>
<th>Control (7)</th>
<th>DLS (6)</th>
<th>DLS&amp;DLF (5)</th>
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</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td></td>
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<tr>
<td>Control</td>
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<tr>
<td>DLF&amp;DLS&amp;DLF</td>
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</tbody>
</table>

- Single Stim.
- Train Stim.
FIGURE 21

LEGEND

Statistical analysis of changes of the silent periods before and after spinal cord lesions. Data were expressed as mean ± standard error of the duration of silent period in sec. Number of recorded preparation is indicated at the bottom. The sequence of lesions was reversed in Fig. B. Dots indicate a significant difference from responses after DLS lesions in Fig. A or after DLF lesions in Fig. B, one dot: P < 0.05, two dots: P < 0.01 and three dots: P < 0.001.
almost disappeared by additional DLF lesions. In Fig 21B, the sequence of the lesions was reversed. After bilateral DLF lesions, the silent periods were abruptly decreased for both single and train stimulations (395 ± 72 msec for Single Stim., 395 ± 81 msec for Train Stim.). After additional DLS lesions, these silent periods were further decreased to 198 ± 50 msec and 144 ± 65 msec for single and train stimulations, respectively.

It is obvious from Fig. 21 that the major decrease of the silent periods occurs whenever DLF lesions were made. This suggests the generation of silent period is mainly influenced by the sympathoinhibitory pathways in the DLF.

c. Total Nerve Activity

The reflexly evoked activity as well as the following silent period are changed independently after various spinal cord lesions as seen in the previous sections. These two factors have opposing effects on total nerve activity which is the most important factor for the effector organ responses (blood pressure change, etc.). Hence, the percent of total number of activity to spontaneous activity (mean ± S.E.) during the stimulation period was compared before and after various spinal cord lesions in Fig. 22. Total nerve activities for prelesion control stimulations were 94 ± 5% and 171 ± 13% of spontaneous activity for single and train stimulations,
FIGURE 22

STATISTICAL ANALYSIS OF THE TOTAL NERVE ACTIVITY

A

% Total Nerve Activity

200

150

100

50

Control (7)  DLS (6)  DLS&DLF (5)

Single Stim.

Train Stim.

B

% Total Nerve Activity

200

150

100

50

Control (7)  DLF (6)  DLF&DLS (5)
FIGURE 22

LEGEND

Statistical analysis of changes of the total nerve activity before and after spinal cord lesions. Data were expressed as mean ± standard error of percent total nerve activity to spontaneous activity. Total nerve activity during stimulation periods was calculated and compared to spontaneous activity for equivalent periods. Number of recorded preparations is indicated at the bottom. The sequence of lesions was reversed in Fig. B. Dots indicate a significant difference from responses in Fig. A or after DLF lesions in Fig. B, one dot: $P < 0.05$, two dots: $P < 0.01$ and three dots: $P < 0.001$. 
respectively. The values after bilateral DLS area lesions were 73 ± 8% for single and 87 ± 7% for train stimulations. When additional lesions were placed in the DLF, total nerve activities did not change significantly (94 ± 10% for Single Stim., 99 ± 15% for Train Stim.) from the DLS area lesions alone. Figure 22B shows total nerve activities when the sequence of the lesions was reversed. The total nerve activity during single stimulation increased significantly after DLF lesions (142 ± 12%) and came back to about control levels by additional DLS area lesions (88 ± 7%). For train stimulation, the total nerve activity did not change significantly (153 ± 20%) after DLF lesions but decreased significantly by additional DLS area lesions (94 ± 8%).

The single and train stimulations are analogous to low and high frequency repetitive stimulations. Although the changes were small, the general pattern of total nerve activity agrees with the blood pressure study in Fig. 7 and the changes of the sympathetic nerve activity in Fig. 11. The reason for the lack of significant change is that the stimulus frequencies in Fig. 22 are not optimal frequencies for the increase (pressor) nor decrease (depressor) of the sympathetic nerve activity (cf. Fig. 7 and Fig. 11).
CHAPTER V

DISCUSSION

A. Sectional Discussion

1. Blood Pressure Study

In the present study the term "dorsolateral sulcus area" is used to describe the location of ascending pressor pathways. Ranson et al. (72, 74) used the term "apex of the posterior horn" which excludes the white matter. Neither the present nor Ranson's study determined whether the pathways are actually located in the gray or white matter.

Ranson and co-workers (72, 74) proposed that pressor responses to peripheral nerve stimulation are mediated by distinct ascending pathways localized bilaterally in the DLS area. Johansson (43), however, failed to confirm Ranson's results. Johansson stimulated the superficial peroneal nerve and could not abolish the pressor response following a lesion of dorsal half of the C3 spinal cord. He suggested that afferent spinal pressor pathways are a rather diffuse system. However, his results were not consistent (Fig. 16 and 20 of ref. 43). The present investigation confirms Ranson's results and contradicts with
Johansson's, in that both the lumbar and cervical spinal cord have ascending pressor pathways in the bilateral DLS regions.

Ranson and Billingsley (71) localized bilateral ascending depressor pathways in the lateral funiculus. However, Johansson (43) suggested that ascending depressor pathways were located in the ventral funiculus. The present investigation failed to confirm either result but localized ascending depressor pathways in the bilateral DLF.

Both DLS area and DLF of the spinal cord contain many ascending and descending pathways in addition to ascending pressor and depressor pathways described in the present study. It is important to note that ascending depressor pathways described in the present work occupy generally the same area as the descending sympathoexcitatory pathways (25, 30, 39, 40).

Ascending pressor pathways are more responsive to higher stimulus frequencies than ascending depressor pathways (Fig. 7). Ascending pressor and depressor pathways compete with each other for final determination of blood pressure responses when dorsal roots are stimulated. Pressor responses dominate at higher frequencies, whereas depressor responses dominate at lower frequencies.

Dorsal roots were stimulated for the blood pressure studies in the lumbar spinal cord because the segmental organization of ascending pathways could be investigated.
Afferent fibers from peripheral nerves enter the spinal cord at more than one spinal segment, making it difficult to investigate segmental organization.

2. Neurophysiological Study

Results of the present neurophysiological study confirmed the localization of ascending pathways. This further suggests that the ascending pressor and depressor pathways are a spinal ascending limb for the somatosympathetic reflexes. That is, ascending pressor pathways are sympathoexcitatory, and depressor pathways are sympathoinhibitory pathways. Coote and Downman (15), while recording evoked responses from renal nerves, reported that the ascending spinal pathways for somatosympathetic reflexes are in both dorsal horns and the dorsal portions of the lateral funiculi. They could not differentiate between the sympathoexcitatory and inhibitory pathways, since evoked responses alone do not indicate the level of spontaneous activity of spinal sympathetic outflow. The present study confirms and extends their conclusion.

3. Ascending Spinal Pathways for Sympathetic A and C Reflexes

The sympathetic C reflex was elicited only when several stimuli were applied in trains with suprathreshold intensities to C fibers, i.e., it required temporal facilitation to be elicited. On the other hand, Sato (77) reported that the spinally mediated sympathetic C reflex did
not require any type of facilitation. This may suggest that the locus which required the temporal facilitation is located beyond spinal cord level, presumably in the brain stem. Not only the sympathetic C reflex but also many other responses (e.g., medullary reticular formation) require temporal facilitation from unmyelinated afferent fibers to be activated (68). It is not known whether the sympathetic C reflex can be elicited by spacial facilitation or not. This question may be answered by simultaneous stimulation of two or more peripheral nerves.

The recruitment phenomenon was originally reported by Mendell and Wall (59). They recorded single unit activity from axons in the dorsolateral funiculus of the spinal cord while stimulating afferent C fibers. The present experiments show the recruitment phenomenon of the sympathetic C reflex similar to the earlier report of Schmidt and Weller (85). It is intriguing that the activation of a neuronal network can influence the response even after many seconds (20-30 sec).

Coote and Downman (15) reported that the conduction velocity of the ascending spinal pathway for the somatosympathetic reflex ranged 20-30 m/sec. The estimated conduction velocity in the present experiments ranged 5-25 m/sec which was similar to the results of Coote and Downman. This apparently wide variation of the conduction velocities is not unusual among ascending spinal pathways. As an example,
conduction velocities of spinothalamic (89) and spinocervical (11) tracts of cats ranged 9-97 m/sec and 17-103 m/sec, respectively.

The latency difference between sympathetic A and C reflexes is due to peripheral afferent conduction velocity differences (85). Therefore, separation between sympathetic A and C reflexes becomes less clear when the stimulation site was closer to the spinal cord. For the first several cats in the sympathetic A and C reflexes study, the radial nerve was stimulated as a control of preparation responsiveness. However, these responses to radial nerve stimulation were difficult to analyze because the sympathetic A and C reflexes were often fused together due to the short afferent conduction distance. Hence, the results were analyzed only from the peroneal nerve stimulation.

Results in Fig. 21 indicate that the silent period of the somatosympathetic reflex is controlled mainly by the sympathoinhibitory pathway in the DLF which is activated by afferent A fibers. Thus, the selective activation of afferent C fibers alone should result in a short silent period. Koizumi et al. (51), while recording mass discharges from the renal nerve, reported that selective excitation of afferent C fibers (after anodal block of A fibers) produced no silent period but only the C reflex excitation. Their measurements of the silent period are not accurate since they recorded mass discharges rather than
single unit activities. The present experiments generally confirmed and revised their results, i.e., an additional excitation of afferent C fibers produces an increment of silent period which is much shorter than the one for A fibers excitation alone (Fig. 21).

Before any spinal lesion, single stimulation of the peroneal nerve elicited a reflex excitation which was followed by a silent period. Total nerve activity (in Fig. 22) was not significantly changed from spontaneous activity, which indicates that the increase and decrease of the sympathetic activity due to the excitation and the silent period compromised each other. On the other hand, total nerve activity was increased during train stimulation (in Fig. 22) despite a longer silent period than during single stimulation. This increased total nerve activity was due to the presence of the additional C reflex which was about twice the size of the A reflex (in Fig. 19). The increase of activity due to the additional C reflex overcame the decrease due to the prolongation of the silent period. These results suggest that the total nerve activity is determined by the relative contributions of the reflex excitation and the silent period. Koizumi and co-workers (51, 52) insisted that the pressor response (increase total nerve activity) elicited by afferent C fibers stimulation is due to reflex excitation which was not followed by a silent period. On the other hand, stimulation of afferent A fibers generated
a long silent period as well as a reflex excitation resulting in a net decrease total nerve activity (depressor response). The results of the present study suggest that both the sympathetic A and C reflexes generate silent periods. With the sympathetic C reflex, however, the contribution of the reflex excitation for the determination of total nerve activity is far greater than the contribution of the silent period, resulting in increased total nerve activity.

In case of train stimulation, total nerve activity was changed significantly only when the DLS area lesions were made (in Fig. 22). The fact that a decrease of total nerve activity by DLS area lesions occurred in spite of a decrease of the silent period (in Fig. 21), indicates this change of nerve activity was accomplished mainly by the abolishment of the C reflex. This suggests that the DLS area is the ascending sympathoexcitatory pathway and the sympathetic C reflex pathway as well.

In Fig. 22B, the total nerve activity was increased during single stimulation after DLF lesions in spite of a partial block of the A reflex excitation (Fig. 20). On the other hand, the silent period was markedly decreased by DLF lesions (Fig. 21B). Figures 20 and 21B suggest that this increase of total nerve activity after DLF lesions was accomplished by decreased silent period. However, the decrease of activity by additional DLS area lesions was due to a further decrease of
the silent period should result in a net increase of the total nerve activity. This partial block of the A reflex by the DLF lesions made a very interesting case, in which the total nerve activity was varied by evoked reflex activity and silent period alternatively. This apparent separate control of total nerve activity by the reflex excitation and the silent period suggests that the generation of the silent period may not necessarily depend on excitation. This evidence favors the proposal of Koizumi and co-workers (52, 53) that independent mechanisms were involved in the generation of the excitation and the silent period of the sympathetic discharge. This evidence does not support the proposal of Polosa (66) who suggested that the silent period is a post-activation depression of the sympathetic preganglionic cell body.

Ascending spinal pathways for the somatosympathetic A and C reflexes have not been previously studied. Results of the present experiments indicate that excitation of afferent A and C fibers selectively activate separate ascending spinal sympathoinhibitory and excitatory pathways, respectively. The present study not only localized the ascending spinal pathways for the sympathetic A and C reflexes, but also made a bridge between the concepts of afferent nerve specificity and ascending spinal pathway specificity regulating blood pressure response during afferent nerve stimulation.
B. General Discussion

Basically qualitative techniques were applied in the present study and all the main conclusions were drawn from comparative data, i.e., based on changes from control responses. Therefore, absolute values were often neither meaningful nor emphasized.

In the present experiments, sympathetic nerve recordings were made from several spontaneously active units. Not all sympathetic units are spontaneously active nor excited by afferent nerve stimulation but generally spontaneously active units are excited by afferent nerve stimulation and vice versa (42). Unit recordings were made instead of mass discharge recording from whole nerve because the silent period could be observed as well as reflex excitation. Sampling of the recorded unit population of the present study was probably skewed toward larger axons which are easier to record, although Jänig and Schmidt (42) claimed their recordings faithfully represented overall population of the axons. These effects may be minimized by recording from several units simultaneously. Multiunit recordings have some additional advantages over single unit recordings for the present experimental purpose. Inhibition of sympathetic nerve activity can be observed easily because the overall spontaneous activity level is higher with multi-unit recordings. Furthermore, it probably represents a better measurement of overall sympathetic outflow without
analyzing numerous single units because sympathetic units having different function may behave differently (41, 52). Therefore, attention can be focused on the central nervous structure with minimized variability of peripheral effects.

Afferent nerve fibers having different origins (muscle, skin, etc.) or sizes (A-alpha, -beta, -delta, C) elicit the somatosympathetic reflexes of different magnitude (41, 51, 52, 80, 84, 85). To activate all the afferent fibers which affect somatosympathetic reflex, mixed nerves (dorsal roots, radial nerve, peroneal nerve) were stimulated with suprathreshold intensity to C fibers in the present experiments. By activating all the afferent fibers, attention can be focused on the central organization without the complexity of sizes or origins of the afferent fibers, which is the ultimate goal of the present study.

Peripheral nerve stimulation can elicit pressor or depressor responses depending on the intensity (55, 69) and frequency (32) of stimulation. Thus, blood pressure responses can be reversed by changing stimulus parameters. Historically, two major theories have been proposed to explain this blood pressure reversal phenomenon. Hunt (38) suggested that some afferent nerve fibers were pressor while others were depressor. Thus, blood pressure responses were determined by the number of activated pressor and depressor afferent nerve fibers. On the other hand,
Ranson and associates (70, 71, 72, 74) proposed that this blood pressure reversal phenomenon was not due to the activation of two different fiber types. They suggested that stimuli of different intensities or frequencies applied to the same afferent fiber types selectively activate a distinct ascending spinal pressor or depressor pathway due to the characteristics of each pathway. The former theory has been studied and well documented by recording blood pressure or sympathetic nerve activity (reviewed in 50 and 82). It is clear that stimulation of different fiber types (A or C fibers) or afferent fibers having different origins (cutaneous, muscle, visceral, etc.) cause different blood pressure responses. However, the concept of dual ascending spinal pathways has received little attention despite the fact that the above two theories are not necessarily mutually exclusive. That is, different afferent nerve fibers may excite separate ascending spinal pathways. The present experiments confirmed Ranson's concept of dual ascending pathways in the cat spinal cord and further demonstrated that different afferent nerve fibers activated separate ascending spinal pathways. Thus, the present study combines these two controversial hypotheses forming a new theory. Figure 23 summarizes the new concept of blood pressure responses to peripheral nerve stimulation. Excitation of afferent A fibers selectively activate ascending sympathoinhibitory pathways in the DLF of the spinal
FIGURE 23
A NEW CONCEPT OF BLOOD PRESSURE RESPONSES TO PERIPHERAL NERVE STIMULATION
FIGURE 23

LEGEND

A diagram showing the new concept of blood pressure responses to peripheral nerve stimulation. Excitation of afferent A and C fibers selectively activate ascending spinal sympathoinhibitory and excitatory pathways, respectively. Sympathoinhibitory pathway has weaker excitatory and stronger inhibitory inputs to the supraspinal sympathetic integrating area. On the other hand, sympathoexcitatory pathway has stronger excitatory and weaker inhibitory inputs to the supraspinal sympathetic integrating area. DLS, dorsolateral sulcus area. DLF, dorsolateral funiculus. DR, dorsal root.
cord. This produces a small reflex excitation (A reflex) followed by a long silent period in the sympathetic nerves resulting in a net decrease of total sympathetic nerve activity (depressor response). On the other hand, excitation of afferent C fibers selectively activate ascending sympathoexcitatory pathways in the DLS area. This produces a large reflex excitation (C reflex) followed by a short silent period resulting in a net increase of total sympathetic nerve activity (pressor response).

Recently, Boivie and Perl (6) reviewed the extensive literature concerning the ascending spinal somatosensory pathways. None of the known pathways conforms to those described in the present study. The spinocervical system generally occupies a position similar to the ascending sympathoinhibitory pathway described. However, these two pathways differ in two aspects. First, the spinocervical system (61) is a unilateral (ipsilateral) system whereas the sympathoinhibitory pathway is a bilateral system. Second, the spinocervical system has much faster conduction velocities than the somatosympathetic pathway. Conduction velocities of the spinocervical system ranged between 17 and 103 m/sec, with a mean of 60 m/sec (11) whereas somatosympathetic pathway ranged between 5 and 30 m/sec (15, Results C4). Thus, the spinocervical and somatosympathetic pathways are apparently different systems although they occupy a similar area in the spinal cord.
Since noxious stimuli elicit sympathetic activity discharge (33, 36, 37, 43, 44), it is probable that the ascending somatosympathetic pathway is a nociceptive pathway. There are two possible relationships between the ascending spinal somatosympathetic pathway and the nociceptive pathway. First, the pathway for pain sensation and the one which elicits autonomic reflexes may be separate in the spinal cord. This notion is supported by Ranson's (71, 74) failure to block the conduction of pain by dorsal hemisection of the spinal cord. This intriguingly suggests that the central nervous system is specialized at the spinal cord level such that one afferent input connects to two different ascending afferent pathways according to their functions and central terminations. However, from an embryological point of view, it is not surprising that separate tracts in the spinal cord are organized according to their central connections. For example, the spinothalamic and spinocervical systems may receive similar afferent input but are destined for different terminations. A second possibility is the ascending spinal pathway for pain sensation and somatosympathetic reflex may be identical, at least in the cat. Based on behavioral studies after spinal lesions, Kennard (47) concluded that the pain pathway in cats is a bilateral system localized in the dorsal half of the spinal cord. This study seems to suggest that the pathways conducting pain sensation and autonomic reflex
may be identical. The question could be answered by a series of experiments comparing pain and autonomic responses to noxious stimuli in cats with selective spinal cord lesions.

Descending impulses originating from various parts of the brain (cerebral cortex, reticular formation, etc.) are known to influence and modulate sensory information processing in the spinal cord (reviewed in 62 and 88). Consequently, the pathways localized in the present experiments may be descending rather than ascending pathways, i.e., descending tonic influence on afferent input was withdrawn by making lesions in the spinal cord. This removal of the descending influences would block the transmission of sensory input to the ascending pathways located in some other area. This possibility can not be ruled out completely in the present experiments. It is reasonable to think that this possible descending influence affects afferent transmission to spinally, as well as supraspinally, mediated somatosympathetic reflexes. However, spinally mediated somatosympathetic reflexes remain even after total removal of descending influences by spinal cord transection (53, 77, 78, 81, 83). Therefore, the pathways described in the present experiments are most likely ascending systems although various descending system may modify the somatosympathetic reflex.

Kirchner et al. (49) reported that the somatosympathetic reflex recorded in the cervical sympathetic trunk
during the hind limb nerve stimulation, similar to the present study, has both spinally and supraspinally mediated components. Therefore, the ascending spinal pathways for the somatosympathetic A and C reflexes localized in the present experiments are probably the pathways for both spinal and supraspinal components of the reflexes. That is, the spinally and supraspinally mediated somatosympathetic reflexes share the same spinal ascending pathway.
CHAPTER VI

CONCLUSIONS

The following observations and conclusions can be obtained from the results of these experiments:

1. There are ascending pathways in the DLS area of the cervical and lumbar spinal cord which mediate pressor responses to afferent nerve stimulation.

2. In addition, ascending depressor pathways are present in the DLF which are anatomically separate from ascending pressor pathways.

3. Both ascending pressor and depressor pathways are bilateral and decussation to the contralateral side is extended about three segments rostral to the site of dorsal root entry for both pressor and depressor pathways.

4. Ascending pressor pathways are more responsive to higher stimulus frequencies than depressor pathways. Ascending pressor and depressor pathways compete with each other for final determination of blood pressure responses when afferent nerves are stimulated. Pressor responses dominate at higher frequencies, whereas depressor responses dominate at lower frequencies.

5. Recordings of sympathetic unit activity during
peripheral nerve stimulation confirmed the localization of ascending pressor and depressor pathways in the bilateral DLS area and DLF, respectively. This further suggested that the ascending pressor and depressor pathways are a spinal ascending limb of somatosympathetic reflexes. That is, ascending pressor pathways are sympathoexcitatory and depressor pathways are sympathoinhibitory pathways.

6. At a slow repetition rate (0.3 Hz), single shock stimulation of both A and C afferent fibers elicited sympathetic A reflex. When short trains of stimuli were given (train frequency 20-30 Hz), an additional C reflex component appeared. This sympathetic C reflex disappeared if the stimulus strength was lowered below the threshold of the afferent C fibers.

7. The size of the sympathetic C reflex increased as stimulus frequency increased. The sympathetic C reflex showed a recruitment phenomenon.

8. Average latency of the sympathetic A and C reflexes were about 73-296 msec and 435-889 msec, respectively.

9. Conduction velocity of the ascending spinal pathways for the somatosympathetic reflex ranged 5-25 m/sec.

10. The sympathetic A and C reflexes are mainly mediated by ascending sympathoinhibitory pathways in the DLF and sympathoexcitatory pathways in the DLS area of the spinal cord, respectively.

11. The silent period is mainly controlled by ascending
sympathoinhibitory pathways.

12. Excitation of afferent A fibers selectively activate ascending sympathoinhibitory pathways in the DLF of the spinal cord and this produces a small reflex excitation (A reflex) followed by a long silent period in the sympathetic nerves, resulting in net decrease of total sympathetic nerve activity (depressor response). On the other hand, excitation of afferent C fibers selectively activates ascending sympathoexcitatory pathways in the DLS area. This produces a large reflex excitation (C reflex) followed by a short silent period, resulting net increase of total sympathetic nerve activity (pressor response).
BIBLIOGRAPHY


APPENDIX A
A method of analysis was developed to express and compare the reflexly elicited nerve activity recorded from preparations which contain different numbers of nerve fibers. A basic assumption was made. Statistically, each nerve fiber of a given system behaves similarly. That is, each unit has the same spontaneous activity and elicits the same number of evoked activity when stimulated. Consequently, the number of evoked responses and the spontaneous activity are proportional to the number of recorded units.

Figure 24 shows an example of the theoretical nerve activity and the resultant poststimulus histogram. The theoretical nerve activity which has both spontaneous and evoked responses is shown in Fig. 24A. From the histogram in Fig. 24B, the evoked and spontaneous portions of the histogram could be averaged so that each portion forms a rectangle as shown in Fig. 24C. Both the evoked and spontaneous responses are proportional to the number of recorded units. That is,

\[ N_r = f_r \times D_r = C_1 n \]  
\[ N_s = f_s \times D_s = C_2 n \]

where \( N_r \) is the number of reflexly evoked activity,
\( N_s \) is the number of spontaneous activity,
\( f_r \) is the frequency of reflexly evoked activity,
\( f_s \) is the frequency of spontaneous activity,
\( D_r \) is the duration of reflexly evoked activity,
\( D_s \) is the duration of spontaneous activity,
\( C_1 \) and \( C_2 \) are the proportionality constants,
\( n \) is the number of recorded units.

Then,

\[ \frac{N_r}{N_s} = \frac{f_r \times D_r}{f_s \times D_s} = \frac{C_1}{C_2} = C \]

for a given \( D_s \), where, \( C \) is a constant.
FIGURE 24
A THEORETICAL NERVE ACTIVITY
An example of the theoretical nerve activity and its poststimulus time histogram. A: theoretical nerve activity including both spontaneous and evoked activity. B: the poststimulus time histogram of the nerve activity. C: evoked and spontaneous activity portions of the histogram were averaged so that each portion forms a rectangle. $D_r$, duration of evoked activity. $f_r$, frequency of evoked activity. $D_s$, duration of spontaneous activity. $f_s$, frequency of spontaneous activity.
From eq. (3),

\[ C \times D_s = \frac{f_r \times D_r}{f_s} \]  

(4)

A factor "Reflex Index (RI)" is defined as \( C \times D_s \).

From eq. (4),

\[ RI = C \times D_s = \frac{f_r \times D_r}{f_s} = \frac{N_r}{f_s} = \frac{N_t - N_s}{f_s} \]  

(5)

where, \( N_t \) is total nerve activity.

Then, the RI is a constant regardless of the number of recorded nerve fibers for a given system. The RI is also independent from frequency and duration of the evoked activity. The RI has a unit of time and indicates the relative size of the evoked activity. Positive and negative values of the RI indicate excitation and inhibition, respectively. RI value of zero means no net change of activity, i.e., continuous spontaneous activity.

To test the RI analysis technique, a series of post-stimulus time histograms (PSTH) for hypothetical nerve recordings were plotted by a PDP-12 computer. The hypothetical nerve recordings were simulated by generating a series of patterned pulses with four squarewave pulse generators (PG1, PG2, PG3 and PG4) and two gate devices (G1 and G2) as shown in Fig. 25. PG1 generates pulses every second as shown in Fig. 25A. The output of PG1 triggers the computer sweep as well as PG2. Then, PG2 generates a long duration pulse after a delay to simulate the evoked response duration. PG3 was used to determine the evoked response frequency and delivers high frequency pulses independently from PG1. Outputs of PG2 and PG3 were fed into G1 which is an AND gate device. The AND gate passes input signals to the output only when signals are simultaneously present in input 1 and 2. Therefore, the output of G1 (Fig. 25D) is a train of pulses with train-duration and impulse frequency determined by PG2 and PG3, respectively. This output was used for the simulated evoked response. The evoked activity was random to the beginning of the response train, as well as the computer sweep, because PG3 ran independent to PG1 and PG2. The simulated spontaneous
FIGURE 25
INSTRUMENTATION FOR SIMULATED NERVE RECORDINGS
Instrumentation for generation of simulated nerve recordings. Simulated nerve recordings were generated using four square wave pulse generators (PG1, PG2, PG3 and PG4) and two gate devices (G1 and G2). The gate devices G1 and G2 are AND and OR gates, respectively. Outputs of PG1, PG2, PG3 and PG4 are shown in A, B, C and E, correspondingly. The outputs of G1 and G2 are shown in D and F, respectively. The output of G2 was used as a response input to the computer for the histogram plotting. The output of PG1 was used as a computer trigger signal. PG2 was driven by PG1 whereas PG3 and PG4 were free running. Difference between evoked and spontaneous activities was emphasized by using dotted line for spontaneous activity.
activity generated by PG4 was random to the evoked response since PG4, PG3, and PG1 ran independently. The outputs of G1 and PG4 were fed into another gate device, G2, which is an OR gate. The OR gate pass input signals to output whenever signals are present in either inputs 1 or 2. Thus, the output of G2 is combination of the evoked response (output of G1) and the spontaneous activity (output of PG4) as shown in Fig. 24F. This G2 output was fed into the computer to plot PSTH.

Figure 26 shows simulated nerve recordings (upper part) and computer plots of PSTH (lower part) from three different conditions. All the PSTH were formed by adding 20 sweeps. Each hypothetical nerve unit was assumed to have a 2 Hz spontaneous activity and responses with one evoked spike for each stimulation. Figure 26A shows a recording from a hypothetical nerve preparation which contains two active units. Therefore, it has spontaneous activity of 4 Hz and gives two impulses for each stimulation. This was done by adjusting PG2, PG3 and PG4 for an evoked response duration of 80 msec, a frequency of 25 Hz and spontaneous frequency of 4 Hz. Figure 26B contains five active units. The spontaneous activity was 10 Hz and five evoked responses are generated for each stimulation. Evoked response duration and frequency were set at 100 msec and 50 Hz, respectively. Figure 26C has the same number of active units as Fig. 26B but has lower evoked response frequency (25 Hz) but a longer evoked response duration (200 msec).

The value of the RI should be same in the three different conditions in Fig. 26A, B and C. The expected and observed values of various parameters are shown in Table 1. Observed values were calculated from the computer readings of PSTH which were formed by adding 20 sweeps. As shown in Table 1, the values of the RI are constant and identical for all expected and observed cases. The RI is independent of the number of recorded units and duration or frequency of evoked activity as long as the basic assumptions are valid.

The RI can be applied not only to reflexly elicited responses but also to direct activated afferent responses. Positive and negative values of RI mean a net gain and loss of nerve activity, respectively. Therefore, the value of RI can be applied to excitatory, as well as inhibitory responses. It is easier to see inhibitory (greater negative RI value) response when higher spontaneous activity is present.

There are some limitations of the use of RI. The basic assumption which was made for the calculation of RI should be satisfied. That is, each unit should statistically
FIGURE 26
SIMULATED NERVE RECORDINGS

A

B

C
Simulated nerve recordings in three different conditions. The stimulus was assumed to be applied at time zero. Evoked and spontaneous activities were generated by a series of square wave pulse generators (see Fig. 24 and text). The upper part shows single sweep responses. For convenience the difference between evoked and spontaneous activity was emphasized using different amplitudes. The lower part shows poststimulus time histogram formed by computer after 20 sweeps were added. Both evoked and spontaneous activities were randomized by using pulse generators which run independently from the computer trigger signal.

A: recording from two active units. This recording has a spontaneous activity of 4 Hz and two evoked impulses with 40 msec interspike interval for each stimulation.

B: recording from five active units. This recording has a spontaneous activity of 10 Hz and five evoked impulses with 20 msec interspike interval for each stimulation.

C: recording from five active units. This recording has a spontaneous activity of 10 Hz and five evoked impulses with 40 msec interspike interval for each stimulation. Imp, number of impulses per bin (20 msec).
TABLE 1

REFLEX INDEX IN SIMULATED NERVE RECORDINGS

<table>
<thead>
<tr>
<th>CASE</th>
<th>NUMBER OF UNITS</th>
<th>$f_s$ (Hz)</th>
<th>$N_r$</th>
<th>$D_r$ (msec)</th>
<th>$f_r$ (Hz)</th>
<th>RI (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>80</td>
<td>25</td>
<td>0.5</td>
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<tr>
<td>B</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>100</td>
<td>50</td>
<td>0.5</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>200</td>
<td>25</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CASE</th>
<th>$N_t$</th>
<th>$N_s$</th>
<th>$N_r$</th>
<th>$D_r$ (msec)</th>
<th>$f_s$ (Hz)</th>
<th>RI (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6(120)</td>
<td>4(80)</td>
<td>2(40)</td>
<td>80</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>B</td>
<td>15(300)</td>
<td>10(200)</td>
<td>5(100)</td>
<td>100</td>
<td>10</td>
<td>0.5</td>
</tr>
<tr>
<td>C</td>
<td>15(300)</td>
<td>10(200)</td>
<td>5(100)</td>
<td>200</td>
<td>10</td>
<td>0.5</td>
</tr>
</tbody>
</table>
TABLE 1

LEGEND

Expected and observed values of Reflex Index in three different simulated nerve recordings. Values of the various parameters in this table are from the corresponding simulated nerve recordings in Fig. 25. The expected values are the parameters set to simulate each recording condition. Observed values are determined from the analysis of the plotted histograms in Fig. 25. Observed values shown in the table are averaged single sweep values which were taken from the direct readings (in parentheses) divided by the number of sweeps (20 times). Note only the value of Reflex Index is consistent in these different conditions.
behave similarly for both spontaneous and evoked activity. Another limitation is the RI value can be calculated only from the system which has spontaneous activity. The smaller the spontaneous level may cause the greater degree of error. The higher spontaneous activity system has two advantages. First, it has less chance of possible error. Second, it is easier to see inhibitory response if there is any.

Absolute value of RI or comparison of RI value in two different systems is meaningless since the intrinsic value of RI is different in every system. It should be used only in comparative sense within a system.
The dissertation submitted by Jin Mo Chung has been read and approved by the following committee:

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 6, 1989
Date  
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Director's Signature