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A Functional Analysis of the Innervation of the Canine Heart

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A FUNCTIONAL ANALYSIS OF THE INNERVATION OF THE CANINE HEART

by

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B I O G R A P H Y

James S. Wechsler was born on June 6, 1943, in New York City, New York. He attended Morgan Park High School, and then continued his studies at Northwestern University at the Evanston campus. After one year at Northwestern, he transferred to the University of Chicago, where he received a Bachelor of Science in Biochemistry in June, 1965.

In the fall of 1965, he began graduate training in the Department of Physiology, under the supervision of Dr. Walter C. Randall, Chairman of the Department. He served as a Graduate Assistant from 1965 to 1969, being supported by a Research Training Grant of the National Institutes of Health, National Institutes of General Medical Sciences.

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A C K N O W L E D G M E N T S

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I want to thank my parents for their support in this venture and I dedicate this dissertation to two people; the boy who said, "I want to be a plain man and work like my daddy does" and his little brother.

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L I S T O F F I G U R E S

STATEMENT OF THE PROBLEM

The heart and circulation can be and have been considered on many levels of organization, from the gross anatomical, through the functional organ-system to the molecular level. Each level of study generates new knowledge and the integration of such knowledge will result in a more comprehensive understanding of the cardiovascular system. It is with this thesis that I approach the functional study of the efferent and afferent innervation of the heart and the vascular system, drawing on information at all levels in order to understand this system.

From the time of Langley it has been known that the cardiac sympathetic preganglionic innervation takes origin from the thoracolumbar levels of the spinal cord. While the general knowledge of the pathways of the cardiac sympathetic efferent innervation has been fairly well documented in the past, lack of specific knowledge of the location of the synaptic stations of these nerves seemed to be a very important problem to which I should address myself. The general concept, not supported by any direct evidence, that all cardiac sympathetic synapses in the dog are made in the stellate ganglion generates the possibility of an error in the anatomical consideration of the autonomic nervous system which could have far reaching consequences. For example, if the synapses were not confined to the stellate ganglion, the value of stellectomy in causing degeneration of post-ganglionic sympathetic fibers would be questionable. Thus all studies based on the premise of this technique would be open to new interpretation.

During a consideration of the terminal pathway of cardiac sympathetic innervation I raised what I consider to be a crucial question. Where exactly are the cardiac sympathetic synapses made and how important is the stellate ganglion as a locale for these synapses? Initially I set out to answer this question. At the time that I was considering the synapses in the efferent cardiac sympathetic nerves I began to wonder as to the relative involvement of these pathways in cardiovascular reflexes. These sympathetic efferent pathways are physically quite close to the pathways taken by efferent vagal (parasympathetic) fibers.

Thus the second part of the study began as an attempt to understand the reflex activation of these efferent pathways. During the course of these experiments I found it possible to study the relative contributions of the cardiac parasympathetic and sympathetic innervation as well as the contributions of the adrenal medulla and the peripheral vasculature.

LITERATURE REVIEW

The history of the study of the efferent and afferent innervation of the cardiovascular system is like a tapestry woven with threads from many different origins yet somehow organized into a unified whole. Thus many different pieces of information obtained from seemingly unrelated experiments provide some understanding of the system.

The first thread in this tapestry involves the understanding of the autonomic nerves and the development of such understanding began with the studies of J.N.Langley. In 1878 Langley (82) noted, "The secretory function of the sympathetic nerve, as well as that of the chorda, is paralysed by atropine." Langley attributed this "paralysis" to nerve endings or gland cells, but this was the first indication that atropine might have an inhibitory effect at sympathetic ganglia. In 1898 Langley, in experiments in which the vagosympathetic trunk of the cat was joined to the severed cervical sympathetic trunk, found the regenerated vagus nerve, on stimulation, gave rise to "sympathetic" pupillary responses (85). He concluded from this experiment that

> "...there is no fundamental difference between the preganglionic fibers of the body, whether they belong to the cranial, the sympathetic or the sacral autonomic systems. And that any preganglionic fiber in the body is capable of forming functional connection with any nerve cell of the sympathetic type wherever found. I conclude further, that the function of any autonomic nerve fiber depends not so much on its inherent properties as upon the nerve cells with which it has an opportunity of becoming connected in the process of development.

> And I have earlier (1897) brought forward some facts to show that the function of the peripheral nerve cell depends upon the peripheral structure in which its axon has an opportunity of ending. So that, the physiological differences depend, in the main, upon the anatomical connections brought about by morphological laws."

While Langley elucidated important facts about the nature of the autonomic neurons, it was the work of Oliver and Schafer (104) that began the study of the neurohumors liberated by such tissues. They found that by freezing the adrenal glands they were able to isolate the active cardiovascular component almost exclusively in the adrenal medulla. They rightly supposed that any cardiovascular action by extracts of the adrenal cortex was due to diffusion of the active principle, known today as epinephrine, from the medulla to the cortex during the freezing process. In 1904 Elliot (55) proposed, on the basis of experiments involving the action of adrenal medullary extract on the innervated and denervated cat dilator pupil muscle and skeletal muscle, that,

> "...it cannot be that adrenalin excites any structure derived from, and dependent for its persistence on, the peripheral neuron. But since adrenalin does not evoke any reaction from muscle that has at no time of its life been innervated by the sympathetic, the point at which the stimulus of the chemical excitant is received, and transformed into what may cause the change of tension of the muscle fiber, is perhaps a mechanism developed out of the muscle cell in response to its union with the synapsing sympathetic fiber, the function of which is to receive and transform the nervous impulse. Adrenalin might then be the chemical stimulant liberated on each occasion when the im-

pulse arrives at the periphery."

Dale (44) added an interesting remark that Elliot's thesis, so nearly correct in principle, raised such an uproar among the physiologists of that day and was held to be in such disrepute that in a 1905 paper, Elliot (56) did not mention that he felt "adrenalin" to be the sympathetic transmitter. The mood of the time may be judged by a paper written by Langley in 1901 when he hypothesised that the action of adrenal medullary extract on denervated organs was due to some type of excitatory action of the active compound on the sympathetic nerve endings (86). At that time he did not believe that the locus of the response was at the postsynaptic site and he indicated that any hypothesis based on the direct action of a transmitter required very careful scrutiny. Apparently Elliot's 1904 paper did influence Langley because in 1905 Langley (87) compared effects of nicotine and curare at the cat neuromyal junction. He hypothesised that the difference in response to the two compounds could be explained in either of two ways; 1) nerve fibers in conjunction with cells give rise to different types of transmitters, 2) the same transmitter could have different effects because of differences in the post-synaptic region of the cell. Thus Langley was beginning to think in terms of transmitter theory. Elliot's 1905 paper represented an extensive study of the action of epinephrine on the bladder, heart, skeletal muscle and nerves of different species. He concluded that; 1) the action of epinephrine on smooth muscle was similar to the action of the thorace-lumbar nerves on smooth muscle, 2) the magnitude of the response was determined by the frequency of sympathetic impulses transmitted to a tissue, 3) any tissue responding to

epinephrine is indicative that the tissue is innervated by sympathetic nerves, 4) denervated smooth muscle shows an increased responsiveness to epinephrine with time (somewhat predating Cannon's demonstration of denervation supersensitivity (31) and 5) sympathetic nerves are not stimulated by epinephrine at their terminations but that exogenously applied epinephrine acts at the muscle cell surface.

Barger and Dale (9) studied the biological activity of various amines and it was they who coined the term "sympathomimetic". In their study they found that a demethylated derivative of epinephrine, later to be known as norepinephrine, had a greater sympathomimetic effect than the methylated parent compound when the similarities of those compounds to sympathetic nerve stimulation were compared. Dale (44) later indicated that it never occurred to Barger and himself that the demethylated compound could be the naturally occurring transmitter of the sympathetic nerves. Cannon and Bacq (28) showed that stimulation of the sympathetic nerves in intact animals caused a sympathomimetic substance to pass into the blood and they named this substance, Sympathin. This compound appeared to be like epinephrine in many ways, so much so in fact, that Bacq (7,8) hypothesized that Sympathin and epinephrine were identical. However, it was becoming clear that epinephrine and Sympathin were not identical. Cannon and Rosenblueth (30) found that after giving ergotoxin, the administration of Sympathin did not give rise to the "epinephrine reversal" which is a depression of blood pressure occurring after epinephrine is infused into an animal pretreated with ergotoxin. Sympathin caused an elevation in pressure. Such obviously different actions of the two compounds caused Cannon and Rosenblueth (30)

to develop their hypothesis of the two Sympathins, Sympathin E and Sympathin I. According to this hypothesis all adrenergic nerves release a common substance which Cannon and Rosenblueth hypothesized to be epinephrine. This epinephrine would then pass into the cells innervated by that neuron and once inside the innervated cell the transition to Sympathin E or Sympathin I would take place. Thus depending on the nature of the innervated cell, the epinephrine would be converted by suitable enzyme systems to either Sympathin E or Sympathin I. After the conversion reaction the Sympathin E or I would either excite or inhibit the target organ. For example, it was felt that most of the adrenergic mechanisms of the gastrointestinal tract involved the Sympathin I enzyme systems because stimulation of adrenergic nerves or the intravenous administration of epinephrine caused the relaxation of gastric smooth muscle. It was hypothesized that the compound released from the liver upon hepatic nerve stimulation was Sympathin E, because this compound caused an increase in blood pressure and heart rate.

Since there are blood vessels in the intestine which are constricted by neural stimulation, according to the sympathin theory, these vessels would contain effluent Sympathin E from the nerves. Sympathin I, passing into the vessels of the gastrointestinal tract, would be contaminated by Sympathin E from the innervated blood vessels. Thus it was hypothesized that pure Sympathin I could never be isolated. Because Sympathin I could act like Sympathin E, for example liver Sympathin E could relax gastrointestinal smooth muscle, it was also necessary to postulate that the two Sympathins could be interconverted by the target organ.

For thirteen years the Sympathin theory held sway and its first great challenge came in 1946 when von Euler (57),

in his classic paper, reported the isolation of the transmitter of adrenergic neurons from several different sources. In all cases the transmitter was norepinephrine, the demethylated derivative used by Barger et al. (9) in 1910 in their structure-activity study of amine action. In 1948 Ahlquist (2) developed his receptor theory which states that there were two post-synaptic sites of action of catecholamine, alpha-sites and beta-sites. These are excitatory and inhibitory sites and the classification of a site on the basis of a tissue response to catecholamine is no less arbitrary than the supposition that there are two enzyme systems changing one type of sympathin to another type of sympathin. The important difference between the Sympathin theory and the current concept is that norepinephrine is the adrenergic transmitter and that epinephrine is released mainly from the adrenal medulla. The receptor theory of Ahlquist (2) may be considered another modification of the receptor concept which dates back to Langley (87) who indicated that there may be post-synaptic "receptors" sensitive to neurohumoral substances.

While it is now generally accepted that the adrenergic nervous transmitter is nor-epinephrine there is currently a controversy as to the steps in the release of this neurohumor from a sympathetic nerve. This controversy had its roots in the work of Dale et al. (45) who found that stimulation of the vagus nerve in the atropinized cat yielded a positive chronotropic effect. Furthermore, twelve days after the removal of the superior cervical, inferior (middle) cervical and the stellate ganglia they were still able to elicit the positive chronotropic response after atropine. Jourdan and Nowak (77) reported the same finding in unatropinized cats, Kabat (78) reported this bivalent behavior of the vagus nerve in the dog and

it was initially explained that two types of fibers, according to Dale's (43) classification, adrenergic and cholinergic in the nerve which had been considered by many to be a "pure" cholinergic nerve tract. The concept that a "pure" adrenergic or cholinergic nerve exists is still held today by some workers and probably hinders understanding to some extent. Hoffmann et al. (71) found that in the isolated, atropinized heart of the dog, cat, rabbit and guinea pig, addition of acetylcholine produced a positive inotropic and chronotropic effect. These responses were abolished by ergotamine, curare and nicotine. After perfusing these hearts with acetylcholine, the perfusate was collected from the hearts and this solution stimulated the hypodynamic frog heart, relaxed the rectal caecum of the fowl and relaxed the small intestine of the rabbit. These are all bioassay techniques for the identification of adrenergic transmitters. Both the injection of acetylcholine and nicotine resulted in the liberation of this compound. It has been shown by Ferry et al. (60) that the stimulatory effect of acetylcholine may be due to a direct stimulation of post-ganglionic adrenergic nerves. Whether this phenomenon occurrs in the natural course of adrenergic neural action is open to debate at present. In 1946 von Euler (57) noted in the same paper which demonstrated the presence of nor-epinephrine in adrenergically innervated tissues that,

> "The presence of the sympathetic ergone in extracts of the vagus nerve...calls for some comment. As to vagus nerves it is known that it contains cardio-accelerator fibers, for instance in the cat, which might be taken as physiological evidence for the presence of adrenergic fibers in this nerve."

As a footnote to this remark, it was subsequently shown by Randall et al. (108, 109) that ventricular force of contraction

was also increased in response to stimulation of the vago-sympathetic trunk in the atropinized dog.

As knowledge of adrenergic fibers in the vago-sympathetic trunk accumulates, it becomes apparent that "pure" sympathetic nerves may not exist. In 1948 Folkow (62) et al. found that stimulation of the stellate ganglion in atropinized, eserinized cats and dogs produced detectable amounts acetylcholine in the coronary sinus effluent blood. Folkow (62) speculated that the acetylcholine might be derived from sympathetic cholinergic vasodilator fibers in the sympathetic outflows to the heart. In the same edition of the journal, Folkow et al. (63) reported the presence of sympathetic cholinergic vasodilator outflows to the hindlimbs of the cat and they reported that this system was atropine resistant. In 1959 Burn and Rand (26) stated,

> "Cholinergic fibers, which seem widespread in the postganglionic sympathetic supply, may liberate noradrenaline from the store at the nerve endings, and thus be adrenergic in effect."

This was the first mention of the hypothesis that acetylcholine causes the release of norepinephrine. In 1961 Brandon and Rand (16) found that after section of the splenic nerve the acetylcholine concentration of the spleen dropped to 78% of control values and the norepinephrine content dropped to 82% of control. Stimulation of the splenic nerves after neostigmine led to the release of a cholinergic-like substance (it caused the contraction of isolated guinea pig ileum and this action was blocked by atropine). Hemicholinium caused the spleen to fail in response to nerve stimulation while the administration of choline reversed this action. Administration of acetylcholine contracted the spleen and this action was not

blocked by atropine but it was reduced by reserpine and abolished by phenoxybenzamine, piperoxan and bretylium. Brandon and Boyd (15) injected acetylcholine intra-arterially into the isolated spleen and found that after treatment with reserpine no norepinephrine was released in response to the injection. After phenoxybenzamine there was an increase in the norepinephrine output of the gland. Brandon and Boyd explained the increased output of norepinephrine after phenoxybenzamine as a result of the weak anticholinesterase effect of the alpha-blocking agents. Blakely et al. (13) showed that physostigmine and neostigmine, anticholinesterase agents, did not increase the output of norepinephrine from the cat spleen while phenoxybenzamine did cause an increase as Brandon and Boyd had reported. Thus Blakely refuted the idea that phenoxybenzamine increased norepinephrine output by increasing the amount of acetylcholine in the vicinity of the adrenergic nerve endings. Arguments about the nature of adrenergic transmission based on the actions of these drugs may be tenuous at best because, as the work of Blaber and Karczmar (11) has shown, the mechanism of drug action in the neuromuscular junction, is not as simple as it first appears, and the action of drugs may not involve mechanisms which have been ascribed to them.

Ferry et al. (60) infused 10-250 mmg acetylcholine into the splenic artery of the cat and noted that these dosages evoked a centripetal discharge of C-fibers in the splenic nerve. This discharge was blocked by hexamethonium but not by atropine or hydergine (an alpha-blocking agent). C-fiber activation was still elicited after afferent denervation of the spleen. The technique of afferent denervation employed involved section of the splanchnic nerves just at the

level of the diaphragm. First one nerve was sectioned and then 10 days later the other nerve was sectioned. Ten days after the last operation the experiment was performed. It may be that the time allowed for degeneration was inadequate and that afferent C-fibers may not have degenerated. Afferent C-fibers of the type found by Hanson et al. (110) in the cat did not respond in this manner. Burn and Rand (27) had already formulated their hypothesis of adrenergic transmission which included the supposition that all post-ganglionic sympathetic fibers were cholinergic and that some other structure, or another part of the post-ganglionic neuron caused the release of norepinephrine. The difficulty in studying the mechanism of action of acetylcholine led Lee et al. to study the action of the cholinergic compounds in producing adrenergic effects on a simpler system. In 1960 Lee et al. (89) found that nicotine (2×10^{-5} g/ml) caused a positive inotropic effect in the four day old embryonic chick heart. This dose of nicotine also produced a transient positive chronotropic effect followed by a negative chronotropic effect. The same response was observed with tetramethylammonium iodide (10^{-4} g/ml) and acetylcholine (10^{-4} g/ml) when the heart was atropinized. Dichloroisoproterenol blocked the nicotine responses. It has been demonstrated histologically by Szepsenwohl and Bron (118) that the sympathetic innervation does not develop until the fifth day of life of the embryonic chick heart. Lee et al. (89) concluded that the positive responses were due to an intermediate structure which contains epinephrine-like materials. They felt that the compound was being released by nicotine, acetylcholine and tetramethylammonium iodide. Ignarro et al. (72) studied various aspects of catecholamine metabolism in the embryonic chick heart and found that catechol-

O-methyl transferase (COMT) and monoamine oxidase (MAO) are first detectable in the four day old chick heart and that these enzymes increase in concentration to the tenth day when their concentrations reach a plateau. The level of COMT in the four day old heart was 0.02 mumol metanephrine/hr/mg protein and the level of MAO was 2.0 mumol indolacetic acid/hr/mg protein. In the three day old chick heart COMT levels were not significantly different from zero and MAO activity was indetectable. In another study (73) they found that dihydroxyphenylalanine (DOPA) and its decarboxylated congener, dopamine were first detected in the embryonic chick heart on the fourth and sixth days, respectively. Cardiac levels of norepinephrine and epinephrine were first detected on the third day of incubation. Tyrosine hydroxylase was first observed on day 1, DOPA decarboxylase was first observed on day 2, dopamine B-oxidase was first observed on day 4 and phenylethanolamine-N-methyl transferase was first observed on day 6. In another paper (74) this group reported that tritiated epinephrine and norepinephrine were taken up by the heart as early as the fourth day of incubation. There was actually a decrease in uptake on the fifth day and then a gradual increase in uptake from the sixth to the tenth day. In embryonic hearts uptake of the labeled compound was exclusively found in the soluble fraction until the fifth day when uptake became apparent in the microsomal fraction. This correlates well with the histological observation that cardiac innervation does not begin until the fifth day of embryonic life. The amount of uptake by the microsomal fraction increased in parallel with the development of the sympathetic innervation of the heart. In the 3-5 day old whole embryo the tritiated norepinephrine was found primarily in the microsomal fraction. Tritiated norepine-

phrine in the microsomes began to increase relative to that in the soluble fraction on the fifth day. Reserpine, cocaine and low temperature markedly altered the ability of five day old hearts to take up norepinephrine but four day old hearts were not so affected.

The embryologic information has indicated that acetylcholine exerts a positive inotropic and chronotropic effect on a heart which does not have adrenergic innervation, but does seem to possess the metabolic mechanisms necessary for some of the reactions which have been regarded as associated with the adrenergic nervous system. Another interpretation of the apparent relation between acetylcholine and norepinephrine has been proposed by Leaders (88). He has postulated a "cholinergic-adrenergic interaction". This theory was based on the assumption that the mammalian vagus nerve is "purely" parasympathetic and the stellate to be "purely" sympathetic, in the sense that the former was cholinergic while the latter was adrenergic. Leaders theorized that there may be an interaction between adrenergic and cholinergic fibers such that acetylcholine from one fiber may act on another fiber to cause the release of norepinephrine and similarly, norepinephrine could affect the release of acetylcholine. Leaders' hypothesis can be explained on the basis of mixed nerve trunks. The point he made, however, is that the interaction occurs at the level of the target organ.

This idea of integration at the target organ level has been given histological confirmation of a sort by the work of Jacobowitz (75) who has shown the presence of chromaffin cells in the hearts of several species and has indicated the location of cholinergic post-ganglionic cell bodies in intimate association with the chromaffin cells.

The apparent interrelationship between acetylcholine

and norepinepharine makes it mandatory to consider acetylcholine itself. Cleghorn (32) found that an aqueous extract of sympathetic ganglia caused the isolated turtle heart to stop beating, caused a depression in blood pressure, but had no effect on cat pupillary muscle. He concluded that the extract from the ganglia bore no relation to the substance liberated from the sympathetic nerves. In 1906 Dixon (48) hypothesized that the heart contained a substance, named "proinhibin", which was converted to "inhibin" after vagal excitation. This compound would then combine with the cardiac muscle resulting in vagal arrest. Dixon believed the chemical action to be similar to that for secretion and pancreatic function. A great step forward in the knowledge of acetylcholine came in 1914 when Dale (42) found, after testing the action of choline derivatives in the cat,

> "In the action of choline, and, with varying degrees of intensification, in the action of certain ethers and esters of choline, two distinct types of action can be detected- a "muscarinic' action, paralysed by atropine, and a 'nicotinic' action, paralysed by excess of nicotine."

This represented the first statement that acetylcholine or other choline derivatives had a dual action. Also Dale speculated that acetylcholine might be the physiologically active compound involved in the organism. In 1915 Burn and Dale (25) found that tetraethylammonium was a nicotinic blocking agent and unlike nicotine, it did not cause a transient stimulation before the onset of blockade. Cowan and Walter (36) found that tetraethylammonium iodide in the perfused frog nerve preparation in concentrations of 15 mM/L or higher caused spontaneous potentials in the nerves, however no evidence of a stimulant action of TEA has been shown in the ganglion. Paton (106) found that in a series of polymethylene

bistrimethylammonium salts, hexamethonium had the greatest action as a ganglionic blocking agent while decamethonium had the greatest action as a neuromuscular blocking agent. The tissues tested were the cat superior cervical ganglion and the cat tibialis muscle.

Koelle and Koelle (80) studied the distribution of acetylcholinesterase in the cat superior cervical ganglion through the use of denervation techniques, ambenonium (a reversible anticholinesterase) and diisopropylfluorophosphate (DFP, an irreversible anticholinesterase). They found that essentially all the functional acetylcholinesterase was present in the presynaptic terminals. They proposed a three-fold function for this acetylcholinesterase; 1) it might prevent post-synaptic activation by acetylcholine during the resting phase, 2) it might prevent the accumulation and spread of acetylcholine during the course of repeated preganglionic volleys and 3) it might protect against antidromic firing of the presynaptic fibers. Utilizing denervation techniques, DFP, and the close arterial injection of acetylcholine and carbamylcholine Volle and Koelle (121) found that acetylcholinesterase appeared to have three roles in sympathetic ganglionic (Superior Cervical ganglion) transmission; 1) limitation, temporally or spatially, of the transmitter action of acetylcholine at the post-synaptic site, 2) protection of the presynaptic nerve terminals against endogenously liberated acetylcholine and 3) prevention of the accumulation and action of acetylcholine liberated during the resting state. From their experiments they felt that presynaptic acetylcholinesterase did not function in the genesis of choline sources for uptake and resynthesis by the terminals of the presynaptic fibers. As Blaber has indicated in a personal communication,

the theory of muscarinic and nicotinic receptors on the postsynaptic membrane of necessity means that acetylcholinesterase could not be present since the acetylcholine has to diffuse from the nicotinic receptor to the muscarinic receptor. Thus the system would not function with postsynaptic acetylcholinesterase present.

It is generally assumed that a majority of cardiac sympathetic synaptic connections in the dog are made in the stellate ganglia. The observations of Bronk et al. (18,19,20) have been interpreted to indicate that the fibers issuing from the cat stellate ganglion are totally post-ganglionic since impulse traffic in the fibers was said to be abolished by painting the stellate ganglion with nicotine. Woollard (130) observed that, "removal of the stellate ganglia alone may be insufficient to remove all the post-ganglionic fibers of the sympathetic system, for in the cat some may come from the cervical region and possibly from the thoracic ganglia." He reported that fibers considered to be sympathetic based on fiber size were still present, although in reduced amounts, in the cardiac tissue of the cat after bilateral stellectomy. Nettleship (98) compared the histological results obtained in the cat heart after bilateral stellectomy and bilateral stellectomy plus bilateral middle cervical ganglionectomy. He noted, "In the latter operation there was no increase in the area over which degenerating nerve trunks were found but individual trunks had a greater fiber loss." Nonidez (103) found that many preganglionic fibers enter the middle cervical ganglion of the dog while many postganglionic fibers leave the ganglion. Recently Brown has shown that a number of preganglionic fibers may "run through" both stellate and caudal cervical ganglia and terminate within or near the walls of the

heart (23). In addition some sympathetic fibers have been shown to synapse within the ventral roots of the spinal cord (3).

Feldberg and Gaddum (59) demonstrated that electrical stimulation of the eserinized, perfused cat superior cervical ganglion resulted in the collection of acetylcholine in the effluent perfusate. The active constituent of the perfusate was indistinguishable from acetylcholine when compared in six bioassay tests. They concluded that acetylcholine was the transmitter at sympathetic ganglia. In 1935 Eccles published a series of three papers dealing with transmission in the cat superior cervical ganglion. In the first paper (49) he recorded four types of potentials which he considered due to the presence of four different types of ganglion cells. More important than this was his demonstration of a late positive and a late negative wave in his extracellular recordings. In the second paper (50) he demonstrated inhibition and facilitation in the ganglion and in the third paper (51) he indicated that the slow negative and positive potentials could be related to inhibition or facilitation of ganglionic transmission. He hypothesized that this action was similar to the inhibitory and excitatory action of the spinal cord which Eccles and Sherrington (53) had suggested in 1930. Eccles concluded that the sympathetic ganglion was more than just a way station for transmission but represents a possible locus for integrative activity. In 1939 Bronk (18) noted that post-ganglionic cell bodies were activated either by spatial summation (simultaneous activation of one post-ganglionic cell body by several different pre-ganglionic impulses) or temporal summation (summation by volleys of pre-ganglionic stimulation).

Marrazzi (93) in 1939 studied the action of pilo-

carpine and atropine on the response of the cat and rabbit superior cervical ganglia to electrical stimulation. He found that pilocarpine increased the response while atropine decreased the response. Marrazzi also found in the cat that,

> "epinephrine in large or in small doses causes depression of the response of the superior cervical sympathetic ganglion to repetitive stimulation of its preganglionic trunk by constant, submaximal shocks." (94)

Bulbring (24) examined the response of the cat nictitating membrane to electrical stimulation of the perfused superior cervical ganglion. She found that at low doses epinephrine facilitated the response, while at high doses the response was inhibited. However, Marrazzi and Marrazzi (95) recorded extracellular potentials and were unable to demonstrate facilitation with even lower doses than used by Bulbring. They indicated that a deteriorating preparation did show facilitation with epinephrine. In 1952 Lundberg (92) found that norepinephrine was one fourth as potent as epinephrine as an inhibitor of ganglionic transmission in the cat superior cervical ganglion. He found that dihydroergotamine (an alpha blocking agent) effectively blocked the action of infused epinephrine in ganglionic transmission. He also showed that dihydroergotamine had no effect on inhibition produced by a tetanic electrical stimulation. Lundberg reasoned that epinephrine did not play a functional role in ganglionic transmission. Reinert (112) found that norepinephrine could be collected from the perfusate of the cat superior cervical ganglion and that the output of norepinephrine increased during orthodromic or antidromic stimulation. He found that monoamine oxidase inhibitors increased the level of norepinephrine but did not alter the recorded extracellular

evoked potentials and that an inhibitory effect of reserpine was related to osmotic changes in the ganglion rather than true excitability changes due to norepinephrine depletion. He agreed with Lundberg's conclusion that catecholamine had no role in ganglionic transmission. DeGroat and Volle (47) found that intraarterial administration of norepinephrine and epinephrine did not alter the ability of the cat superior cervical post-ganglionic cell body to fire in response to acetylcholine or nicotine. However, the catecholamines enhanced a late occurring atropine sensitive discharge. This after-discharge was potentiated by alpha adrenergic blocking agents and antagonized by beta adrenergic blocking agents. They hypothesized that norepinephrine and epinephrine caused hyperpolarization and depolarization, respectively. They noted that isoproterenol (a beta adrenergic stimulant) reduced the efficacy of a KCL induced depolarization but not that of a $BaCl_2$ depolarization. They hypothesized that the beta sensitive inhibition was related to a change in postsynaptic membrane permeability to K^+. Most of the drug doses were quite high and how much the results reflect a normal physiological response is open to question.

In 1961 Eccles and Libet (54) investigated the properties of ganglionic transmission in the curarized superior cervical ganglion of the rabbit. They developed a model for ganglionic transmission based on the action of different drugs on observed evoked extracellular potentials. The model involves the hypothesis of three different receptor sites on the postganglionic cell body, a muscarinic site, a nicotinic site and a catecholamine sensitive site. Chromaffin cells associated with the post-ganglionic cell body are hypothesized to contain atropine sensitive sites and release epinephrine upon

stimulation. In the Eccles-Libet model epinephrine produces the N potential (fast depolarization) and the LN potential (slow depolarization). Takeshige and Volle (119) recorded surface potentials from the superior cervical ganglion of the cat and responses to acetylcholine and tetramethylammonium administered via intraarterial injections. They found that the surface potential was altered by both acetylcholine and tetramethylammonium (TMA) but that the TMA effect was blocked by hexamethonium but not by atropine while acetylcholine was blocked by both compounds. They expanded their study in another paper (120) in which they studied the alteration of ganglionic responses to cholinomimetic agents following conditioning of the ganglion (cat SCG) by preganglionic stimulation or pilocarpine or physostigmine. Responses to injected acetylcholine, tetramethylammonium and methacholine were the same except that pilocarpine conditioning resulted in no change in the late post-ganglionic discharge while the other conditioning processes increased this discharge. They postulated that the atropine sensitive sites, their H and LD sites (corresponding to Eccles' (54) N and P sites) were important as modulators of transmission. Riker (113) showed that the response of the isolated frog VIIth ganglion to acetylcholine infusion differed from the response obtained to orthodromic electrical stimulation. He demonstrated that acetylcholine induced spikes were antidromic in nature. It will be recalled that Ferry (60) demonstrated that acetylcholine infusion elicited antidromic firing in C fibers in the cat spleen. Gebber (63) studied the perfused cat superior cervical ganglion and found that the positive after-potential recorded by bipolar electrodes appeared to be inversely related to the production of C_6 sensitive discharge evoked by infused acetylcholine. As the preganglionic fibers

were electrically stimulated at varying parameters, only those parameters which decreased the positive afterpotential resulted in a facilitation of the response to infused acetylcholine. Atropine did not affect these discharges.

Gebber (62) in another study employed close arterial injections and bipolar electrodes recording from the cat superior cervical ganglion. He found that: 1) tetanic preganglionic stimulation antagonised the block occurring during depolarisation evoked by nicotine, 2) d-tubocurare antagonised depolarisation evoked by nicotine but enhanced the simultaneously occurring block of transmission, 3) the intravenous infusion of nicotine (30-100 micrograms/kg/min.) evoked a postganglionic discharge which was maintained when transmission was abolished and 4) discharges evoked by non-nicotinic stimulating agents (5-hydroxytryptamine, methacholine) were enhanced during the blockade of transmission and depolarisation evoked by nicotine. Gebber concluded that the blockade of transmission and the simultaneously occurring ganglionic depolarisation by nicotine were not causally related.

Gebber and Snyder (64) compared the effect of the close arterial injection of tetramethylammonium (TMA) and the constant infusion of TMA in the cat superior cervical ganglion. They found that the injection of TMA produced a response blocked by C_6 but not by atropine, confirming the findings of Takeshige and Volle (119). The constant infusion of TMA produced a response blocked by both C_6 and atropine. Varying the infusion rate did not alter the character of the response. They concluded that infusion of TMA initiated an interaction between the C_6 sensitive and atropine sensitive excitatory sites. They proposed that the spread of depolarisation from the C_6 sensitive site markedly enhanced a weak muscarinic

stimulating action of TMA. With an injection of acetylcholine, the response was blocked by C_6. The infusion of acetylcholine produced a discharge blocked by atropine but not by C_6. The conclusion they draw is that both sites must be activated to have successful transmission.

In none of the three sets of experiments do the authors indicate whether infusion of the agents caused stimulation of presynaptic fibers. If Riker's finding of the antidromic nature of acetylcholine-induced spikes are applicable, it may be that some of the results to infusion and injection might be due to the "unphysiologic" release of acetylcholine by the preganglionic neuron in response to TMA or nicotine. The weak muscarinic effect of TMA might be caused by acetylcholine release.

Much of the informative work on ganglionic transmission has been derived through the use of intracellular recordings of ganglionic potentials. In 1953 Blackman et al. (12) showed that there appeared to be a quantal discharge of transmitter from the pre-ganglionic sympathetic fiber of the frog to orthodromic electrical stimulation. Nishi et al. (102) extended Blackman's work and on the basis of intracellular potential measurements of the bullfrog sympathetic ganglion and bioassay techniques, calculated quanta and the quantal size associated with pre-ganglionic fibers of the sB and sC type. The bullfrog is interesting in that preganglionic B fibers synapse only with postganglionic B fibers and preganglionic C fibers synapse only with postganglionic C fibers. Nishi et al. calculated that sB synaptic knobs yielded approximately 2 quanta per impulse with 8,000 molecules of acetylcholine per quantum. Similarly they calculated that sC preganglionic synaptic knobs yielded approximately 5 quanta

per impulse with 12,000 molecules of acetylcholine per quantum. Libet (90) analyzed slow potentials in the rabbit superior cervical ganglion and he showed the presence of long latent periods for the onset of slow inhibitory and excitatory post synaptic potentials. He found that the onset time for the IPSP was 35 msec. and the onset time for the EPSP was 200-300 msec. These slow potentials were enhanced by a specific anti-acetylcholinesterase (B.W.284) and little affected by diisopropyl fluorophosphate (used as a specific inhibitor of butyrlcholinesterase). He concluded that the slow potentials were cholinergic in origin. Libet indicated, "The slow post synaptic potentials provide models of slow neuronal responses which may have relevance in the autonomic and central nervous systems." Brown (23) demonstrated that atropine partially blocked synaptic transmission in cardiac sympathetic adrenergic pathways of the cat, indicating the importance of the muscarinic receptor site in neural function. Nishi and Koketsu (101) recorded from bullfrog sympathetic ganglia using the sucrose gap technique and looked specifically at the slow inhibitory post synaptic potential. They found that this P potential was independent of Cl^-, enhanced by an increase in Na^+ and depressed by a decrease in K^+. They concluded that the P potential may be produced by the synaptic activation of an electrogenic sodium pump in the ganglion cells. They also demonstrated that the P potential originates in the proximal portion of the post-ganglionic axon rather than somewhere in the cell body. In another study Nishi and Koketsu (100) studied the genesis of early and late afterdischarges in bullfrog sympathetic ganglia with the sucrose gap technique. They determined that the afterdischarge in postganglionic sympathetic fibers after preganglionic stimulation was composed of

two separate components differing in origin; 1) EAD (early after discharge) which is cholinergic in nature and triggered by the LN potential and 2) LAD (late after discharge) which is non-cholinergic in nature and is triggered by a very slow post-synaptic depolarization which is the LLN potential of the ganglion. They concluded that the LN potential was a slow EPSP which triggered the early after discharge, the LLN was a late slow EPSP which triggered the late after discharge and was non-cholinergic in nature. In another study Koketsu and Nishi (81) demonstrated that preganglionic sympathetic fibers of the bullfrog are depolarized by the action of acetylcholine released from the presynaptic neurons. In view of this finding by Koketsu and Nishi it is interesting to compare the results of Riker (113) who showed that transmission block may occur at unmyelinated portions of the presynaptic sympathetic fiber of the bullfrog. These two bits of evidence may lend credence to the "percussion" theory of acetylcholine release as initially proposed by Koelle (79). It is therefore possible that blockade of transmission could occur at the presynaptic fiber by blocking the action of acetylcholine on the presynaptic nerve terminal.

Libet and Tosaka (91) have successfully recorded intracellular potentials from ganglion cells of the rabbit superior cervical ganglion. They have demonstrated that post-ganglionic cells have three separate sites resulting in fast EPSP's, slow EPSP's and slow IPSP's. They have also demonstrated that preganglionic fibers can be specific in eliciting either slow EPSP's or slow IPSP's. Jacobowitz (76) has shown histologically that htere appear to be collaterals of sympathetic post-ganglionic fibers ending in the region of other postganglionic cell bodies. It is tempting to speculate that

there may be inhibitory action of one postganglionic neuron upon another postganglionic neuron.

Whether this speculation will be justified in the future is uncertain but it should be noted that Libet and Tosaka's demonstration of three sites does offer support to the model proposed by Eccles and Libet in 1961.

Hanson et al. (111) found that the nodose ganglion of the vagus nerve in the cat showed no evidence of any synaptic connections but did contain bipolar and unipolar cell bodies. Heinbecker and O'Leary (68) found that the vagal cardiac efferent fibers were derived from cells within the central nervous system and they confirmed the work of Hanson et al. in that they did not find any evidence of synaptic connections in the nodose ganglion and they hypothesized that the cell bodies in the nodose ganglion were predominantly afferents. They also concluded that the cervical sympathetic trunk contributed little to the non-myelinated fibers of the vagus nerve but recently Nielsen et al. (99) reported that a significant number of cardiac adrenergic fibers do cross over to the vagal part of the cat's vagosympathetic trunk. They also showed that bilateral superior cervical ganglionectomy resulted in a 22-27% decrease in the level of cardiac norepinephrine.

Foley et al. (61) compared normal cats and vagal rhizotomized cats and determined, histologically, that 65-80% of the fiber population are afferent and 20-35% of the fibers are efferent at the cervical level. Agostoni et al. (1) confirmed these values in a study of the distribution of the cat vagosympathetic trunk to the heart, lungs and abdominal viscera.

Heymans and Ladon (70) demonstrated the action of

the aortic baroreceptors and this was confirmed by Anrep and Segall (5). The aortic chemoreceptors were demonstrated first by Heymans and Heymans (69) and localized by Comroe (33). Boss and Green (14) reported the presence of ancillary baroreceptors in carotid arteries of the dog and cat. They indicated that these baroreceptors send afferent fibers which ascend in the vagosympathetic trunk. Aviado et al. (6) demonstrated the presence of pressoreceptors in the right atrium which, when stimulated, resulted in a reflex bradycardia and a vasodilitation. Ventricular stretch receptors have been demonstrated by Whitteridge (128) and confirmed by Paintal (105). The timing of these fibers has been related to electrocardiographic tracings while it seems that they should be related to ventricular pressure curves if they are to be meaningfully interpreted. Sleight and Widdicombe (115,116) have provided evidence for epicardial and pericardial stretch receptors. It should be stressed at this time no involvement of ventricular, epicardial or pericardial stretch receptors has been demonstrated in cardiovascular reflexes.

Brodie and Russell (17) electrically stimulated the central ends of the cut pulmonary vagi in the cat and demonstrated profound reflex bradycardia and hypotension. Anrep et al. (4) later found that some efferent vagal fibers looped down into the pulmonary vagi and then passed up and back down toward the heart.

DeBurgh Daly et al. (46) have shown that pulmonary inflation results in a reflex hypotension but their records do not show nor do they report an associated bradycardia.

Comroe and Mortimer (34) have stressed the differences in the responses of the aortic and carotid chemoreceptors. The aortic chemoreceptors, upon stimulation resulted in tachy-

cardia, arterial hypertension and slight hyperpnea while the carotid chemoreceptors result in bradycardia, hypotension and marked hyperpnea.

Brown (22) has demonstrated the presence of a reflex bradycardia and hypotension resulting from distension of the left coronary artery in the cat. He found that the vagus nerve carried the afferent part of the reflex loop.

Glick and Covell (67) demonstrated in a cross circulation experiment that when either the carotid or aortic baroreceptors increase their firing rate, the animal responds to the positive stimulus (increase in receptor firing) over the negative stimulus (decrease in receptor firing) when the two receptor areas are compared.

METHODS

The major tool of measurement in this study is the strain gauge arch. It is therefore crucial to examine the development of this instrument and by examining this development an understanding of exactly what such a device measures may be determined.

The development of the strain gauge arch as applied to the study of the heart owes its origin to the studies of Roy and Adami (114) who developed the myocardiograph, a device used in conjunction with a kymograph to study the contractile responses in the in situ heart. Cushny (38,39,40,41) made extensive use of this device and the device became known as the "Cushny Myocardiograph". Essentially the myocardiograph consisted of two limbs which were sutured on either side of an area of myocardium or epicardium to be measured. One limb of the apparatus acted as a fulcrum and the other limb acted as a lever which was mechanically linked to the recording drum. Ignoring the resistance of the lever to movement, the machine measured the change in length of the particular myocardial segment under study, the lever system amplifing this change.

In a study of the effects of different drugs on right ventricular contractility, Walton and Brodie (122) introduced a modification of the myocardiograph which employed a calibrated spring which could be stretched to oppose the development of tension by the segment of myocardium under study and thus the force or tension applied by the spring was used as an index of the tension of the myocardium. At the peak of

the response to an infusion of epinephrine, for example, the spring was tightened to such an extent that it represented an opposing force of 98 grams, therefore they felt that the heart, under the limbs of the gauge, developed a force of 98 grams. Walton et al. (123) demonstrated the same device at the A.P.S. in 1949, but with one noteworthy change. The spring element had been replaced with a strain gauge element. The introduction of the strain gauge element made the strain gauge an apparently isometric device. "Apparently" because on closer examination one observes that in order for the gauge system to indicate a change in force the resistance element of the strain gauge arch must be deformed.

Walton, Leary and Jones (125) compared the myocardiograph with a strain gauge element to a strain gauge arch and reported that there was no appreciable difference between them. However, these workers did not consider the strain gauge arch to be giving an absolute value for the force generated by the muscle under the gauge. Also they chronically implanted the strain gauge arches for three months in dogs and reported only slight fibrosis around the gauge. Walton, Cotten, Brill and Gazes (124) found no appreciable difference in the response of the two gauges to the same strains generated by right ventricular myocardium in response to epinephrine infusion. In the light of the work done by Szentivanyi et al. (117) the responses of two separate areas of a single ventricular chamber would not be expected to give rise to the same responses when fine branches of the autonomic nervous supply to the heart are stimulated but the agreement between two separate gauges can be expected to be closer in the face of more generalized interventions such as stellate stimulation or catecholamine infusion. Cotten (35) studied the relationship of various parameters of

cardiac function to the force recordings and he noted that stretching the muscle under the gauge by 50% seemed to produce recordings which were free of artifacts. In my studies this "stretching" procedure was routinely followed. Also Cotten showed that the depth of suture placement used to anchor the gauge seemed to be important in that the deeper the sutures the greater was the magnitude of the force recording. In my studies I have attempted to standardise the depth of my suture placement to about 2 millimeters but there is of course some variability. Cotten also noted the maximum control contractile force occurred when the underlying muscle was stretched to 50% over its "resting" length. Any investigator who claims that his strain gauge measurements are recording the absolute force developed by the myocardium is making a grave error in judgement if one accepts the previously quoted work. It is much more sensible to state that a strain gauge can be used to monitor changes in the force of contraction of the heart. It is meaningless to speak of measuring the force of contraction in grams and using that value as a common factor in a table of data since the previously mentioned variables can so alter the quantitative nature of the response. Thus any attempt to compare the experimental results on the basis of grams of force is indefensible. Quantitation of the strain gauge response is not possible but utilization of the per cent change of a response from a control level as a means of making comparisons is more useful. Since comparisons of per cent change are only valid if the strain gauges respond in a linear fashion, a strain gauge calibrator was built to test the linearity of response. The calibrator consists of a heavy brass plate which was machined such that one limb of a strain gauge arch could be firmly clamped to the plate. The other limb was then attached

to a thread which passed over a pulley and weights from 1 to 200 grams could be suspended from the thread to test the gauge response. The gauges were tested at different amplifications of the Grass model 7 polygraph which was utilized in actual experiments. The amplifications were from 0.5 mv/cm pen deflection to 20 mv/cm pen deflection and in this range the gauges were found to respond linearly. Since the same gauges were always applied to the same regions of the heart in experiments utilized in the tables, while the slopes of different gauges varied slightly (that is the change in pen deflection per unit weightchange), the responses of a particular region of the heart could be compared on a per cent change from control basis for all dogs of a series. It should be noted that the strain gauges are linear over a wide range of distention. Thus while the gauges may be fixed at various tensions when sutured to the epicardium the applied tension may be determined on the basis of the offset voltage required to bring the strain gauge arch to the same baseline present before the gauge was sutured in place. In practice, at a sensitivity of 20 mv/cm when full span was equivalent to forces in excess of 200 grams, the offset voltage or alternatively, the shift in baseline was never more than equivalent to 40-50 grams. Of course there was variability from preparation to preparation but the offset technique indicates that the gauges are being stressed over a linear portion of their performance.

In all the experiments of a series incorporated into a table, I attempted to be certain that gauge placement was comparable from experiment to experiment. Thus the gauge placed on the right ventricular sinus region was always placed in the same ventral-lateral position and perpendicular to the division of the right and left ventricles.

The experiments were conducted in three separate modes of anesthesia, 1) phencyclidine HCl (2.0 mg/kg) and a-chloralose (80 mg/kg), 2) alpha-chloralose (100 mg/kg), 3) phencyclidine HCl (2.0 mg/kg) and alpha-chloralose (60 mg/kg).

The first anesthetic combination was used in the initial phases of the study on the localization of the synaptic connections in the cardiac sympathetics. This combination was used until Priola (107) reported that phencyclidine HCl acted as a beta-blocking agent and then the second anesthetic combination was used. However, there did not appear to be any noticeable difference between animals anesthetized with the first combination and animals anesthetized with the second combination so the first anesthetic combination was utilized in later experiments. In the study of afferent pathways it it was decided that the anesthetic dose of chloralose should be reduced so as to make sure that deep anesthesia, which might seriously depress reflex responses, would not be a problem. Thus the 60 mg/kg dose of alpha-chloralose in conjunction with the phencyclidine HCl produced a lighter level of anesthesia but not so light that the animal gave the appearance similar to that of being "anesthetized" with an agent like d-tubocurare.

While phencyclidine HCl may exert its anesthetic effects through some form of beta-blockade the mechanism of action of chloralose is not clear at this time but if, as Eccles (52) had indicated, it acts by prolonging the phenomenon of presynaptic inhibition then it is possible that such a prolongation of presynaptic inhibition might affect some of the responses which are mediated at least in part through the mechanism of presynaptic inhibition.

Blood pressure was measured by means of a P23AC

pressure transducer with a catheter of PE 50 polyethylene tubing. In the experiments dealing with the cardiac sympathetic synapses blood pressure was taken from the right femoral artery while in the study of afferent vagal pathways the aortic pressure was taken by means of a catheter passed through the internal thoracic artery into the ascending aorta. While the responsiveness of the P23AC transducer is admittedly inferior to that of a transducer such as a P23DB it should be noted that the model 7 polygraph used in all the experiments reported here has a maximum frequency of 45 cycles per second. Thus the analysis of fast transient responses might be in error. However, in these experiments fast transients such as the rate of rise of aortic pressure or the rate of rise of the strain gauge recordings were not measured and thus this criticism cannot be leveled in this instance. It might be argued that the measurement of a peak response with these techniques is open to the same criticism and this may be valid. The major points in this dissertation, however, are of a more qualitative nature and involve changes which are more of vector quantity than a scalar quantity. Thus direction is far more important in determining the nature of the response than the magnitude of the response.

The responses were initiated by means of electrical stimulation delivered through bipolar stainless steel electrodes which were constructed especially for the purpose. A Grass SD-5 stimulator was used to supply supramaximal stimulating voltages and these stimulations were continuously monitored with a cathode ray oscilloscope so that this investigator was satisfied as to the consistency of his stimulation parameters. For the studies involved in the determination of synaptic stations 10 cycles per second, 5 msec. duration

and 5 volts were used. These stimulation parameters have been used by Szentivanyi et al. (114) and have proved to be optimal parameters for the gross stimulation of efferent cardiac sympathetic pathways. The parameters employed in the stimulation of afferent vagosympathetic pathways were 30 cycles per second, 5 msec. duration and 5 volts. Voltages higher than 5 volts did not produce any greater response and so 5 volts was used throughout the study. The frequency of stimulation is, of course, critical in the study of afferent-induced responses of this kind. There seems to be more in the literature about the conduction velocities of various afferent vagal fibers (useful as an index of the type of fiber carrying the afferent impulses) but less about the normal frequency of response of various afferent vagal neurons. However, Whitteridge (125) has found that some cardiovascular afferent pathways conduct normal impulse traffic at frequencies of 30 to 90 cycles per second and Widdicombe (126) has given evidence that some pulmonary stretch receptors may fire at frequencies up to 300 cycles per second. It is difficult to obtain single unit recordings of these afferent fibers and thus I am not certain that the higher frequencies represent normal physiological responses. The range of responses which may obtain are such that any one choice of stimulation parameters may not be optimal for all afferent fibers and this must be admitted at the outset. It is the purpose of this dissertation, however, to report the findings elicited with the stimulation parameters previously mentioned. It is recognized that different afferent pathways may have different optimal frequencies of stimulation.

The animals employed in the series of experiments dealing with the localization of the cardiac sympathetic synapses were placed on positive pressure respiration and a bilateral thoracotomy was performed between the third and fourth ribs. Strain gauges were sutured to the epicardial surface and right femoral artery was canulated for blood pressure. Then the thoracic autonomic nerve trunks were dissected free and the cervical vagosympathetic trunks were sectioned in the neck to eliminate reflex responses originating from the thoracic level. The T4 segment was decentralized from the thoracic chain as was the stellate ganglion. The heart was almost completely decentralized with the exception of aberrant cervical inflows as reported by Weisman et al. (127). Stimulations were carried out at locations on the left and right sympathetic and vagosympathetic trunks.

The first series of experiments involved the localization of the cardiac sympathetic synaptic connections. In these experiments the anesthetic agents were either phencyclidine HCl (2.0 mg/kg) and alpha-chloralose (80 mg/kg) or alpha-chloralose alone (100 mg/kg), the chest opened, and Walton-Brodie strain gauge arches sutured to the ventral surface of the myocardium. Five to six strain gauges were employed, gauges being sutured to the apical and basal regions of both ventricles as well as on the right and sometimes left atria. The metal arch of the gauge was routinely placed under tension by slight compression of its limbs.

The left and right sympathetic trunks at the level of the stellate ganglion and the fourth thoracic segment, as well as both thoracic vagosympathetic trunks, were carefully dissected free. The decentralized T4 segments, the stellate ganglia, the anterior ansae, the left ventromedial or ventro-

lateral cervical cardiac nerve and the right stellate cardiac nerve were stimulated consecutively by rectangular pulses from a Grass model SD-5 stimulator with continuous monitoring on a cathode ray oscilloscope. To eliminate the possibility of thoracic afferent stimulation leading to reflex activity, the cervical vagosympathetic trunks were sectioned in the neck. Atropine sulfate (0.25 mg) and either tetraethylammonium Cl (2.5 mg) were administered by successive injections directly into the stellate and caudal cervical ganglia, stimulations being carried out before and after each injection. Finally, atropine sulfate (0.5 mg/kg) and one of the three named nicotinic blocking agents were given systemically (0.5 mg/kg) and the nerve stimulations repeated after each intravenous injection.

The second series of experiments involved the study of afferent vagal pathways and in this series the animals were intubated, maintained on positive pressure respiration and a lateral thoracotomy was performed with the removal of the first six ribs. All connections of the vagosympathetic trunk on the operated side were severed from the diaphragmatic level to above the caudal cervical ganglion. The contralateral vagosympathetic trunk was untouched. A Walton-Brodie strain gauge arch was sutured to the right ventricular epicardium. It was felt at the time of the experiments that the response of the right ventricle to a massive afferent stimulation would result in a total change in right ventricular contractile force. Unpublished data indicates that this may not be true but this information was not available at the time the experiments were conducted. The true nature of a differential right ventricular response to afferent stimulation has therefore not been determined. Aortic blood pressure was recorded

with a catheter via the internal thoracic artery connected to a P23AC Statham pressure transducer. Right ventricular contractile force and aortic pressure were recorded on a Grass model 7 polygraph.

The vagosympathetic trunk was stimulated and all stimulations were continuously monitored on a cathode ray oscilloscope.

Four experimental steps were employed: 1) control stimulations were carried out in which selected branches or portions of the vagosympathetic trunk were electrically stimulated, 2) the contralateral vagosympathetic trunk was then sectioned in the neck and the stimulations repeated, 3) the contralateral stellate ganglion was completely removed and the stimulations repeated and 4) the animal was given propranolol (0.25 mg/kg) and the stimulations repeated.

RESULTS

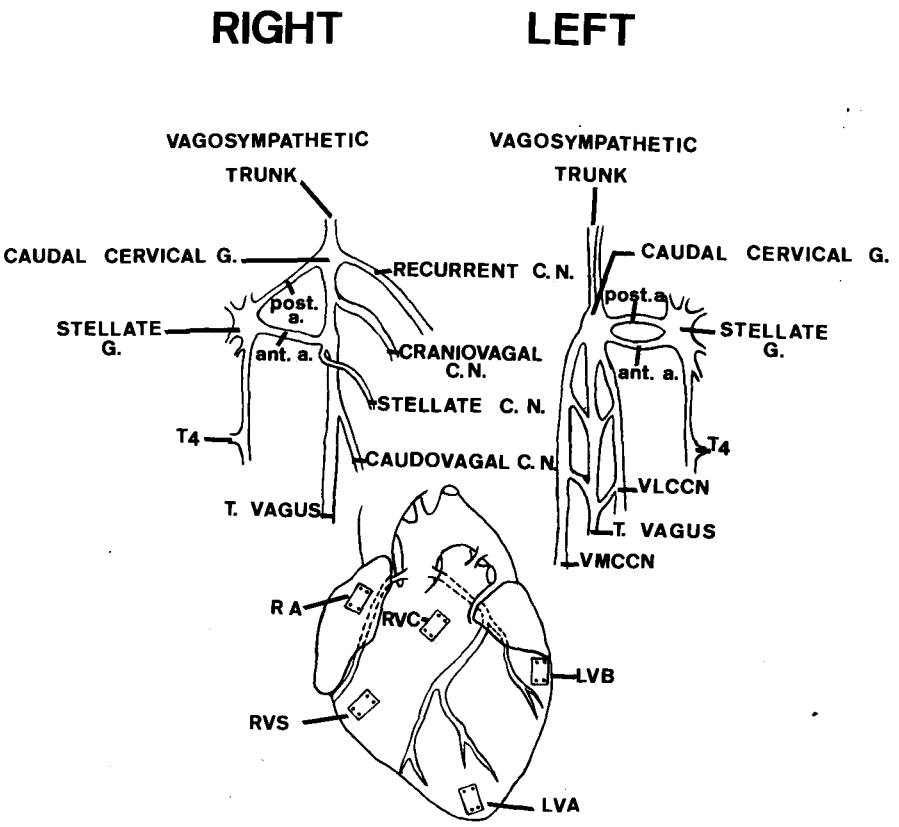
Synaptic Stations-Sympathetic Cardiac Pathways

Figure 1 illustrates the arrangement of the sympathetic and vagosympathetic trunks of the dog whose responses are illustrated in Figures 2 and 3. The nerve tracts are identified according to the terminology developed by Miserem (97). The ventral view of the heart in Figure 1 illustrates the placement of the five strain gauge arches in the dog, and the contractile responses from these areas are illustrated in Figures 2 and 3. Thus, Figure 2 illustrates response to electrical stimulation at four selected portions of the right thoracic autonomic supply to the heart; the T4 segment of the thoracic sympathetic trunk, the stellate ganglion, the right anterior ansa and the stellate cardiac nerve. Each location was stimulated first under control conditions, next after the injection tetraethylammonium Cl (TEA) into the stellate ganglion and finally after the injection of TEA into the caudal cervical ganglion.

Stimulation of the T4 segment under control conditions yielded a generalized increase in the force of contraction as measured at all five areas of the heart, an associated increase in pulse pressure as recorded in the femoral artery, as well as a 60 beat/minute increase in heart rate. Stimulation of this same segment after the injection of TEA into the right stellate ganglion resulted in a 13% reduction in the inotropic response with only a 38 beat/minute increase in heart rate. Blood pressure elevation was correspondingly reduced. Stimulation of the T4 segment after the injection

FIGURE 1

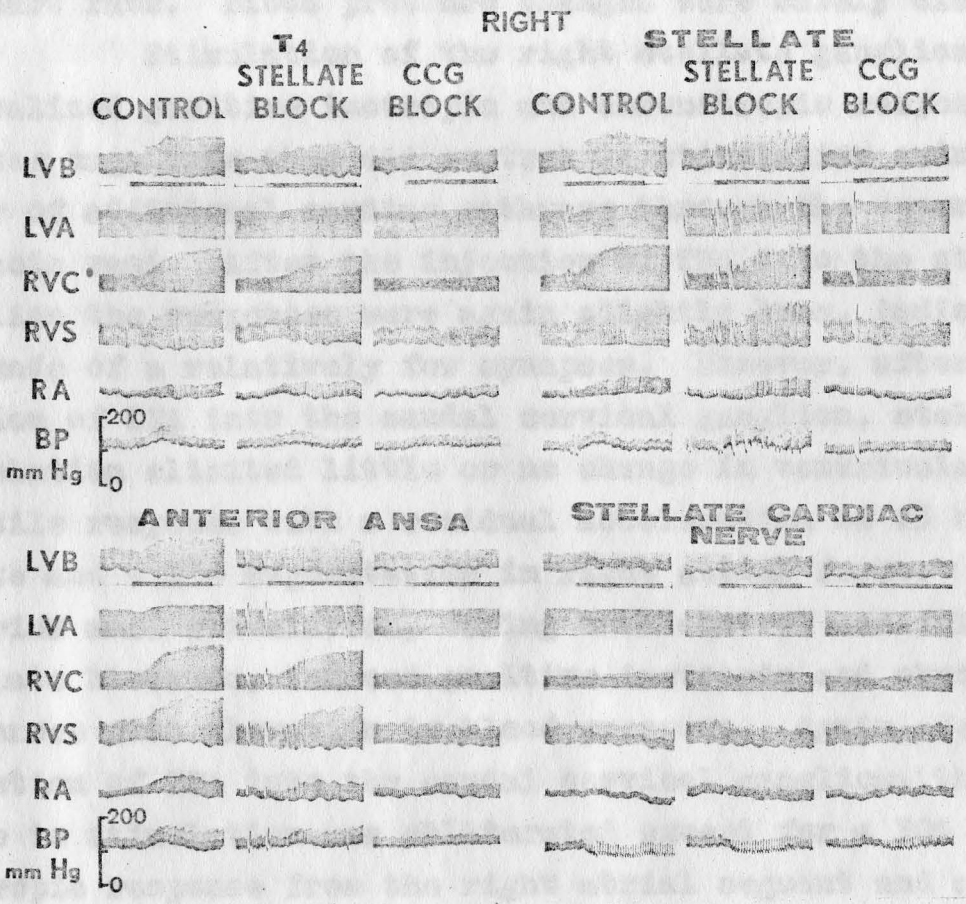
ANATOMY OF THE THORACIC AUTONOMIC NERVOUS SYSTEM AND STRAIN GAUGE PLACEMENT



Above, Sketch of the left and right sympathetic and vagosympathetic trunks of the dog whose responses are shown in figures 2 and 3. Terminology is after Mizeres (97). Below, Sketch of the anterior surface of the heart illustrates the location of strain gauge arches from which records shown in Figures 2 and 3 were made.

FIGURE 2

RESPONSE TO STIMULATION OF THE RIGHT CARDIAC SYMPATHETIC NERVES



Responses of the heart to electrical stimulation of four neural segments (right T4, right Stellate ganglion, right anterior ansa and right Stellate cardiac nerve) under three conditions (Control, after injection of TEA into right Stellate ganglion and after injection of TEA into right caudal cervical ganglion). Responses are recorded from strain gauge arches to the epicardial surface of the left ventricular base (LVB), left ventricular apex (LVA), right ventricular conus (RVC), right ventricular sinus (RVS) and right atrium (RA). Femoral arterial pressure (BP) is also shown. All stimuli were constant at 10 cycles/second, 5 msec and 5v (the latter adjusted during each stimulation as monitored by cathode ray oscilloscope).

of TEA into the right caudal cervical ganglion yielded only slight increases in contractile force from the left ventricle and the right atrium with a residual 38 beat/minute increase in heart rate. Blood pressure changes were barely discernible.

Stimulation of the right stellate ganglion yielded generalized positive inotropic and chronotropic responses of greater magnitude than did control T4 stimulation owing to the entry of additional cardiac pathways through the upper three thoracic rami. After the injection of TEA into the stellate ganglion the responses were again slightly less, indicating blockade of a relatively few synapses. However, after the injection of TEA into the caudal cervical ganglion, stellate stimulation elicited little or no change in ventricular contractile response with a residual acceleration of 25 beats/minute and a 25% augmentation in right atrial force. Right anterior ansa stimulation, during both control and following stellate blockade, induced positive inotropic and chronotropic responses with elevation in blood pressure. Again, following injection of TEA into the caudal cervical ganglion, the response to stimulation was obliterated except for a 30% positive inotropic response from the right atrial segment and a 30 beat/minute acceleration.

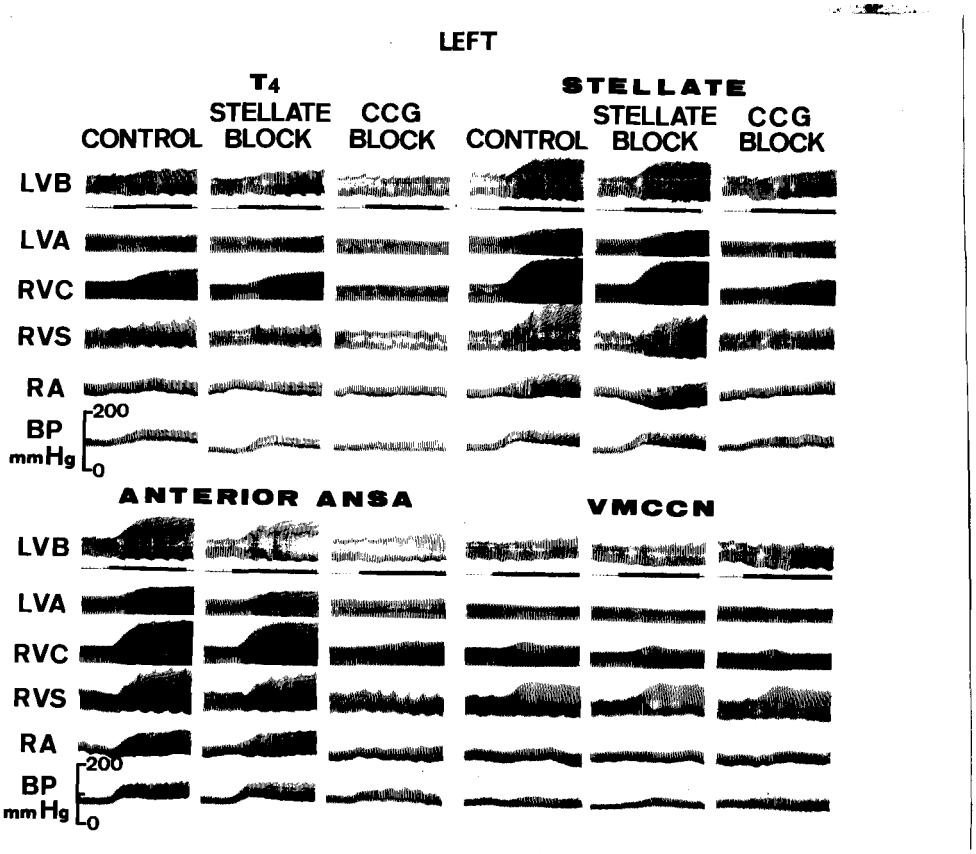
Stimulation of the stellate cardiac nerve (See Figure 1) yielded little or no inotropic response but induced a profound increase in heart rate (from 143 to 218 b/m), with accompanying pulsus alternans. This chronotropic response was only slightly changed by ganglionic blockade. Thus, a small number of fibers at the level of the T4 segment make synaptic connections in the stellate with a major proportion in the caudal cervical ganglion. Even after TEA blockade of both ganglia, positive inotropic and chronotropic responses per-

sisted to T4 stimulation with four alternative explanations; 1) synaptic connections may be made near either ganglion but not in the structure itself, 2) some pre-ganglionic fibers may synapse at the level of the heart as suggested by Brown (23), 3) sympathetic fibers may synapse central to the T4 segment as suggested by Alexander et al. (3), or 4) the ganglionic blockade may have been incomplete. The fact that the response to stellate ganglion stimulation is not much affected by the administration of TEA into the stellate ganglion indicates that its neural and functional capacity is not compromised and preganglionic fibers continue to respond to electrical excitation. Synaptic connections blocked in the stellate may not show reductions in contractile force response provided the postganglionic fibers are stimulated directly. Since the anterior ansa is distal to the first injection, there should be no difference in response providing the blocking agent did not diffuse into the systemic circulation and induce generalized blockade. Thus most of the synaptic connections appear to be made in the caudal cervical ganglion with a lesser number in the stellate ganglion. Stimulation of the stellate cardiac nerve which is distal to the points of injection shows no change from control which indicates the lack of any deterioration of the preparation.

Figure 3 illustrates responses to electrical stimulation before and after injections of TEA into the left stellate and caudal cervical ganglia. As on the right side, the magnitude of change in contractile force indicated relatively few cardiac pre-ganglionic fibers were present at the fourth thoracic segment. Nevertheless, blockade of the left stellate ganglion resulted in slight depression of the response to T4 stimulation, which was further depressed after blockade of the caudal cervical ganglion. Control stimulations of the stellate

FIGURE 3

RESPONSES TO STIMULATION OF THE LEFT CARDIAC SYMPATHETIC NERVES



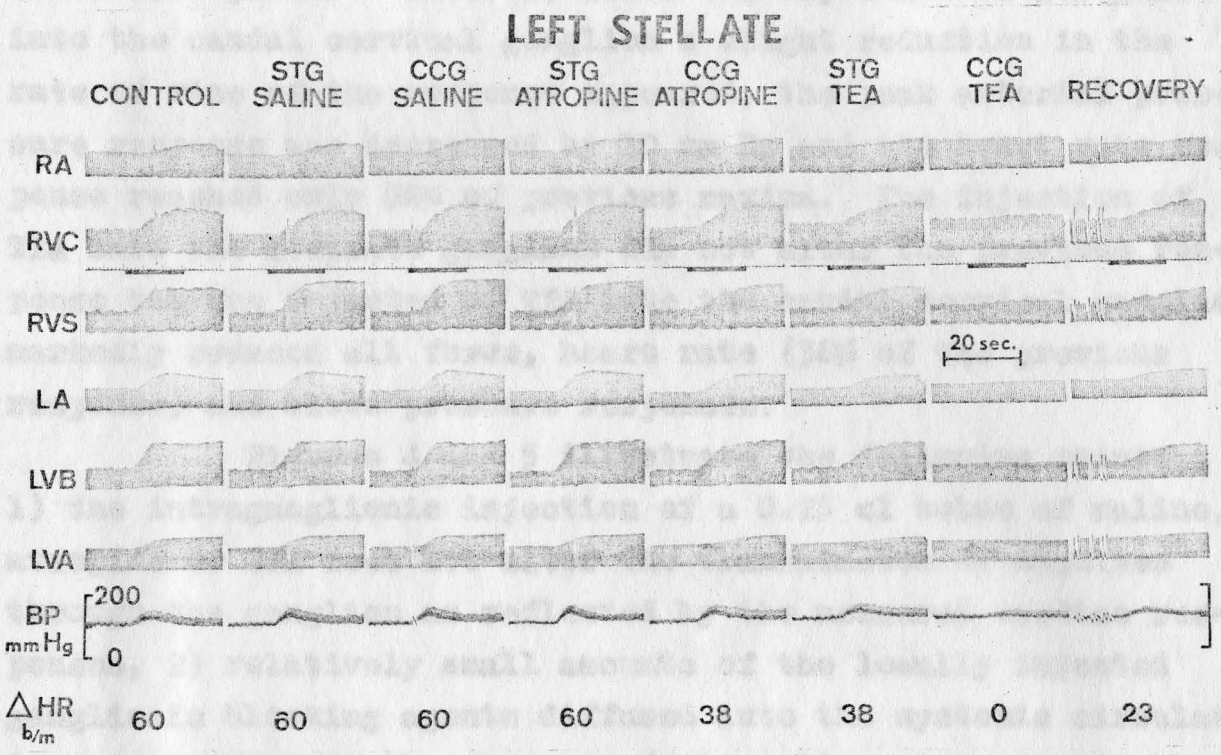
Responses of the heart of the same dog as in Figure 2 to electrical stimulation of four neural segments (left T4, left Stellate ganglion, left anterior ansa and ventromedial cervical cardiac nerve) under three conditions (Control, after injection of TEA into left stellate ganglion and after injection of TEA into left caudal Cervical ganglion). Stimulation parameters same as Figure 2.

ganglion and the left anterior ansa yielded maximum inotropic responses and acceleration of 80 beats/minute. These responses were only slightly altered by stellate injection of TEA. However, following additional blockade of the caudal cervical ganglion, the responses were markedly reduced. These influences were essentially confirmed during comparable manuevers at the left anterior ansa. Responses to comparable excitation of the ventromedial cervical cardiac nerve, which carries a variable fraction of cardiomotor post-ganglionic fibers, remained intact in spite of ganglionic blockade.

Figure 4 illustrates the responses of six areas of the heart together with aortic pressure during electrical stimulation of the decentralized left stellate ganglion. The first vertical panel represents the simultaneous responses to control stimulation. Injection of 0.25 ml of 0.9% saline into the stellate ganglion (panel 2) or caudal cervical ganglion (panel 3) produced no significant alterations in response to stimulation. Injection of 0.25 mg atropine sulfate into the stellate ganglion also resulted in little or no change. However, after injection of atropine into the caudal cervical ganglion there was a decline in the rate of development of the strain gauge response to stimulation as well as a decrease in the magnitude of the contractile responses of RA and LA. Also, the increase in heart rate attained only 63% of the control response. The injection of 2.5 mg TEA into the stellate ganglion was without apparent effect. Finally, the injection of TEA into the caudal cervical ganglion profoundly attenuated the response to stellate stimulation. Thirty-five minutes after the injection of TEA into the caudal cervical ganglion stimulation of the stellate ganglion yielded positive responses indicating initial recovery from the ganglionic blockade.

FIGURE 4

RESPONSES TO STIMULATION OF THE LEFT STELLATE GANGLION



Responses of the heart to electrical stimulation of the left stellate ganglion under eight conditions (Control, after injection of 0.25 ml saline into left stellate ganglion, after injection of 0.25 ml saline into left caudal cervical ganglion, after injection of 0.25 mg atropine into left stellate ganglion, after injection of 0.25 mg atropine into left caudal cervical ganglion, after injection of TEA into left stellate ganglion, after injection of 25 mg TEA into left caudal cervical ganglion and 35 minutes after the injection of 2.5 mg TEA into left caudal cervical ganglion). Responses are recorded from strain gauge arches stitched to the epicardial surface of the right atrium (RA), right ventricular conus (RVC), right ventricular sinus (RVS), left atrium (LA), left ventricular base (LVB) and left ventricular apex (LVA). Aortic blood pressure (BP) and the change in heart rate, in beats/minute, is shown during each procedure. Stimulation parameters same as Figure 2.

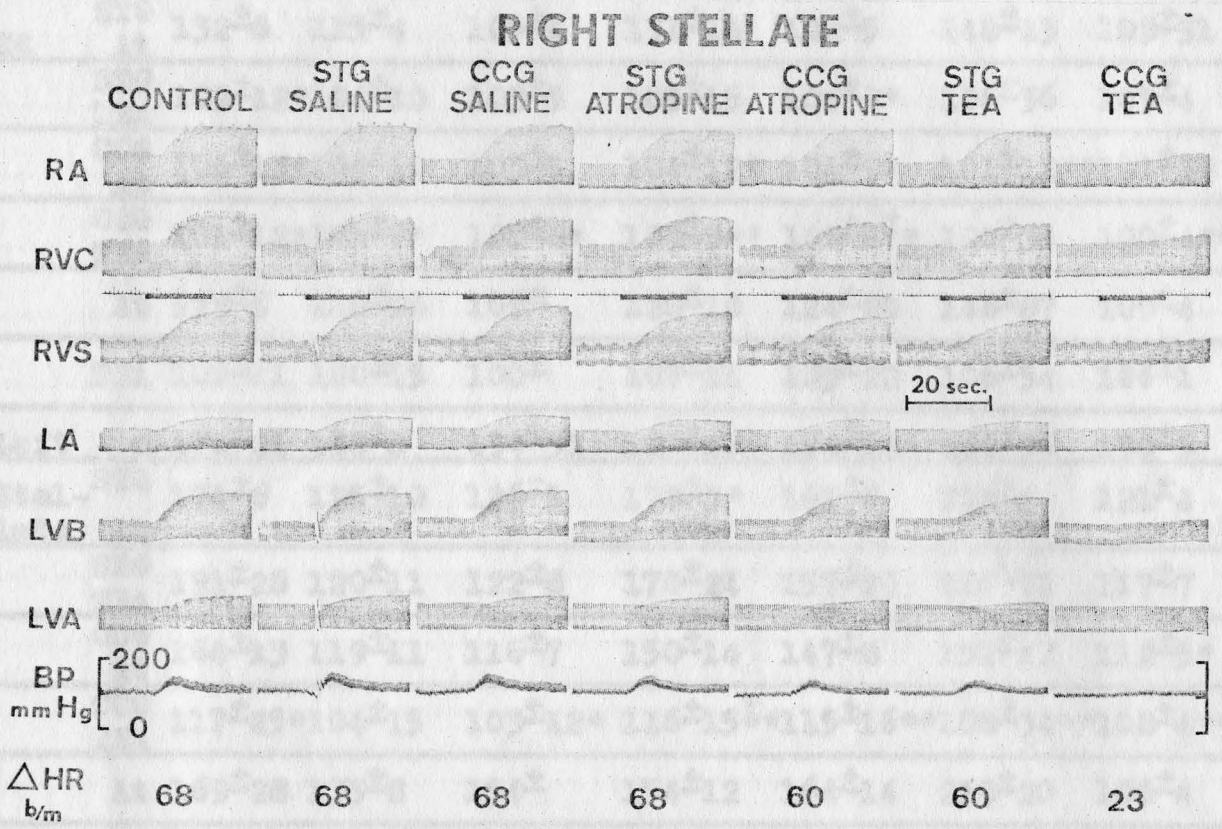
Figure 5 illustrates the response of the same animal to right sympathetic stimulation. Little or no change was apparent in the response to stellate stimulation in the first four panels. However, after the injection of atropine into the caudal cervical ganglion a slight reduction in the rate of rise of the response appeared, the peak arterial pressure response was decreased by 10 mm Hg and the heart rate response reached only 88% of previous maxima. The injection of TEA into the stellate ganglion did not alter the previous response but the injected of TEA into the caudal cervical ganglion markedly reduced all force, heart rate (38% of the previous response) and blood pressure responses.

Figures 4 and 5 illustrate the following points; 1) the intraganglionic injection of a 0.25 ml bolus of saline, atropine or TEA does not alter the transmission of impulses through the ganglion as reflected by the measured cardiac responses, 2) relatively small amounts of the locally injected ganglionic blocking agents diffused into the systemic circulation as revealed by the absence of alterations in systemic blood pressure, 3) injection of TEA into the stellate ganglion does not markedly alter the response to T4 stimulation (Figure 5) indicating that TEA itself does not alter axonal conduction, either by compression block or through action on the axon itself.

Table I summarizes data in which the complete series of observations were made from twelve animals. The values represent the per cent change from prestimulation contractile force and heart rate during stimulation of eight neural segments (four on the left and four on the right). These responses were tabulated for six areas of the heart and heart rate. Thus stimulation of the left T4 segment before the administration of blocking agents resulted in an average increase in the

FIGURE 5

RESPONSES TO STIMULATION OF THE RIGHT STELLATE GANGLION



Responses of the heart of the same dog shown in Figure 4 to electrical stimulation of the right stellate ganglion under seven conditions (Control, after injection of 0.25 ml saline into right stellate ganglion, after injection of 0.25 ml saline into right caudal cervical ganglion, after injection of 0.25 ml saline into right caudal cervical ganglion, after injection of 0.25 mg atropine into right stellate ganglion, after injection of 0.25 mg atropine into right caudal cervical ganglion, after injection of 2.5 mg TEA into right stellate ganglion and after injection of 2.5 mg TEA into right caudal cervical ganglion). Stimulation parameters same as Figure 2.

TABLE I

		LVB	LVA	LA	RVC	RVS	RA	HR
Left	C	147±9	125±20	119±20	147±30	142±25	177±33	115±14
T4	STG At	132±6	115±4	105±4	131±14	122±5	148±13	109±31
	STG TEA	109±12*	104±10	103±5	105±15	104±8*	106±36	103±4
	CCG At	106±13*	104±10	103±5	104±12*	104±9*	101±12**	101±3*
	CCG TEA	101±12*	101±7*	100±8*	103±9**	101±8**	100±34	100±4***
	At	113±4	110±26	109±	128±10	124±20	146±87	100±4
	TEA	102±11	100±15	100±	107±11	103±22	106±54	106±1
Left	C	178±21	138±8	134±35	187±16	174±15	234±35	130±5
Stel-late	STG At	174±6	125±10	126±5	178±3*	161±6	224±4	121±4
	STG TEA	191±28	120±11	122±8	170±24	157±20	180±21	117±7
	CCG At	164±13	119±11	116±7	150±14	147±8	151±47	112±5*
	CCG TEA	117±25*	104±15	103±12*	116±15**	115±16**	108±34**	108±4***
	At	169±28	133±8	154±	154±12	164±14	218±20	106±4
	TEA	117±86	121±32	147±	121±33	134±50	157±61	111±12
Left Ant	C	175±19	139±10	129±8	179±14	175±17	233±33	123±4
Area	STG At	195±6	126±11	116±3	179±7	166±5	221±10	121±2
	STG TEA	188±6	126±9	116±	168±15	152±14	218±19	118±5
	CCG At	151±12	111±13	108±6	133±11*	134±15	142±13	112±5
	CCG TEA	110±20**	104±10*	103±7**	107±13**	111±19*	108±36**	103±3***
	At	144±61	120±20	156±	144±11	146±19	210±40	105±6
	TEA	110±73	110±26	147±	118±28	126±24	161±82	111±12

TABLE I (CONT'D)

		LVB	LVA	LA	RVC	RVS	RA	RR
VLCCH	C	119±5	124±6	107±2	103±1	115±3	121±8	108±4
	STG At	125±4	135±8	107±3	107±3	122±7	110±4	107±2
	STG TEA	131±12	135±8	103±2	107±5	123±7	115±5	108±3
	CCG At	122±7	128±6	104±3	118±2	111±6	122±4	107±4
	CCG TEA	129±10	132±6	112±3	109±3	119±5	139±31	107±2
	At	115±21	126±11	124±	110±6	113±1	112±30	105±7
	TEA	109±20	128±0	131±	107±4	113±8	104±40	118±12
Right T4	C	170±24	112±14	129±17	164±16	155±5	199±36	132±3
	STG At	137±14	114±6	117±3	147±5**	140±3**	126±3*	122±2**
	STG TEA	158±7	117±5	121±3	172±9	145±7	143±3	127±6
	CCG At	150±12	109±14	120±6	150±9	128±8*	124±1**	121±8
	CCG TEA	107±14**	104±7	101±8**	105±10**	103±5**	105±21**	105±4**
	At	137±28**	110±4	111±	112±10*	114±12	140±39	124±4
	TEA	104±94	100±3	100±	100±7	100±9	107±43	106±19
Right Stel-late	C	183±24	120±13	143±15	175±9	183±12	259±38	139±7
	STG At	147±4**	109±3**	127±5*	162±5**	148±5**	156±10**	126±2**
	STG TEA	141±5	123±10	120±3	184±4	147±3	175±2	136±1
	CCG At	143±6**	109±2*	127±5**	151±5**	139±4**	155±8**	126±2**
	CCG TEA	128±7**	107±2**	112±5**	117±5**	117±4**	128±14**	121±2**
	At	164±28	114±5	143±	147±7	134±10	196±30	159±4
	TEA	112±57	104±4	148±	115±11	115±20	137±104	112±9

TABLE I (CONT'D)

		LVB	LVA	LA	RVC	RVS	RA	RR
Right Ant	C	170±24	116±4	143±15	164±9	150±11	238±41	137±6
Anes	STG At	151±7*	114±1**	129±7*	165±5	156±2*	164±10*	129±2**
	STG TEA	160±17	132±1	132±21	195±9	173±5	192±7	140±2
	CCG At	136±5**	111±2**	123±5**	142±5**	128±6**	162±9**	126±2**
	CCG TEA	120±6**	105±2**	111±5**	113±14**	114±4**	122±13*	118±2**
	At	139±45	116±2	140±	155±6*	147±10	183±62	160±4
	TEA	112±58	101±5*	138±	126±3	119±13	175±36	160±7
Right Stell. Card. Nerve	C	127±9	108±9	118±29	123±8	117±4	182±25	130±18
	STG At	122±1	115±2	103±1	116±2	120±2	129±7	129±2
	STG TEA	121±2	121±10	108±6	114±6	114±6	122±2	140±6
	CCG At	124±2	128±3	104±2	124±4	124±3	125±5**	124±3
	CCG TEA	117±1	112±2	115±1	122±4	118±2	143±7**	153±1
	At	122±2	100±6	179±	109±6	114±3	162±6	140±3
	TEA	122±2	101±4	167±	106±2	118±7	146±7	123±2

* = .05
** = .01
*** = .005+.001
± "x"= Standard Error

force of contraction of the region of the left ventricular base (LVB) of 47%. Similarly for the left ventricular apex (LVA) the average response achieved 125% of control.

The Fisher t-test was employed to determine the level of significance of the results and the asterisks after the figures denote the degree of significance calculated and the standard error is indicated.

Injection of atropine into the stellate ganglion frequently resulted in a significant reduction in response to T4 stimulation. This was further reduced by atropine in the caudal cervical ganglion. However, the major blocking action occurred upon injection of TEA directly into the caudal cervical ganglion with responses attenuated on all test portions of the heart. Successive stimulation of the sympathetic outflows at the stellate ganglion and anterior ansa induced much greater contractile and heart rate changes due to the entry of large numbers of pre-ganglionic fibers from the T3-T1 segments. The major attenuation in response followed TEA injection into the caudal cervical ganglion indicating this ganglion is the primary synaptic site for the cardiac sympathetics. It appears that synaptic connections are made in the left stellate ganglion since there was a decrease in response to stimulation of the left T_4 segment. It seems, however, that the left stellate ganglion (composed of a fusion of the first three thoracic rami in the dog) does not contain all the cardiac sympathetic synapses since the injection of TEA into the caudal cervical ganglion markedly reduced the response to left stellate and left anterior ansa stimulation. There is evidence that cardiac sympathetic fiber tracts terminating in specific areas of the heart (RA), pass through the T4 level and may be blocked by atropinization of the caudal cervical ganglion. After the T4

contribution combines with cardiac sympathetic tracts derived from the T3-T1 segments, as in the stellate or the anterior ansa, the influence of the T4 derived fibers on the response to stimulation of larger trunks is reduced.

Injection of atropine into the right stellate induced significant changes in the response of the right ventricle and heart rate to electrical excitation at the T4 segments, thus indicating a distinct difference in location of synapses and distribution of post-ganglionic fibers when compared with the left side. However, the predominant mass of synapses for sympathetic pathways to the entire heart reside in the caudal cervical ganglion. Both atropine and TEA induced significant blockade of the right caudal cervical ganglion, with the action of TEA invariably inducing the more complete blockade. Stimulation of the right stellate cardiac nerve resulted in little significant change indicating that most of the fibers in this particular tract are post-ganglionic. The systemic administration of atropine following localized ganglionic blockade often resulted in somewhat greater responses to sympathetic nerve stimulation, but the alteration was not sufficiently consistent to establish significance. Systemic administration of TEA often produced a small additional attenuation in contractile and heart rate response, suggesting the existence of more distal synaptic sites although additional blockade at the stellate and caudal cervical ganglia cannot be ruled out.

Vagal Afferent Pathways in Cardiac Reflex Control

Figure 6 illustrates the cardiovascular responses of a dog to right vagal afferent stimulation. It should be noted that after left vagisection there was little change in either the control level of right ventricular contractile force

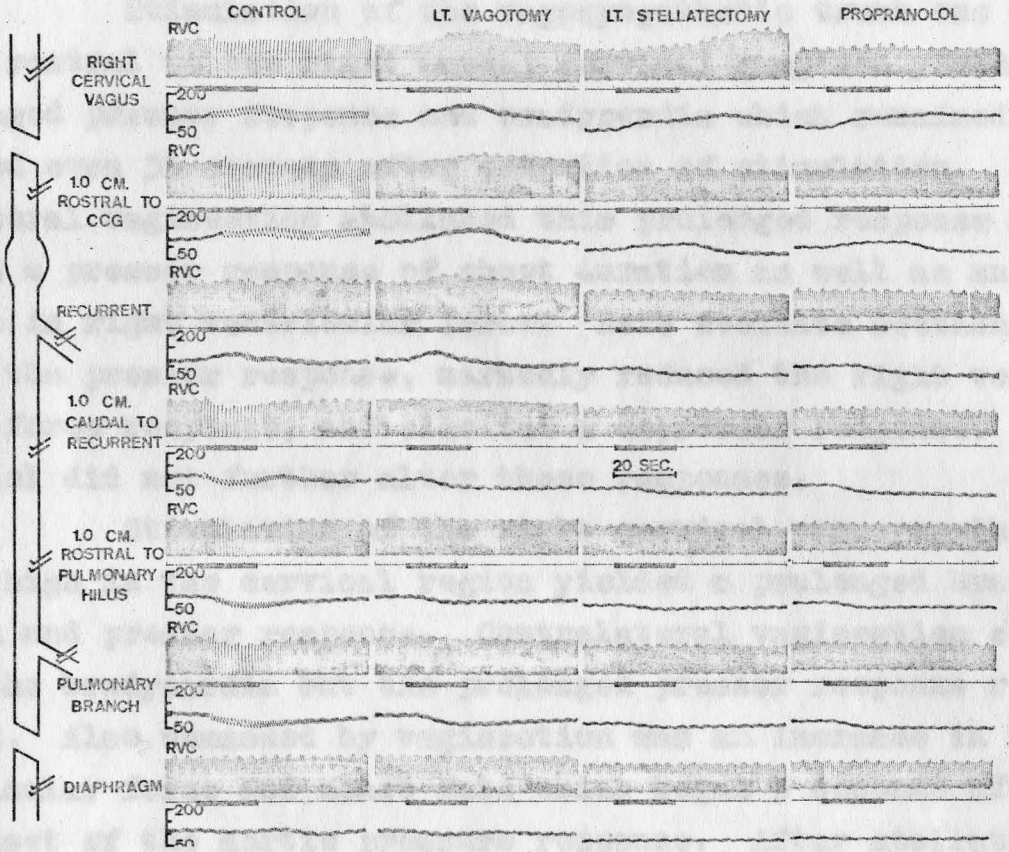
or aortic blood pressure. After left stellate ganglionectomy, however, both control recordings were depressed. After propranolol there were only slight changes in contractile force and systemic blood pressure.

Stimulation of the vagosympathetic trunk at the level of the diaphragm yielded little or no response and in most instances this was characteristic. Stimulation of a branch arising from the level of the pulmonary hilus showed a depressor response with bradycardia. The bradycardia was eliminated by contralateral vagisection but the depressor response remained during stimulation. After left stellate section the rate of recovery was decreased and the addition of propranolol did not alter this response. Stimulation of the vagosympathetic trunk one centimeter rostral to the pulmonary hilus yielded responses essentially identical to those elicited from the pulmonary branch. Stimulation of the vagosympathetic trunk 1.0 centimeter caudal to the recurrent cardiac nerve yielded responses of the same nature as the two previous stimulations.

Stimulation of the recurrent cardiac nerve caused, in order of appearance; a pressor response, a slight bradycardia and a slight depressor response. After contralateral vagisection the bradycardia was abolished but the pressor and depressor responses were still elicited by electrical excitation. In addition, after vagisection an increase in right ventricular force occurred during stimulation but this increase did not occur following stimulation after left stellate section. It should also be noted that stellectomy markedly reduced the pressor component of the response to electrical stimulation as reflected by the aortic pressure recording. Propranolol did not affect this response. Another point of interest concerns a comparison of the onset of the depressor response after re-

FIGURE 6

RESPONSES OF THE HEART AND BLOOD PRESSURE TO RIGHT AFFERENT STIMULATION



Responses of the right ventricular conus (RVC) and aortic blood pressure to stimulation of the isolated right vagosympathetic trunk. All stimuli were constant at 30 cycles/sec, 5 msec, and 5 v (the latter adjusted during each stimulation as monitored by cathode ray oscilloscope). Seven stimulation points were stimulated under four different conditions, control, after cervical section of the contralateral vagosympathetic trunk, after section of the contralateral stellate ganglion, and after 0.25 mg/kg propranolol.

current cardiac nerve and pulmonary branch stimulation. The onset of the depressor response after pulmonary branch stimulation was 4-8 seconds while the onset after recurrent cardiac nerve stimulation was 10-12 seconds (comparison after propranolol).

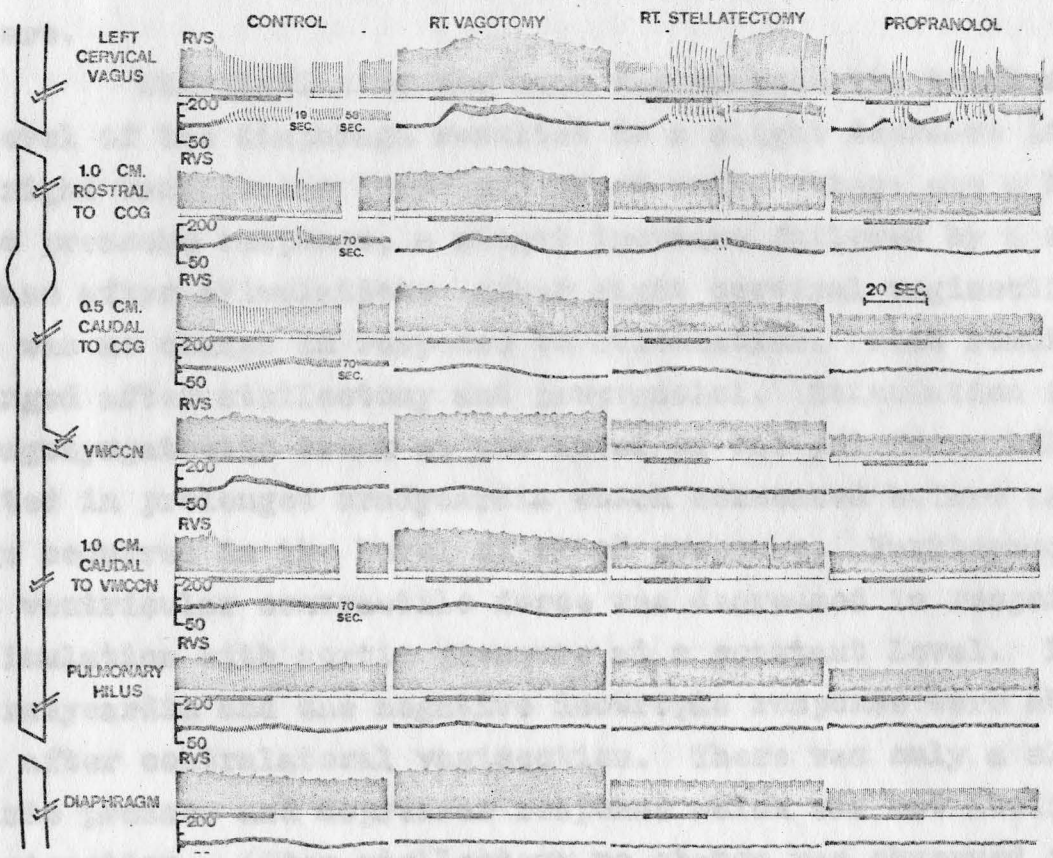
Stimulation of the vagosympathetic trunk one centimeter rostral to the right caudal cervical ganglion yielded a prolonged pressor response and bradycardia which remained pronounced even 35 seconds after cessation of stimulation. Contralateral vagisection abolished this prolonged response and unmasked a pressor response of short duration as well as an increase in right ventricular force. Left stellate section reduced the pressor response, markedly reduced the right ventricular force response, and elicited a depressor response. Propranolol did not further alter these responses.

Stimulation of the right cervical vagosympathetic trunk high in the cervical region yielded a prolonged bradycardia and pressor response. Contralateral vagisection eliminated the bradycardia but the prolonged pressor response remained. Also unmasked by vagisection was an increase in right ventricular force and heart rate which began 3 seconds after the onset of the aortic pressure response. After stellate section both the onset of the increase in right ventricular force and the peak of the pressor response were delayed. Propranolol abolished the increase in right ventricular force and eliminated the tachycardia accompanying stellate section. The magnitude of the pressor response was not further affected by propranolol.

Figure 7 illustrates the cardiovascular responses of a dog to left vagal afferent stimulation. It should be noted that after right vagisection there was a slight increase

FIGURE 7

RESPONSES OF THE HEART AND BLOOD PRESSURE
TO LEFT AFFERENT STIMULATION



Responses of the right ventricular sinus (RVS) and aortic blood pressure to stimulation of the isolated left vagosympathetic trunk. Stimuli were the same as Figure 1. Seven stimulation points were stimulated under four different conditions, control, after cervical section of the contralateral vagosympathetic trunk, after section of the contralateral stellate ganglion, and after 0.25 mg/kg propranolol.

in the control values of right ventricular force and heart rate but little effect on aortic blood pressure. After right stellectomy control right ventricular force and heart rate fell but control blood pressure remained approximately the same. After intravenous administration of propranolol, control right ventricular force and heart rate decreased with little change in blood pressure.

Stimulation of the left vagosympathetic trunk at the level of the diaphragm resulted in a slight decrease in both right ventricular force and heart rate. There was a biphasic pressure response, a slight increase followed by a slight decrease after stimulation. After right cervical vagisection there was no change in response to stimulation. This remained unchanged after stellectomy and propranolol. Stimulation of the vagosympathetic trunk at the level of the pulmonary hilus resulted in prolonged bradycardia which commenced before any change occurred in the level of blood pressure. Furthermore, right ventricular contractile force was decreased in response to stimulation with aortic pressure at a constant level. Both the bradycardia and the negative inotropic response were abolished after contralateral vagisection. There was only a slight biphasic pressor and depressor response which was not abolished by vagisection. After stellectomy no change was observed in response to electrical stimulation and this was unaffected by propranolol.

Stimulation of the vagosympathetic trunk 1 centimeter caudal to separation of the ventromedial cervical cardiac nerve resulted in bradycardia rapid in onset (within 1 second of the onset of stimulus) and prolonged in duration (91 seconds after the cessation of stimulus) which was abolished by contralateral vagisection. A slight pressor response was maintained

after vagisection but this was markedly reduced after stellectomy and absent after propranolol. After contralateral vagisection there was a progressive decrease in right ventricular force which did not occur after right stellectomy and propranolol.

Stimulation of the ventromedial cervical cardiac nerve resulted in a pressor response while both heart rate and right ventricular force declined. These decreases were not elicited after right vagisection. The magnitude of the pressor response was most affected by vagisection while stellectomy did not alter the response further. After propranolol there was little response to stimulation.

Stimulation of the vagosympathetic trunk 0.5 centimeters caudal to the caudal cervical ganglion resulted in a prolonged bradycardia and pressor response which were abolished after contralateral vagisection. During the initial phase of the bradycardia, right ventricular force fell while aortic pressure remained constant. After vagisection the pressor response became biphasic, an initial increase in pressure followed by a decrease. The biphasic response was diminished after stellectomy and there appeared to be little response after propranolol.

Stimulation of the vagosympathetic trunk 1.0 centimeter rostral to the caudal cervical ganglion resulted in a rapidly developed, prolonged pressor response, a prolonged bradycardia and a decrease in right ventricular force. The prolonged responses and the decreased force were abolished after contralateral vagal section and right ventricular force became increased. The pressor response showed a fast rising initial component followed by a plateau or secondary component. Stellectomy resulted in a decrease in the fast rising component

with a delay in the onset of the force increase. Propranolol abolished the latent increase in force and decreased the magnitude of the pressor response.

Stimulation of the left cervical vagosympathetic trunk produced responses similar in nature to those seen in the previously stimulated segment but of a much greater magnitude. After stellectomy there was a prolonged pressor response which was not abolished by propranolol although right ventricular force was decreased. It should be noted that this prolonged response was not elicited after vagisection. The arrhythmic nature of the trace may be due to the afterload increase due to the increase in aortic pressure due to a peripheral vascular constriction.

Table 2 summarises the average responses of fifteen animals to those procedures illustrated in Figures 6 and 7. Right ventricular force, systolic pressure, diastolic pressure, pulse pressure and heart rate were measured in response to electrical stimulation of six different points on the left and right vagosympathetic trunks. The points were; 1) cervical vagosympathetic trunk, 2) 1 cm above the caudal cervical ganglion, 3) either the recurrent (right) or ventromedial (left) cervical cardiac nerves, 4) 1 cm below the caudal cervical ganglion, 5) at the level of the pulmonary hilus and 6) at the diaphragmatic level. Four different experimental conditions obtained; 1) control stimulation, 2) stimulation after contralateral vagotomy, 3) stimulation after contralateral stellectomy and 4) stimulation after propranolol (0.25 mg/kg IV). The standard error was calculated and accompanies each value in the table. The responses are given in terms of percent of the prestimulation control contractile force, pressure, or rate. Thus the response of right ventricular force to control stimulation

TABLE 2

L E F T

	CERVICAL VAGUS	ABOVE CCG	VMCCN	BELOW CCG	PULMONARY HILUS	DIA-PHRAGM
CONTROL						
RIGHT VENT F.	81±8	109±15	104±11	107±23	85±4	99±6
SYSTOLIC B.P.	139±15	141±8	134±9	116±20	98±6	108±4
DIASTOLIC B.P.	127±2	135±8	127±5	111±22	92±5	107±4
PULSE PRESS.	201±84	175±20	143±9	148±17	142±20	113±4
HEART RATE	46±9	69±8	72±6	66±6	70±5	93±4
VAGOTOMY						
RIGHT VENT F.	140±8	158±19	166±26	125±18	108±12	106±3
SYSTOLIC B.P.	170±3	173±13	156±16	124±19	87±10	105±3
DIASTOLIC B.P.	161±2	171±13	151±15	121±19	86±12	105±3
PULSE PRESS.	213±3	180±20	177±26	141±26	91±15	103±3
HEART RATE	117±3	118±2	117±8	106±5	98±2	101±1
STELLECTOMY						
RIGHT VENT F.	274±94	184±33	100±0	112±27	106±6	98±2
SYSTOLIC B.P.	220±51	202±29	122±14	93±12	97±4	96±3
DIASTOLIC B.P.	319±130	213±32	123±16	90±12	97±3	97±2
PULSE PRESS.	226±31	171±20	117±5	111±13	95±6	95±3
HEART RATE	132±19	116±6	109±9	104±4	103±3	102±2
PROPRANOLOL						
RIGHT VENT F.	100±0	140±33	83±0	128±28	100±0	100±0
SYSTOLIC B.P.	141±11	182±25	125±25	119±34	89±11	100±0
DIASTOLIC B.P.	142±7	192±28	130±30	121±40	86±14	100±0
PULSE PRESS.	194±94	148±19	110±10	105±9	100±0	100±0
HEART RATE	100±0	104±4	103±3	108±8	98±1	100±0

± "x" = Standard Error

100% = Pre Stimulation Control Value

TABLE 2 (CONT'D)

R I G H T

	CERVICAL VAGUS	ABOVE CCG	REC. CARD.N.	BELOW CCG	PUL. HILUS	DIA-PHRAGM
CONTROL						
RIGHT VENT P.	109±7	111±9	134±22	91±3	86±2	109±3
SYSTOLIC B.P.	138±14	131±10	127±12	84±5	83±2	111±4
DIASTOLIC B.P.	148±10	133±11	137±9	81±8	76±4	114±5
PULSE PRESS.	142±20	150±13	126±10	101±9	112±6	105±3
HEART RATE	85±6	78±6	89±4	89±11	80±5	101±1
VAGOTOMY						
RIGHT VENT P.	143±7	149±11	119±10	92±5	90±7	109±8
SYSTOLIC B.P.	165±5	161±9	122±11	89±5	80±11	115±6
DIASTOLIC B.P.	174±8	170±11	134±11	84±17	77±11	114±9
PULSE PRESS.	130±6	131±12	109±3	99±6	95±10	103±2
HEART RATE	120±7	116±7	104±2	99±1	96±4	102±2
STELLECTOMY						
RIGHT VENT P.	138±8	132±7	105±8	105±7	93±3	97±3
SYSTOLIC B.P.	169±9	146±11	103±19	89±13	77±6	103±3
DIASTOLIC B.P.	174±10	159±14	106±28	82±18	106±28	103±3
PULSE PRESS.	156±20	115±11	96±6	109±1	96±9	103±3
HEART RATE	109±4	106±3	102±2	100±0	100±0	100±0
PROPRANOLOL						
RIGHT VENT P.	115±7	115±4	100±5	95±11	94±2	100±0
SYSTOLIC B.P.	167±9	151±12	102±21	85±31	80±9	106±6
DIASTOLIC B.P.	174±8	154±10	102±26	83±39	77±10	107±7
PULSE PRESS.	146±31	123±16	104±4	98±2	95±4	100±0
HEART RATE	100±0	100±0	100±0	100±0	100±0	100±0

± "x" = Standard Error

100% = Pre Stimulation Control Value

of the left cervical vagosympathetic trunk was 81±8 percent of the prestimulation control response. Similarly stimulation of the left vagosympathetic trunk 1 cm above the caudal cervical ganglion under the same conditions resulted in a 109±15 percent response or 9±15 percent over prestimulation control.

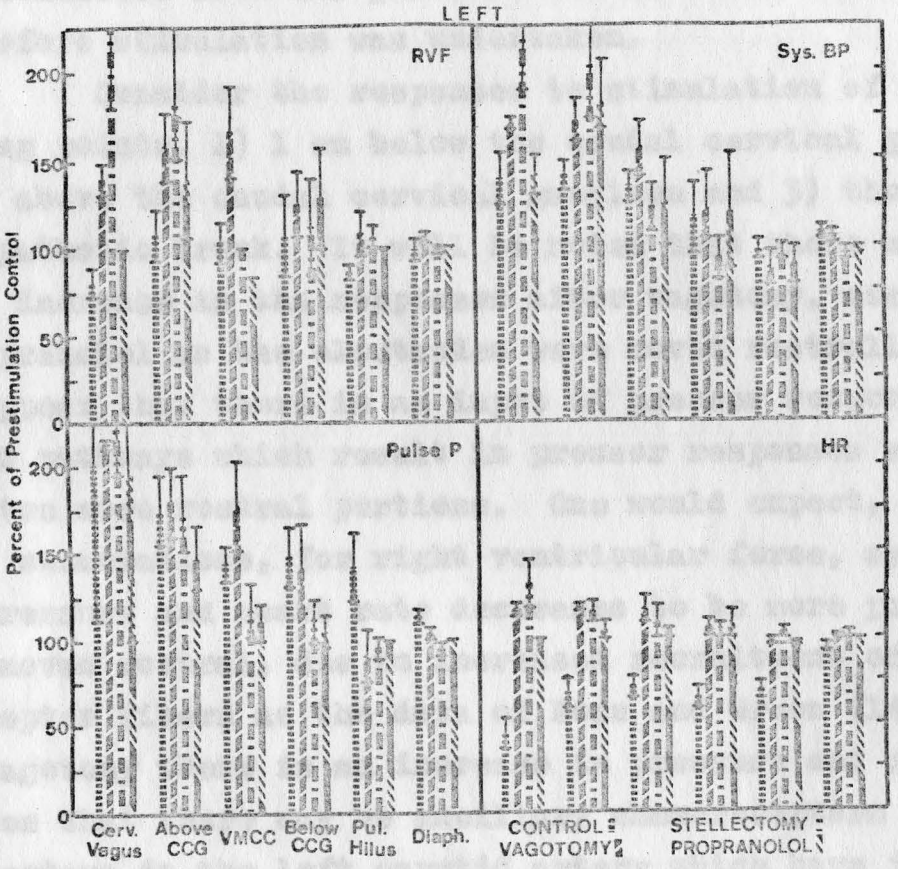
The responses of right ventricular force, systolic blood pressure, pulse pressure and heart rate as listed in table 2 are graphed in Figures 8 and 9.

Figure 8 illustrates the responses to stimulation of the left vagosympathetic trunk. Stimulation at the level of the diaphragm resulted in only slight changes from control levels, indicating that few if any afferent pathways with cardiovascular effects are present at the diaphragmatic level. A slight but definite bradycardia was generally elicited in the control as well as a slight increase in systolic and pulse pressure. Since after contralateral vagotomy the pulse pressure and systolic pressure fall with a commensurate rise in heart rate, it would appear that the change in pressures are due to the induction of the bradycardia. Stimulation at the level of the pulmonary hilus resulted in a marked bradycardia with an increase in pulse pressure but no change in systolic pressure and a decrease in right ventricular force. After vagotomy systolic and pulse pressures fell in response to stimulation. After stellectomy all parameters except heart rate were slightly depressed. After propranolol there was a slight decrease in systolic pressure but no other decreases.

It would appear from this graph that there is a definite depressor reflex whose afferent input is at the level of the pulmonary hilus. This confirms the work of DeBurgh Daly who found a depressor reflex originating from pulmonary stretch (46). In their records they do not show a bradycardia of vagal

FIGURE 8

RESPONSES OF SEVEN DOGS TO
LEFT AFFERENT STIMULATION



Responses of right ventricular force, systolic blood pressure, pulse pressure and heart rate to stimulation of the left vagosympathetic trunk at six locations: 1) cervical vagosympathetic trunk, 2) 1 cm rostral to the caudal cervical ganglion, 3) ventromedial cervical cardiac nerve, 4) 1 cm distal to the caudal cervical ganglion, 5) at the level of the pulmonary hilus and 6) at the level of the diaphragm. Stimulations were carried out at each point under four consecutive procedures; 1) control stimulation, 2) stimulation after contralateral vagotomy, 3) stimulation after contralateral stellectomy and 4) stimulation after propranolol (0.25 mg/kg IV). All stimulations were at 30 cycles/sec, 5 msec duration and 5 volts (the latter being adjusted during stimulation as monitored by a cathode ray oscilloscope). All data and standard errors taken from Table 2. Ordinate represents percent of prestim.control.(100%=no change)

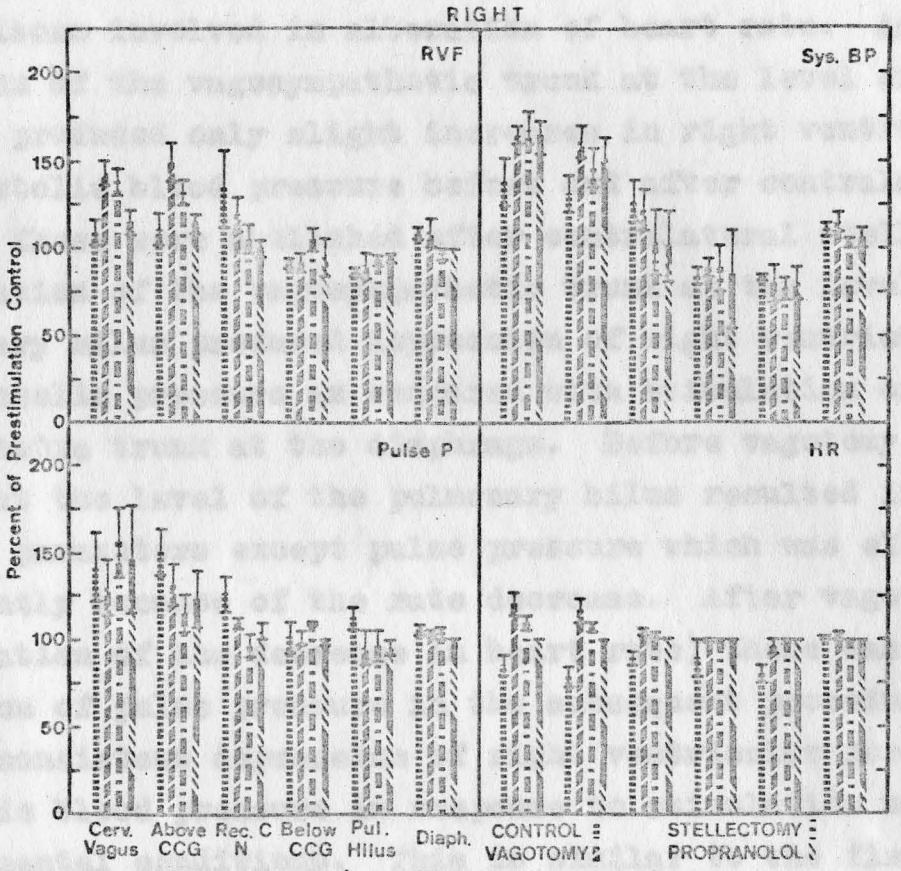
origin which is present in these experiments. The bradycardia confirms observations made by Brodie and Russel (17), which were challenged by Anrep et al. (4). Anrep's criticism can not be applied to the present work since the left vagosympathetic trunk was disconnected from all possible efferent connections to the heart before stimulation was undertaken.

Consider the responses to stimulation of the three following points, 1) 1 cm below the caudal cervical ganglion, 2) 1 cm above the caudal cervical ganglion and 3) the cervical vagosympathetic trunk. It will be noted that there was a general increase in the responses after vagotomy, stellectomy and propranolol as the electrodes were moved rostrally. It would appear that there is an input of pressor responses or afferent pathways which result in pressor responses as one stimulates more rostral portions. One would expect, in the control stimulations, for right ventricular force, systolic blood pressure and heart rate decreases to be more pronounced as one moves rostral, due to increased recruitment of ancillary baroreceptor fibers as the data of Boss and Green (14) suggest. After vagotomy there is an increase in pressure and force which indicates that there may be ancillary chemoreceptors as well as baroreceptors in the left carotid artery which have inputs into the left vagosympathetic trunk.

Figure 9 summarizes the responses of the right vagosympathetic trunk to stimulation. All neural segments are at the same levels as in the previous figure except that the recurrent cardiac nerve was stimulated instead of the ventromedial cervical cardiac nerve. Stimulation of the right vagosympathetic trunk generally produced responses which were of a lesser magnitude than those found after left vagosympathetic trunk stimulation. Changes in heart rate resulting from right

FIGURE 9

RESPONSES OF EIGHT DOGS TO
RIGHT AFFERENT STIMULATION



Responses of right ventricular force, systolic blood pressure, pulse pressure and heart rate to stimulation of the right vagosympathetic trunk at six locations: 1) cervical vagosympathetic trunk. 2) 1 cm rostral to the caudal cervical ganglion, 3) recurrent cardiac nerve, 4) 1 cm distal to the caudal cervical ganglion, 5) at the level of the pulmonary hilus and 6) at the level of the diaphragm. Stimulations were carried out under the four consecutive procedures listed in Figure 8. Stimulation parameters were the same as in Figure 8. All data and standard errors were taken from Table 2.

vagosympathetic trunk stimulation (ie, efferent limb via the left vagosympathetic trunk) were less than that seen when the right vagosympathetic trunk carried the efferent limb of the response arc. This follows from the work of Randall et al. (108) indicating that the right vagosympathetic trunk carries more fibers involved in alteration of heart rate. Again stimulation of the vagosympathetic trunk at the level of the diaphragm produced only slight increases in right ventricular force and systolic blood pressure before and after contralateral vagotomy. These were abolished after contralateral stellectomy. Stimulation of the vagosympathetic trunk at the level of the pulmonary hilus produced depression of right ventricular force and systolic pressure as compared with stimulation of the vagosympathetic trunk at the diaphragm. Before vagotomy, stimulation at the level of the pulmonary hilus resulted in a decrease in all parameters except pulse pressure which was elevated predominantly because of the rate decrease. After vagotomy (and elimination of the decrease in heart rate) there was slight depression of pulse pressure in the subsequent procedures. There was a consistent depression of right ventricular force and systolic blood pressure in response to stimulation under all experimental conditions. This is similar to the findings during stimulation of the left vagosympathetic trunk at pulmonary hilus. Again, as the stimulating electrodes were moved rostrally, the response to stimulation increased.

Comparison of the response to stimulation of the more rostral branches of the vagosympathetic trunk and the response to stimulation of either the ventromedial cervical or recurrent cardiac nerves shows that the responses are not additive, at least as far as the recording techniques employed in this study may be evaluated. Thus it is difficult to assess

the quantitative contributions of different nerve tracts to the afferent input. However, the qualitative increase in the number of fiber tracts entering the vagosympathetic trunk as it ascends toward the head is evident from figures 8 and 9.

DISCUSSION

The technique of localised injection does not adversely affect the physical or structural ability of the nerves to conduct impulses since injections of saline did not alter the response of the heart to nerve stimulation. Furthermore, Langley (83) using nicotinic depolarisation blocking agents (ie. nicotine sulfate) frequently recorded initial autonomous increases in cardiac rate and force of contraction which indicated that the blocking agent was exerting an initial stimulatory effect.

The classical observations of Langley (82), Cannon et al. (29) and others suggested possible inhibitory effects of atropine on sympathetic responses. Marrazzi (93) in 1939 established the role of atropine sensitivity in ganglionic transmission and Eccles et al. (54) provided a test model for this system. Eccles et al. hypothesised that muscarinic receptors have an important role in transmission through the rabbit superior cervical ganglion. They showed the presence of a late negative potential which could function in altering the excitability of the postganglionic cell body by partially depolarising it. Also they indicated a muscarine sensitive site on associated chromaffin cells which would release catecholamine causing the development of an inhibitory potential in the post ganglionic sympathetic cell body. More recently, Libet (90) showed that long lasting inhibitory post synaptic potentials (IPSP's) and slow excitatory post synaptic potentials (EPSP's) are abolished by atropine in the rabbit superior cervical ganglion. He stated: "The slow post synaptic potentials provide models of

slow neuronal responses which may have relevance for the slow activities in the autonomic and central nervous systems."

The present data conclusively demonstrate a great number of cardiac sympathetic synapses in the dog's caudal cervical ganglia. Furthermore, there appear to be atropine sensitive synapses thus confirming the work of Brown (23). He showed that intravenous administration of atropine abolished synchronous sC elevations in extracellular recordings of cardiac sympathetic nerve trunks in the dog and cat. He also showed that the ability of pre- and post-ganglionic fibers to conduct impulse traffic were unaffected. He concluded that atropine (in doses as low as 30 micrograms/kg) partially blocked ganglionic transmission. In the work of Libet and Eccles, atropine sensitivity was considered important in the modulation rather than the blockade of transmission.

The results obtained from the ganglionic injection technique have shown that the majority of canine cardiac sympathetic synapses are made in the caudal cervical ganglia. A minority are made in the stellate ganglion and it appears, as Brown (23) indicated, that some fibers may "run through" both the stellate and caudal cervical ganglia to synapse near or within the heart. On the left side the majority of sympathetic nerves in T_4 appear to synapse in the stellate which differs from the results obtained from the right ganglia. The T_1 to T_3 thoracic rami, which fuse to make up the stellate ganglion on the left side, appear to make a large number of synaptic junctions in the left caudal cervical ganglion. Brown showed that B fiber activity associated with cardiac function could be recorded in what have been considered purely post-ganglionic nerves. He proposed that some preganglionic sympathetic fibers "run through" both the stellate and caudal cervical ganglia.

The data in this dissertation lends some support to this hypothesis.

This author has shown earlier (126) that the caudal cervical ganglia are major loci for cardiac sympathetic synapses and this observation has been confirmed by Parr et al. (58). The importance of this observation lies in the consideration of techniques employed to produce post-ganglionic denervation in dogs. Chronic removal of the stellate ganglia cannot be considered an adequate technique for post-ganglionic denervation of the heart, and studies based on the assumption that bilateral stellectomy results in total degeneration of post-ganglionic sympathetic fibers must be examined in a new light. For example, studies of "denervated" hearts and their catecholamine levels may reflect the catecholamine content of adrenergic nerves rather than the heart.

Boss and Green (14) reported the presence of ancillary baroreceptor areas in the carotid arteries of the cat and dog. They indicated that the afferent fibers from these areas entered the vagosympathetic trunk and traveled up the trunk into the brainstem. The data presented here supports this view in that supramaximal stimulation of the upper cervical vagosympathetic trunk yields a more pronounced bradycardia than stimulation of the thoracic vagosympathetic trunk just rostral to the caudal cervical ganglion when the centralateral vagus is intact. This effect is more prevalent when the right vagus is intact and the left vagus is being stimulated.

After section of the centralateral vagosympathetic trunk, supramaximal stimulation of the cervical vagosympathetic trunk yielded a greater presser response than supramaximal stimulation of the vagosympathetic trunk at a point immediately rostral to the caudal cervical ganglion. Thus, there may be

accessory chemoreceptors as well as accessory baroreceptors along the length of the dog's carotid artery. Bergmann et al. (10) have suggested that there may be a baroreceptor in the cat carotid sinus which increases its firing rate with decreased distension of the baroreceptor area. However, they did not record neural traffic in the sinus nerve but used pharmacological agents as tools to study the sinus area. Even if some influence of this type of receptors were cited as a possible mechanism to explain the increase in response at the cervical levels, Bergmann et al. have indicated that this receptor yields a small response. Thus, if such receptors exist, their contribution to the response would be expected to be smaller than the data presented in this dissertation would indicate. Furthermore, Bronk et al. (19) have shown that stimulation of carotid sinus afferents causes a decrease in impulse traffic through the stellate ganglion, presumably to the heart. Thus if the primary baroreceptor (the classical receptor which increases its firing rate with increases in distension of the receptor area) is activated by stimulation, inhibition of sympathetic outflow should be expected and this should predominate over the weaker facilitation response.

Frequently, stimulation of the vagosympathetic trunk from the level of the pulmonary hilus and cephalad gave rise to a prolonged bradycardia which lasted up to 110 seconds after the termination of the electrical stimulus. McDowell (96) reported a similar finding. Daly et al. (46) studied a cardiovascular depressor reflex generated by lung inflation and stimulation of the pulmonary hilus branches elicited this depressor response. However, in addition to the peripheral vascular response reported by those workers, with the contralateral vagosympathetic trunk intact at this level we also obtained

a reflex bradycardia. In a number of instances this bradycardia could be obtained after pulmonary level stimulation without a depressor response as in Figure 7. This indicates, since Daly's records do not show a depression of heart rate, that this bradycardia may be associated with some other reflex phenomenon.

The depressor response which Daly reported was abolished by division of the nerves entering the vagosympathetic trunk at the pulmonary hilus level which is where stimulation of the vagosympathetic trunk produced a depressor response in our experiments. Furthermore, the response was not affected by division of the thoracic vagus caudal to the pulmonary hilus in their experiment and in our experiments the depressor response could not be evoked by stimulation of the thoracic vagosympathetic trunk below the level of the pulmonary hilus.

Brown (22) reported the presence of a depressor reflex arising from the left coronary artery of the cat under chloralose anesthesia. Associated with this reflex was a bradycardia and he indicated that this response was abolished by vagotomy. Thus the bradycardia seen with stimulation of the vagosympathetic trunk at the level of the caudal cervical ganglion may in part be due to this reflex mechanism.

Stimulation of the vagosympathetic trunk at the level of the caudal cervical ganglion produced a biphasic pressure response with an initial increase in pressure followed by a decrease in pressure. Stellectomy and propranolol diminished the cardiogenic pressor response with little effect on the decrease in pressure. Since the depressor response is first elicited at the level of the pulmonary hilus it would seem that this serves as a good example of the mixing of afferent fibers as the electrodes are moved rostral toward the head.

In 3 dogs stimulation of the cervical vagosympathetic trunk after contralateral vagisection resulted in predominantly a sympathetic response manifested by pathways coursing through the stellate ganglion since after its removal the positive inotropic response was delayed and was probably due to secretion from the adrenal medulla. The fact that the adrenal medullary response did not manifest itself until after the stellate was removed indicates that the adrenal response may have been inhibited due to the presence of the stellate response. The carotid sinus baroreceptors were intact in these animals and the response of these baroreceptors to the rise in pressure generated by the reflex stellate stimulation may have inhibited the action of the late induced adrenal medullary stimulation. This indicates that the reflex regulation of blood pressure is more complicated than a simple on-off response from one system.

CONCLUSIONS

By means of injection of atropine, tetraethylammonium (TEA) directly into the sympathetic ganglia while recording contractile force, heart rate and arterial blood pressure, the location of sympathetic synaptic connections was studied in fifteen dogs. Cardiac responses were elicited by supramaximal electrical excitation at points both proximal and distal to the stellate and caudal cervical ganglia. A majority of synapses were found to be in the caudal cervical ganglion with significantly smaller numbers in the stellate ganglion. Atropine sensitive, muscarinic synapses, as well as TEA sensitive nicotinic synapses were demonstrated in both ganglionic locations. Evidence is offered in support of the concept of sympathetic fibers "running through" both ganglia to synapse within or near the heart itself.

Cardiovascular responses to afferent vagosympathetic electrical stimulation while recording aortic blood pressure, heart rate and right ventricular force were studied in fifteen dogs. Four experimental conditions were imposed; 1) control stimulation of various portions of the vagosympathetic trunk completely isolated from all efferent connections, 2) stimulation of the trunk after contralateral cervical vagotomy, 3) stimulation of the trunk after contralateral stellectomy and 4) stimulation of the trunk after propranolol. Few afferent fiber tracts give rise to cardiovascular responses are at the level of the diaphragm. Stimulation at the level of the pulmonary hilus elicited a depressor response with a bradycardia and stimulation of the vagosympathetic trunk with the intact contralateral vagosympathetic trunk often resulted in a pro-

longed bradycardia. Pressor responses increased in magnitude as the stimulating electrodes were moved rostrally along the vagosympathetic trunk and the responses to afferent stimulation were broken down as to the levels of the vagosympathetic trunk at which different cardiovascular responses were elicited.

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A P P R O V A L S H E E T

The dissertation submitted by James Stephen Wechsler has been read and approved by the following five members of the dissertation 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 necessary changes have been incorporated, and that the dissertation is now given final approval with reference to content, form and mechanical accuracy.

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

May 26, 1969
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