Peptidergic Modulation of Efferent Sympathetic Neurons in Intrathoracic Ganglia Regulating the Canine Heart (42890)

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Abstract. **When either substance P or vasoactive intestinal peptide was injected into an acutely decentralized intrathoracic sympathetic ganglion, short-lasting augmentation of cardiac chronotropism and inotropism was induced. These augmentations were induced before the fall in systemic arterial pressure occurred which was a consequence of these peptides leaking into the systemic circulation in enough quantity to alter peripheral vascular resistance directly. When similar volumes of normal saline were injected into an intrathoracic ganglion, no significant cardiac changes were induced. When substance P or vasoactive intestinal peptide was administered into an intrathoracic ganglion, similar cardiac augmentations were induced either before or after the intravenous administration of hexamethonium. In contrast, when these peptides were injected into an intrathoracic ganglion in which the β-adrenergic blocking agent timolol (0.1 mg/0.1 ml of normal saline) had been administered no cardiac augmentation occurred. These data imply that in the presence of β-adrenergic blockade intraganglionic administration of substance P or vasoactive intestinal peptide does not modify enough intrathoracic neurons to alter cardiac chronotropism and inotropism detectably. When neuropeptide Y was injected into an intrathoracic ganglion, no cardiac changes occurred. However, when cardiac augmentations were induced by sympathetic preganglionic axon stimulation these were enhanced following the intraganglionic administration of neuropeptide Y. As this effect occurred after timolol was administered into the ipsilateral ganglia, but not after intravenous administration of hexamethonium, it is proposed that the effects of neuropeptide Y are dependent upon functioning intrathoracic ganglionic nicotinic cholinergic synaptic mechanisms. Intravenous administration of either morphine or [D-ala^2, D-leu^5]enkephalin acetate did not alter the capacity of the preganglionic sympathetic axons to augment the heart when stimulated. Following the intravenous administration of naloxone, the positive inotropic cardiac responses induced by efferent preganglionic sympathetic axonal stimulation were enhanced minimally in control states and significantly following hexamethonium administration. Thus, it appears that enkephalins are involved in the modulation of intrathoracic ganglion neurons regulating the heart, perhaps via modification of β-adrenergic receptors. Taken together these data indicate that substance P, vasoactive intestinal peptide, neuropeptide Y, or enkephalins modify intrathoracic ganglionic neurons which are involved in efferent sympathetic cardiac regulation.** [P.S.E.B.M. 1989, Vol 191]

In addition to classical nicotinic cholinergic receptors, muscarinic cholinergic ones exist in intrathoracic autonomic ganglia which are involved in the efferent sympathetic regulation of the heart (1–3). α- and β-adrenergic receptors have also been demonstrated in these ganglia (1), as have peptidergic ones (4). A number of different peptides has been proposed to be associated with neurons in sympathetic ganglia as a result of immunohistochemical studies (5–7). Substance P-like, vasoactive intestinal peptide (VIP)-like, and neuropeptide Y (NPY)-like immunoreactivities have been associated with neuronal somata in the canine middle cervical and stellate ganglia (8). In addition, some axons in these ganglia demonstrate enkephalinergic-like immunoreactivity. Peptidergic synaptic mechanisms have been proposed to be involved in the activation of stellate, middle cervical, and mediastinal ganglionic neurons by efferent preganglionic sympathetic axons (3, 9–11). It is not known if these synaptic mechanisms in intrathoracic autonomic ganglia are involved in the efferent sympathetic regulation of the

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heart and, if so, to what degree they are so involved. In the present experiments the ability of substance P, VIP, NPY, and opiate agonists and antagonists to modify the function of intrathoracic neurons regulating the heart was investigated in order to determine whether peptides can directly or indirectly alter intrathoracic efferent sympathetic neurons regulating the heart.

Materials and Methods

Animal Preparation. A total of 64 mongrel dogs of either sex, weighing 16–24 kg, were tranquilized with sodium thiopental (12–15 mg/kg iv) and anesthetized with α-chloralose (100 mg/kg iv). α-Chloralose (25 mg/kg iv) was administered as a bolus every 2 or 3 hr throughout the experiments or more frequently, as required. Following intubation, positive-pressure ventilation was initiated and maintained using a Bird Mark 7A ventilator. A bilateral thoracotomy was made in the fourth intercostal space and the pericardium incised to expose the heart. Walton Brodie strain gauge arches were sutured to the right and left atria. Miniature solid-state pressure transducers (Konigsberg Instruments, model P16C-12) were inserted into the midwall regions of the right ventricular conus, as well as the ventral and lateral walls of the left ventricle and the interventricular septum to record regional intramyocardial pressures (12). These sensing devices were used because intraventricular systolic pressure is not a sensitive index of the efferent sympathetic tone to the heart (13). Left ventricular chamber pressure was measured using a Bentley Trantec model 800 transducer connected to a catheter which was inserted into that chamber via a femoral artery. All data, including an electrocardiogram, were recorded on a curvilinear eight-channel Beckman dynograph.

In all experiments the right and left middle cervical and stellate ganglia were acutely decentralized by sectioning the vertebral nerves, as well as the T1, T2, and T3 rami and the sympathetic chains bilaterally. The cervical vagosympathetic complexes were sectioned bilaterally. When neural stimulations were performed, the following acutely decentralized structures were stimulated in turn with supramaximal stimuli (10 V, 5 msec, 10 Hz) for 20 sec using a Grass SD-9 square wave stimulator, the output of which was monitored on a Telequipment D-54 oscilloscope: the left sympathetic chain caudal to the left stellate ganglion plus the left T2 rami; the left T1 rami; the left C8 rami; the left subclavian ansae ~1 cm from the left middle cervical ganglion; and the left caudal pole nerve. Enough time occurred between stimulations to allow the preparation to return to basal conditions. Maximum heart rate, atrial forces, intramyocardial pressures, and left ventricular chamber pressure were measured for five consecutive beats before and at the peak of the responses elicited.

Experimental Protocol for the Intraganglionic Administration of Peptides. In the first group of experiments, consisting of 12 dogs, 0.1 ml of normal saline was injected via a 1-ml syringe attached to a 27-gauge needle into one of the four *in situ* major acutely decentralized intrathoracic ganglia (the right and left stellate and middle cervical ganglia), the sheaths and connective tissue of these ganglia being intact. The effects, if any, of these injections on cardiodynamics were monitored. Thereafter, once the preparation had returned to basal states a similar injection was made into another of these ganglia until all four major intrathoracic ganglia had been investigated in turn. Then 50 μg of substance P (peptide content ~85%) in 0.1 ml of normal saline was injected via a 1-ml syringe attached to a 27-gauge needle individually into each of these four major acutely decentralized intrathoracic ganglia, enough time being allowed to elapse between each injection for the preparation to return to control states. Thereafter, in six of these dogs the nicotinic cholinergic blocking agent hexamethonium was administered intravenously (10 mg/kg iv in a bolus form followed by a constant infusion of ~1 mg/min iv). Then substance P was reinjected into each ganglion, one at a time, with enough time being allowed to elapse between each injection for the preparation to return to control states. In the other six dogs timolol maleate (0.1 mg in 0.1 ml of normal saline) was injected into one of the four major intrathoracic ganglion. Timolol was employed because it is a β-adrenergic blocking agent with little, if any, membrane stabilizing properties in the central nervous system (14) or in the intrathoracic autonomic nervous system (unpublished results). Then substance P was reinjected into that ganglion and, after the preparation was stable, the ganglion was extirpated. Each of the four major intrathoracic ganglia was so investigated, with enough time being allowed to elapse between each injection for the preparation to return to control states. In a number of instances, injections were also made into the capsule of a ganglion in order to test the effects of an injection into non-neuronal tissues. Preliminary experiments had demonstrated that when 10 or 25 μg of substance P were injected into a ganglion, lesser cardiac responses were elicited and it was harder to identify the ganglionic region where injections resulted in cardiac changes.

In the second group consisting of 12 dogs, the same protocol was followed except that 200 μg of VIP in 0.1 ml of normal saline (peptide content ~75%) was administered individually and in turn into each major intrathoracic ganglion. In six of these dogs VIP was administered after hexamethonium administration had begun and in the other six dogs VIP was administered individually into each of the four major intrathoracic ganglia, one at a time, after timolol maleate (0.1 mg in 0.1 ml of normal saline) had been administered into each ganglion. Preliminary experiments had demon-

strated that when 100 μg of VIP were injected into a ganglion, lesser cardiac responses were elicited and it was harder to identify the ganglionic region where injections resulted in cardiac changes.

In a third group, consisting of 14 dogs, the neural structures described above with bipolar electrodes placed around them were stimulated in turn before and after the local administration of normal saline into the left middle cervical and left stellate ganglia, as well as following the administration of 100 μg of neuropeptide Y into both of these ganglia. Thereafter, in eight of these dogs hexamethonium was administered intravenously and the nerve stimulations described above repeated before and after neuropeptide Y had been reinjected into the ipsilateral intrathoracic ganglion. In the other six dogs timolol was administered into the ipsilateral ganglia; after waiting 1 min, neuropeptide Y was reinjected into the ganglia and the stimulations described above repeated. Preliminary experiments had demonstrated that when 50 μg of neuropeptide Y were injected into a ganglion no detectable changes were noted.

Experimental Protocol in which Pharmacologic Agents were Administered Intravenously. In a fourth group consisting of eight animals, the acutely decentralized neural structures described above were stimulated individually before and after the following pharmacologic agents had been administered intravenously in the order described: morphine sulfate (1 mg/kg), [D-ala^2, D-leu^5]enkephalin acetate (DADLE) (0.1 mg/kg), and naloxone hydrochloride at two different doses (0.1 and 1 mg/kg). Enough time occurred between administrations and stimulations for the preparations to reach steady-state baselines.

In a fifth group consisting of 12 animals, the neural structures described above were stimulated in turn. Afterward hexamethonium was administered and then each of these neural structures were restimulated in turn. Thereafter, naloxone hydrochloride (1 mg/kg iv) was administered and the various neural structures were restimulated as described above while hexamethonium continued to be administered. In a sixth group, consisting of six animals, the neural structures described were stimulated in turn before and after the administration of normal saline and subsequently timolol maleate (0.1 mg in 0.1 ml of normal saline) into the ipsilateral stellate and middle cervical ganglia. Thereafter, naloxone hydrochloride (1 mg/kg iv) was administered and the various neural structures were restimulated as described above.

Data Analyses. Heart rate and peak systolic forces and pressures were measured for five consecutive beats and their means ± SEM calculated. The cardiac responses elicited in each group were evaluated by comparing augmentations induced immediately prior to each intervention with maximal changes elicited following each intervention. Data obtained from the same animals during different interventions were compared using a two-way analysis of variance with repeated measures, followed by Fisher's protected least significant difference test (significance of $P < 0.01$ utilized).

Results

Cardiac Responses Induced by Intraganglionic Administration of Peptides. When 0.1 ml of normal saline was injected into the ipsilateral stellate and middle cervical ganglia of 24 dogs, no significant change of cardiac rate or force occurred except for the fact that in two animals right ventricular intramyocardial pressure was augmented by ~5 and 15%. Such injections did not alter cardiac augmentations induced by electrical stimulation of ipsilateral sympathetic efferent preganglionic axons in the 20 dogs in which preganglionic axons were stimulated before and after saline injections (cf. Fig. 5).

When substance P was injected into an acutely decentralized stellate or middle cervical ganglion, cardiac chronotropism and/or inotropism were augmented ~5–15 sec after the injection, maximum values being achieved ~10–20 sec thereafter. These changes occurred before systemic vascular pressure fell, the hypotension presumably being the result of leakage of the peptide into the systemic circulation and thereby causing vasodilation (Fig. 1). When substance P was injected into the capsule of a ganglion or a ganglionic region that did not elicit cardiac augmentation, systemic vascular hypotension still was produced. Such systemic vascular hypotension frequently lasted 15 min or more. In the 12 dogs studied, when the right middle cervical ganglion was injected with substance P there was an immediate increase in heart rate (137 ± 8 to 174 ± 15 beats/min; $P < 0.01$), atrial force of contraction (100 ± 10% to 150 ± 18%; $P < 0.0001$), right ventricular conal intramyocardial pressure (17 ± 1 to 36 ± 3 mm Hg; $P < 0.001$), and left ventricular ventral intramyocardial pressure (96 ± 6 to 119 ± 10 mm Hg; $P < 0.01$). Following hexamethonium administration, similar augmentations were elicited when substance P was reinjected into ganglia (Fig. 2). After injection of timolol into a ganglion, no changes occurred in the cardiovascular parameters monitored. Thereafter, repeat administration of substance P into that ganglion failed to elicit any cardiac responses other than reductions in left ventricular intramyocardial and chamber systolic pressures concomitant with the late fall in systemic vascular systolic pressure which such injections induced. When an injection of substance P was made into the capsule of a ganglion or the caudal pole of a stellate ganglion systemic vascular hypotension was induced without any cardiac early cardiac changes. Usually cardiac acceleration accompanied the systemic hypotension.

When VIP was injected into an acutely decentralized intrathoracic ganglia, cardiac chronotropism and/

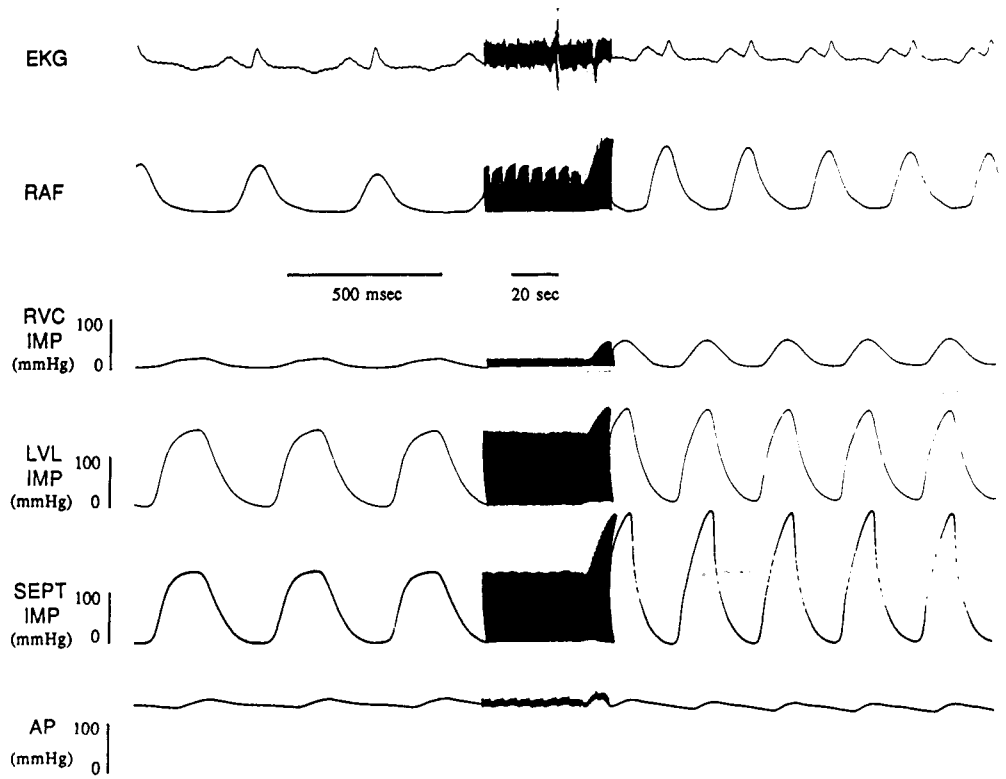

Figure 1. Injection (arrow at top) of 50 μg of substance P in 0.1 ml of normal saline into the cranial pole of an acutely decentralized right stellate ganglion resulted in increases in heart rate, right atrial force (RAF), right ventricular conus intramyocardial systolic pressure (RVC IMP), as well as left ventricular lateral wall (LVL) and septal (SEPT) intramyocardial systolic pressures. Aortic pressire (AP) was elevated briefly and then fell below control levels.

or inotropism were augmented briefly. For instance, injection of VIP into the right middle cervical ganglion resulted in an augmentation of heart rate (129 ± 8 to 176 ± 15 beats/min, $P < 0.01$), atrial force (100 ± 8 to 208 ± 16%, $P < 0.001$), as well as intramyocardial pressures in the right ventricular conus (14 ± 3 to 28 ± 6 mm Hg, $P < 0.01$) and left ventricular ventral wall (104 ± 9 to 145 ± 18 mm Hg, $P < 0.05$). A modest and transient hypotension occurred in two of the six dogs after cardiac augmentations had peaked; in the other dogs systemic pressure was unaltered. In four of the dogs, when VIP was injected into a stellate or middle cervical ganglion similar cardiac changes were induced after hexamethonium administration as before (Fig. 3). For instance, when VIP was injected into the right middle cervical ganglion after hexamethonium had been administered intravenously, there was an increase in heart rate from 123 ± 9 to 159 ± 23 beats/min ($P < 0.05$ using a one-tailed t test and not significant using ANOVA), atrial force from 100 ± 16 to 168 ± 21 ($P < 0.05$ using a one-tailed t test and not significant using ANOVA), as well as right ventricular conal intramyocardial pressures (16 ± 4 to 31 ± 8 mm Hg, $P < 0.05$ using ANOVA). In six dogs, following timilol administration into the major intrathoracic ganglia, repeat injection of VIP did not produce any detectable cardiac changes. When VIP was injected into a ganglionic capsule or the caudal pole of a stellate ganglion, no cardiac augmentation occurred.

Cardiac Responses Induced by Stimulation of Acutely Decentralized Sympathetic Preganglionic Axons before and after the Intraganglionic Administration of Neuropeptide Y. Cardiac chronotropism and inotropism were consistently augmented when the left chain and T2 rami were stimulated simultaneously in the 14 dogs investigated. Less cardiac augmentation occurred when the T1 rami were stimulated. When the C8 rami were stimulated, augmentation occurred in ~15% of the cases and when present was much less than that induced by stimulations of T1 rami. Thereafter, following the intraganglionic administration of normal saline into the ipsilateral stellate and middle cervical ganglia the induced augmentations were unchanged. Then, following administration of neuropeptide Y into the ipsilateral stellate and middle cervical ganglia the induced augmentations of cardiac inotropism were enhanced, the greatest changes occurring in right ventricular conal intramyocardial pressures (Figs. 4 and 5). The enhancement by neuropeptide Y of the augmentations induced by preganglionic axonal stimulation was also present in those animals in which timolol was subsequently injected into the ipsilateral stellate and middle cervical ganglia, but it was absent in those animals in which hexamethonium was admin-

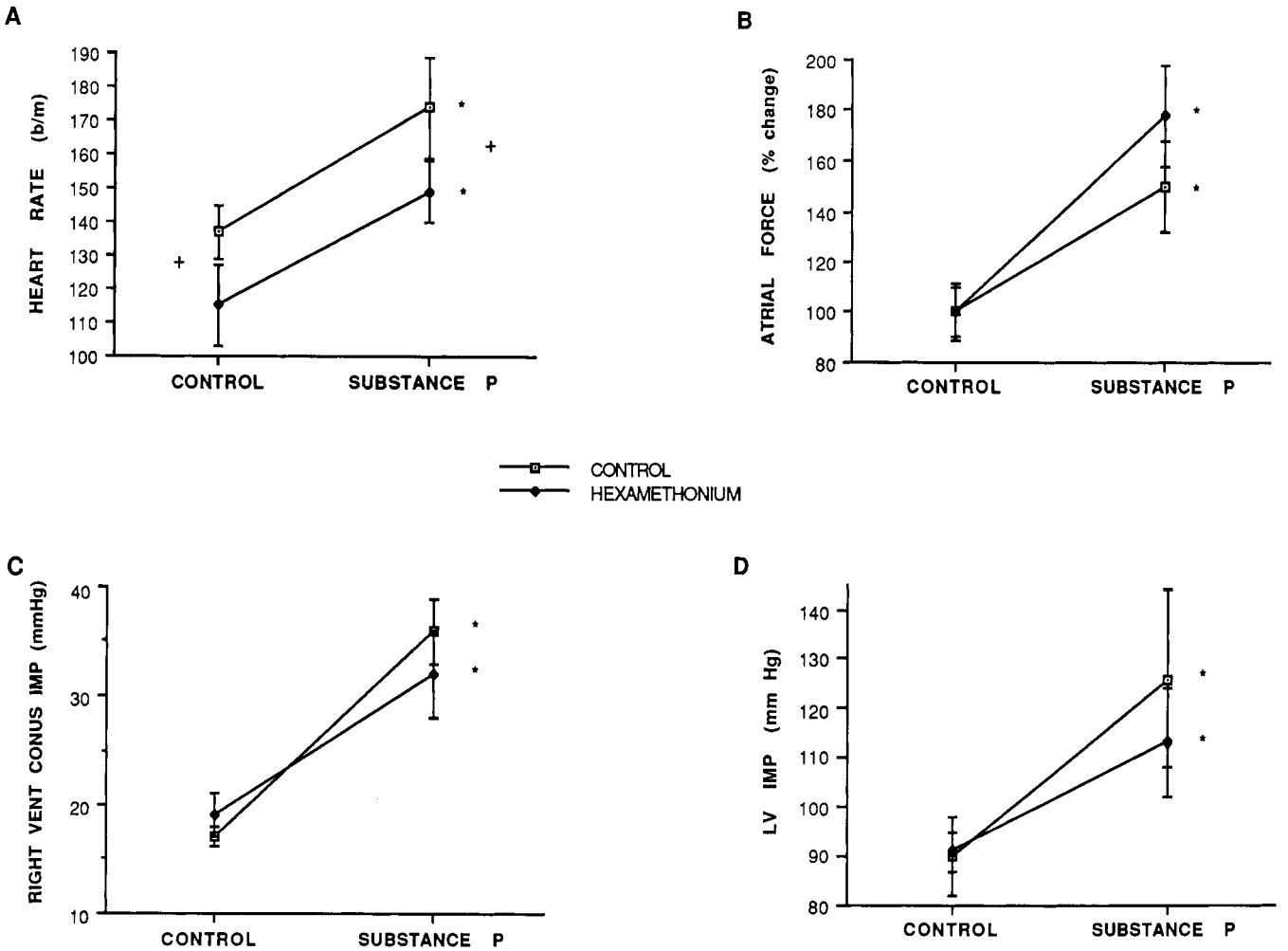

Figure 2. Injections of 50 μg of substance P into the right middle cervical ganglia of six dogs before (control) and after (hexamethonium) hexamethonium administration induced augmentations in heart rate (A), atrial force (B), and intramyocardial pressures in the right ventricular conus (C) or left ventricular ventral wall (D). Note that following hexamethonium administration heart rate was reduced significantly both before and after substance P administrations. When normal saline was injected first into these ganglia no cardiac changes were induced (not shown). * indicates significant differences comparing control to stimulation date; + indicates significant differences obtained before and after drug administration ($P < 0.05$). Similar symbols in the other figures of graphs represent similar comparisons.

istered intravenously. Injections of neuropeptide Y into an acutely decentralized intrathoracic ganglion did not by themselves elicit any detectable change in the cardiac parameters monitored. The cardiac augmentations induced when the efferent postganglionic sympathetic axons in cardiopulmonary nerves were stimulated were not altered following intraganglionic administration of neuropeptide Y.

Cardiac Responses Induced by Stimulation of Acutely Decentralized Thoracic Sympathetic Preganglionic Axons before and after the Intravenous Administration of Naloxone. The cardiac augmentations induced by stimulation of preganglionic sympathetic axons in the sympathetic chain and T2 rami or the T1 rami were not altered significantly after the intravenous administration of naloxone. Following hexamethonium administration the cardiac augmentations induced by stimulation of preganglionic sympathetic axons in 12 dogs were depressed significantly; when naloxone was then administered intravenously there was a significant enhancement of the induced augmentations of cardiac inotropism, particularly that of right ventricular conal intramyocardial pressure (Fig. 6). The cardiac augmentations elicited by preganglionic sympathetic nerve stimulations were suppressed after the intraganglionic administration of timolol. In these six animals the subsequent intraganglionic administration of naloxone resulted in no detectable changes in the induced cardiac augmentations.

No significant changes occurred in the cardiac augmentations elicited by stimulation of preganglionic sympathetic axons in the sympathetic chain or thoracic rami following the intravenous administration of morphine or DADLE, either before or after hexamethonium administration or timolol administration. In addition, administration of morphine or DADLE did not

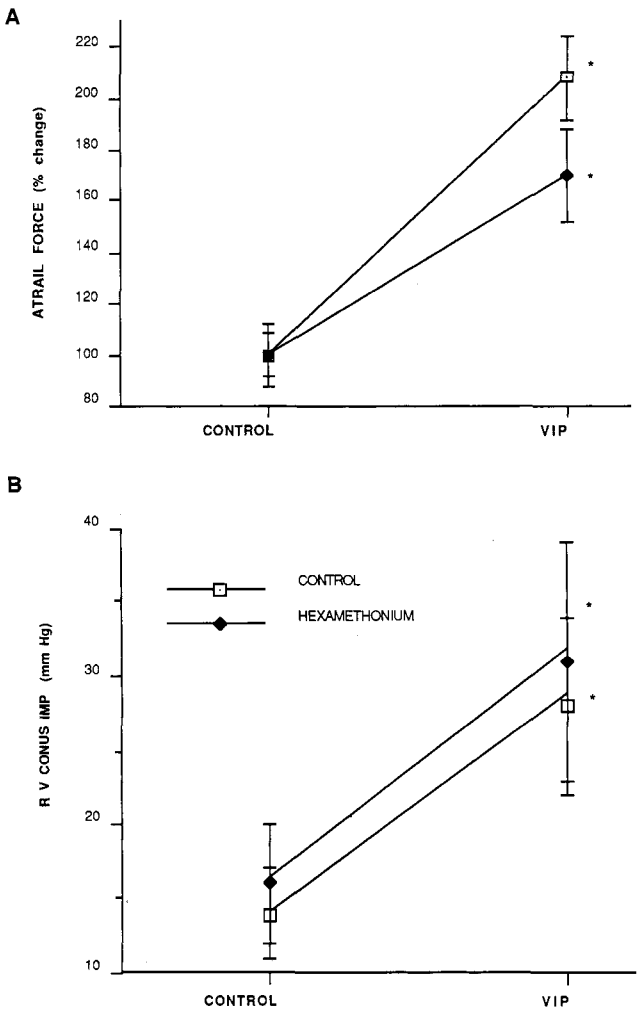

Figure 3. Injection of 200 μg of VIP into right middle cervical ganglia of six dogs in control states (control) resulted in an augmentation of right atrial forces (A) and right ventricular conus intramyocardial pressures (B) (RV conus IMP). Similar augmentations were induced after hexamethonium administration (hexamethonium).

alter the cardiac augmentations induced when axons in cardiopulmonary nerves were stimulated either before or after hexamethonium or timolol administration.

Discussion

These experiments demonstrate that administration of substance P or vasoactive intestinal peptide into an acutely decentralized middle cervical or stellate ganglion induces a transient and significant augmentation of heart rate and force. Using immunohistochemistry, different peptide-like substances have been identified

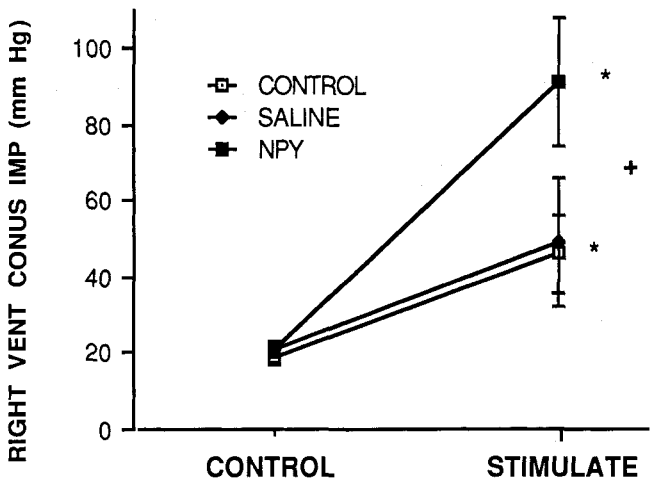

Figure 5. Stimulation (stimulate) of acutely decentralized axons in the left T1 rami augmented right ventricular conus intramyocardial pressures similarly before (control) and after (saline) administration of 0.1 ml of normal saline into each of the left stellate and left middle cervical ganglia in eight dogs. Following subsequent injections of 100 μg of NPY into each of these ganglia, the induced augmentations of right ventricular conus intramyocardial pressure were enhanced significantly.

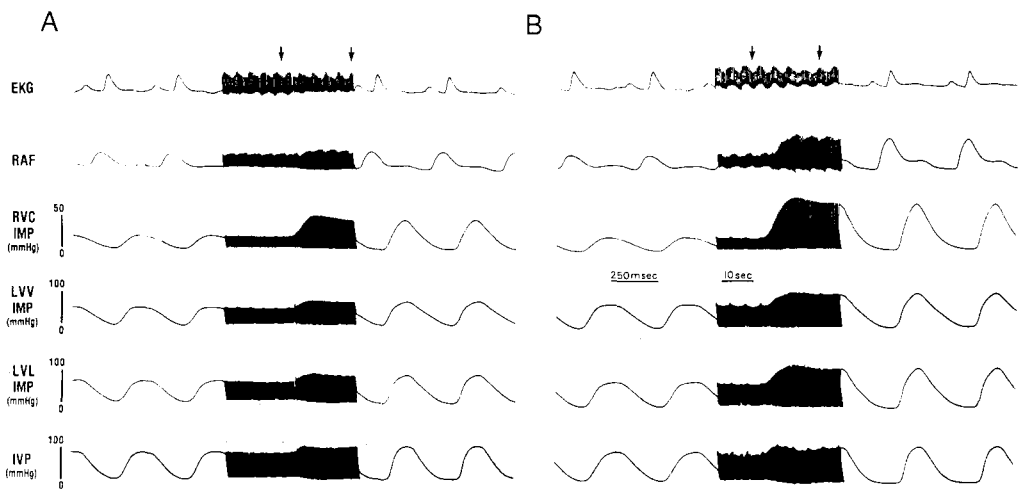

Figure 4. (A) Stimulation (10 V, 5 msec, 10 Hz) (between arrows) of the acutely decentralized left T1 ramus of a dog induced augmentation of right atrial force (RAF) as well as intramyocardial systolic pressures (IMP) in the right ventricular conus (RVC) and the left ventricular ventral (LVV) and lateral (LVL) walls. Left ventricular chamber systolic pressure (IVP) also increased. (B) Following administration of 100 μg of neuropeptide Y into the ipsilateral stellate and middle cervical ganglia, repeat stimulations of the T1 ramus resulted in augmentations which were greater than those generated previously (i.e., RVC IMP increased from 17 to 51 mm Hg before (A) and from 17 to 83 mm Hg after (B) the NPY administration).

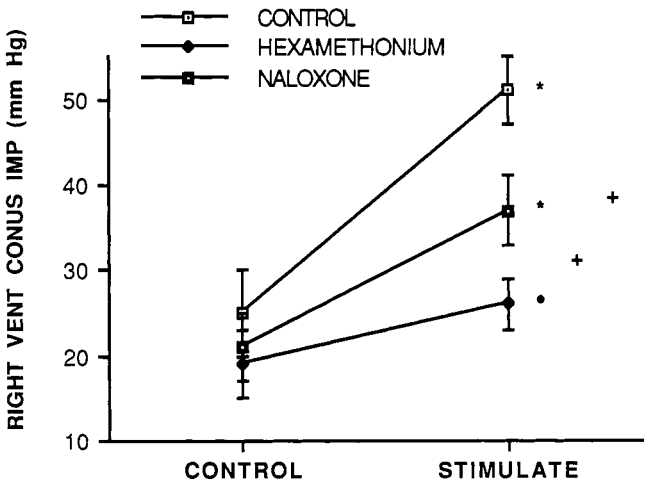

Figure 6. Augmentations of right ventricular conus intramyocardial pressures induced by stimulation of the left sympathetic chain and T2 rami in 12 dogs (control) was reduced significantly following hexamethonium administration (hexamethonium). Subsequently, after the intravenous administration of 1 mg/kg of naloxone (naloxone) the induced augmentations of right ventricular intramyocardial pressures were enhanced.

in autonomic ganglia (6), including the major intrathoracic ganglia of cats (5) or dogs (8). Substance P- and VIP-like immunoreactivity have each been associated with ~5–10% of the perikarya of neurons on canine stellate and middle cervical ganglia. Since such immunoreactivity is found in chronically decentralized intrathoracic autonomic ganglia (6), the presence of these peptides presumably does not depend wholly on the influences of central nervous system neurons.

Intraganglionic administration of either substance P (50 μg) or VIP (200 μg) induced an augmentation of cardiac chronotropism and inotropism (Figs. 1–3). Overall, injections of equal volumes of normal saline into such ganglia failed to elicit cardiac responses, although in a few animals such injections did result in modest cardiac augmentation. That intraganglionic administration of substance P produces tachycardia is not in accord with the fact that this substance has been reported to produce bradycardia when administered into the perfusate of an isolated mammalian heart (15). The induced cardiac augmentations always preceded the systemic hypotension that occurred when these peptides were administered intraganglionically, the systemic vascular pressure falling a few seconds after cardiac augmentations had been initiated. These data indicate that when these peptides are administered into intrathoraic ganglia in sufficient quantities to induce cardiac changes, they leak also into the circulation in sufficient quantities to cause systemic vascular vasodilation. Substance P and VIP are known to modify systemic vascular resistance, producing systemic hypotension when administered intravenously (16–18). When these agents were administered in lesser doses, the cardiac augmentations induced were of lesser magnitude. As a matter of fact, because these peptides exert significant effects on systemic vascular resistance even when administered in relatively small doses and because such hypotension might elicit tachycardia via reflex mechanisms, it was not possible to study the effects of these peptides with confidence when administered into the arteries which supply these intrathoracic ganglia due to the frequently profound systemic vascular effects of these agents when so administered. Such hypotensive changes lasted for a number of minutes. Therefore, it was deemed necessary to choose intraganglionic injections of these peptides to study their effects on intrathoracic neurons regulating the heart. Since injections of similar volumes of the vehicle (i.e., normal saline) into these ganglia did not alter cardiac mechanisms significantly overall, it is presumed that the transient cardiac augmentations induced immediately after intraganglionic administration of either substance P or VIP were primarily due to direct or indirect activation by these peptides of the efferent sympathetic neurons innervating the heart.

That the cardiac augmentations induced by intraganglionic administration of either substance P or VIP were not a direct consequence of the systemic vascular hypotension induced by these peptides is presumed for a number of reasons. First, because when administered intraganglionically the cardiac effects always preceded by a few seconds the induced systemic vascular hypotension (Fig. 1). In addition, because augmentations occurred in atrial force, an inotropic index relatively unaffected by systemic vascular resistance changes, such augmentations presumably are due to direct neuronal activation. Similarly, since right ventricular intramyocardial pressures are not directly affected by systemic vascular pressure changes and left ventricular intramyocardial pressure is not enhanced, but rather reduced, when systemic vascular hypotension occurs (12), it is unlikely that the induced cardiac augmentations were directly dependent on systemic vascular resistive changes. Finally, after intraganglionic administration of timolol repeat injections of substance P or VIP into ganglia failed to elicit any cardiac responses; however, systemic vascular hypotension of similar magnitudes was produced as those elicited previously. Thus, it is unlikely that the cardiac changes which occurred immediately after administration were a direct consequence of the resultant hypotension. However, since the ensuing systemic vascular hypotension frequently persisted for 5 or more minutes after intraganglionic administration, longer lasting cardiac changes like acceleration of heart rate were probably due to reflex changes elicited by the systemic vascular hypotension.

That intraganglionic administration of substance P induced an augmentation of cardiac chronotropism and inotropism (Figs. 1–3) is in accord with the fact that heart rate can be augmented by substance P administration after hexamethonium administration (16). Be-

cause the effects of either VIP or substance P persisted after nicotinic cholinergic blockade (Figs. 2 and 3), presumably these effects were not related primarily to nicotinic cholinergic transmission. However, since their effects were not elicited after intraganglionic β-adrenergic blockade, it is presumed that transmission via β-adrenergic receptors in intrathoracic ganglia involved in cardiac regulation (1, 9, 10) can be modulated by these peptides. This occurred despite the fact that stimulation of efferent postganglionic sympathetic axons in cardiopulmonary nerves elicited similar cardiac augmentations before or after the timolol injections, indicating that any lack of elicited response to peptide administration was not due to the reduction of function in the efferent postganglionic sympathetic axons or cardiac myocyte β-adrenergic receptors. Perhaps these peptides act to release catecholamines from SIF cells or other intraganglionic cells. This is in accord with the fact that VIP and noradrenaline can act synergistically on central nervous system neurons (19).

Neuropeptide Y is known to be involved in cardiovascular regulation (20–24) and has been associated with ~85% of the neurons in canine middle cervical and stellate ganglia (8). Although intraganglionic administration of NPY did not produce any detectable cardiac changes, its intraganglionic administration did result in an enhancement of cardiac augmentation induced by efferent sympathetic preganglionic axon stimulation (Fig. 5). Neuropeptide Y has been proposed to act presynaptically on neurons in the central nervous system (25). Since it did not exert any detectable effect on cardiodynamics when administered into an intrathoracic ganglion but did alter cardiac augmentations produced by stimulation of sympathetic preganglionic axons, it does not appear to act directly on efferent neurons involved in cardiac regulation but rather may act presynaptically, perhaps reducing inhibitory mechanisms. As this peptide did not produce any detectable effects after hexamethonium administration, but did after timolol administration into the ganglia, it may modulate nicotinic transmission in the intrathoracic efferent sympathetic nervous system regulating the heart.

Opiate receptors are involved in cardiovascular regulation (26–28) and have been shown to modify synapses in autonomic ganglia (3, 29). Enkephalinergic-like immunoreactivity has been associated with axons, but not perikarya, in canine intrathoracic ganglia (8). Because enkephalinergic-like immunoreactivity associated with axons in canine intrathoracic ganglia is virtually eliminated following chronic decentralization of the ganglia (8) and because enkephalinergic-like immunoreactivity has been identified in spinal cord neurons (30), it has been postulated that enkephalinergic axons in intrathoracic ganglia arise from perikarya in the spinal cord. Intraganglionic administration of the opiate antagonist naloxone, as well as the opiate agonists morphine or DADLE, did not induce cardiac changes. In contrast, opoids can modulate the efferent sympathetic innervation of feline hearts (3) and the cardiac augmentation induced by canine intrathoracic cardiopulmonary-cardiac reflexes (31). In the present experiments cardiac augmentations induced by preganglionic efferent sympathetic axonal stimulation were enhanced following the intravenous administration of naloxone. That such enhancement of augmentation persisted after hexamethonium administration (Fig. 6) but not after intraganglionic administration of the β-adrenergic blocking agent timolol indicates that enkephalinergic axons may act to modify the intrathoracic ganglionic β-adrenergic neurotransmission involved in the efferent sympathetic modulation of the heart. Because of the lack of effect of morphine, DADLE, or naloxone when injected intraganglionically, it is presumed that the effects of naloxone are indirect, perhaps functioning via presynaptic inhibitory mechanisms (32). Due to the fact that the larger dose of naloxone, and not the smaller one, produced these changes, it is presumed that delta opiate receptors (28) account for the majority of the effects noted.

The results of these experiments demonstrate that substance P and VIP act either directly or indirectly to modify intrathoracic efferent postganglionic sympathetic neurons which regulate the heart, thereby augmenting cardiac chronotropism and inotropism. Since their actions persist following nicotinic cholinergic pharmacologic blockade, but not following intraganglionic β-adrenergic blockade which does not modify cardiac myocyte β-adrenergic receptors, it appears that these peptides modify the β-adrenergic transmission in intrathoracic ganglia involved in cardiac regulation. In contrast, NPY may act to modulate directly or indirectly efferent sympathetic neurons innervating the heart, perhaps via nicotinic cholinergic synaptic transmission. Finally, enkephalinergic receptors in intrathoracic ganglia may modulate the β-adrenergic receptor transmission involved in cardiac regulation. As additional peptides like neurotensin have been also identified in autonomic ganglia (5, 7, 8, 11), presumably other peptides can modulate intrathoracic efferent sympathetic neurons which regulate the heart.

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