Differential effects from parapyramidal region and rostral ventrolateral medulla mediated by substance P

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1Department of Veterinary Biosciences, University of Illinois, Urbana, Illinois 61801; 2The Copenhagen Muscle Research Center and Department of Neurology, National University Hospital, Rigshospitalet, DK-2100, Copenhagen, Denmark; 3Department of Internal Medicine, Washington University, St. Louis, Missouri 63110; and 4Department of Physiology, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Swiatkowski, Kenneth, Lynn M. Dellamano, John Vissing, Kenneth J. Rybicki, Gerald P. Kozlowski and Gary A. Iwamoto. Differential effects from parapyramidal region and rostral ventrolateral medulla mediated by substance P. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1120–R1129, 1999.—Rostral ventrolateral medulla (rVLM) and parapyramidal region (PPr) serve as important medullary control sites for sympathoexcitation. rVLM and PPr have direct projections to the intermediolateral cell column (IML) that are thought to be important in maintaining mean arterial blood pressure (MAP). Substance P (SP) is found in PPr neurons and in and near the subretrofacial area of the rVLM. At least some of these cells project to the IML. We investigated the involvement of SP at the IML in mediating rVLM- and PPr-evoked pressor responses in the chloralose-anesthetized cat. Pressor responses to electrical and chemical PPr stimulation were altered after intrathecal injection, at the level of the T1-T3 spinal cord, of either SP antagonist [D-Pro2, D-Phe7, D-Trp9]-SP, SP antagonist CP 96,345, or SP antiserum. Although MAP and heart rate responses to PPr stimulation were attenuated by intrathecal SP antagonists or antiserum, MAP responses to rVLM stimulation were augmented. Previous studies have revealed differences in transmitters associated with these two areas, even though the general response of both areas is sympathoexcitatory. The present study implies that the identical substance may increase or decrease the MAP response depending on the pathway activated.

brain stem; cats; CP 96,345; intermediolateral cell column; pressor responses

Several medullary regions have been identified that give rise to sympathoexcitation on stimulation. Two of the better-studied sites are the rostral ventrolateral medulla (rVLM) and the parapyramidal region (PPr). Both sites have projections directly to the intermediolateral cell column (IML) (1, 4, 18, 46). Despite similarities, these areas have separate neurochemical identities (6, 7, 35, 46–49) and are thus thought to exert actions independently (43).

The extensively studied rVLM may be defined in the cat as extending rostrally from the rostral pole of the lateral reticular nucleus (external division) to the vicinity of the subretrofacial nucleus (8) and is sometimes identified as the subretrofacial nucleus (40). This area projects directly to the IML of the thoracic spinal cord (4, 8, 46, 47). Stimulation of the rVLM gives rise to pressor effects, and the area is important both for the maintenance of resting blood pressure (4, 16, 34, 39, 44, 47) and reflex responses (4, 8). rVLM appears to be topographically organized into neuronal subpopulations corresponding to specific functional target tissues (type of vascular bed) (39).

Immunocytochemical studies have indicated that the rVLM has neurons that contain tyrosine hydroxylase (46), dopamine β-hydroxylase (7), phenylethanolamine N-methyltransferase (PNMT) (7, 46–49), 5-hydroxytryptamine (5-HT) (6, 35), and substance P (SP) (6, 35). However, the extent of SP localization in the subretrofacial area of the rVLM can be debated on the basis of the appearance of immunocytochemical data (6) and reports that SP is not found in cells of the subretrofacial nucleus (39). SP and PNMT appear to be colocalized at least to some degree in rVLM neurons of the rat (34, 42, 45).

A review by Helke et al. (22) has defined the PPr in the rat as close to the ventral surface, lateral to the pyramidal tract, bordering the lateral aspects of the inferior olivary nuclei, including portions of the B1 and B3 cell groups and medial aspects of the nucleus paragigantocellularis lateralis. The cat PPr has not been as specifically defined. However, the SP-containing cells of the cat are not a compact group, but rather diffuse in their distribution (6).

In both the cat and the rat, SP and serotonin are present in PPr perikarya (6, 21, 34) and to some extent colocalized (25). There is evidence for the presence of several other neuropeptides (6, 22) and to some extent catecholamine-synthesizing enzymes (7, 34, 46–48). Stimulation of the PPr produces elevations in mean arterial pressure (MAP) (27, 43).

SP is thought to be partially responsible for PPr sympathoexcitation at the IML. SP cells from ventral medulla project to IML (18). SP receptors are localized on sympathetic preganglionic neurons (17). These cells are excited when SP is applied microiontophoretically (14, 40) but with a very slow time course (11). Intrathecal injections of SP agonists give rise to cardioacceleratory responses in rats (20, 31). In the rat, intrathecal SP antagonists were shown to inhibit cardiovascular responses initiated by ventral medullary activation and cause resting MAP to fall to levels observed in spinal
animals (32, 33, 52) with attendant reduction of sympathetic nerve activity (52). Some of the effects seen with the SP antagonists were reversible (32). These results support the idea that SP is involved in tonic maintenance of MAP. However, this has been complicated by several reports, which have suggested that the SP antagonists may have nonspecific neurotoxic and vasocostrictor effects in the rat spinal cord (19, 21, 26).

Other studies have shown that 1) at least two SP antagonists apparently had no neurotoxic effects when introduced intrathecally (23, 29), and 2) a polyclonal antibody to SP (also intrathecally) was biologically active in immunoneutralizing SP in the cat (30). These studies suggested that intrathecal immunoneutralization as well as the use of antagonists could discriminate the effects of SP from those of other transmitters in the spinal cord. If SP is used mainly as an excitatory transmitter at the IML, one might possibly expect that an agent that would interfere with its transmission would be likely to reduce a pressor response regardless of the source. Thus in the current study we used intrathecal injections of SP antagonists and SP antiserum to investigate the involvement of SP at the thoracic IML in cardiovascular responses evoked from stimulation of the rVLM and PPR in the cat.

METHODS

General. The use of animals for these experiments was approved by the University of Illinois Urbana/Champaign Office of Laboratory Animal Care Advisory Committee.

Anesthesia was induced in adult cats (2.2–4.2 kg) with halothane (1–5%) in a mixture of one-third oxygen and two-thirds nitrous oxide. Cannulas were placed in one external jugular vein and one common carotid artery for infusion of fluids and monitoring of blood pressure, respectively. The trachea was also intubated, and the cat was placed on a ventilator for the remainder of the experiment. The anesthesia was maintained by the addition of α-chloralose (65 mg/kg iv). At this time the gaseous anesthetics were terminated. Anesthesia levels were monitored through corneal reflex, limb withdrawal to toe pinch, pupillary diameter, and blood pressure. The chloralose was supplemented as needed. Rectal temperature was monitored and maintained at 37 ± 0.5°C with the aid of a heating pad and a heat lamp. The bladder was catheterized to assure that it remained empty.

The cat was then mounted in a Kopf stereotaxic frame equipped with an attachment to tilt the head ventrally 45 degrees. The head was maintained at a level ranging from 15 to 18 cm above table level to ensure that the caudal end of the cat was well below the head. An external fixation dama was placed on the spinous process of the C2 vertebra to ensure stability of the caudal brain stem and to keep it in a "normal" position with respect to the cranium to facilitate pipet or electrode placement, even though the remainder of the body was markedly slanted ventrally. A craniectomy was carried out to expose the cerebellum. A small portion of the caudal cerebellum was removed by suction to allow direct visualization of the obex.

A cannula made of PE-50 tubing was introduced into the subarachnoid space at the level of C1. This cannula was passed caudally to the level of T1–T3. Patency of the cannula was verified by injecting Ringer solution until the cerebrospinal fluid (CSF) level was raised sufficiently to cover the medulla. In most cases after completion of the experiments, Fast green dye was injected into the cannula to verify placement and determine the extent of migration of the injections. The cat was removed from the stereotaxic device, and a laminectomy from cervical vertebrae to the lumbar vertebrae was performed. Upon dissection, dye was observed to be present along the entire spinal cord. We cannot rule out migration of the dye cranially to the brain stem over time (due to natural flow of the CSF), but no dye was immediately apparent on the brain stem from the dorsal aspect.

Electrical stimulation. Semimicroelectrodes (Rhodes SNE 300) were introduced into the rVLM and PPR stereotaxically. According to Berman's coordinate system (3) the rVLM area is located at ~P10–11, L3.5–4, and is ~4–5 mm from the dorsal surface, and the PPR is at ~P11–12, L2.5–2.85, and is 4.9–5.4 mm from the dorsal surface. Expressed in terms of distance from the obex, for the rVLM this is 2.25–2.3 mm rostral, 3.7–4.0 mm lateral, and 5.0–5.75 mm ventral, and for the PPR this is 2.0–2.3 mm rostral, 2.5–2.85 mm lateral to midline, and 5.5–5.9 mm ventral.

The rVLM was stimulated with 0.7-ms rectangular pulses at 50–200 µA in trains of 30 s duration. Because activation frequency appears to be an issue with the release patterns of SP (24), we controlled this aspect in most of the experiments (the exceptions being in those cases in which chemical stimulation was used). Two stimulus frequencies (2–5 and 30–100 Hz) were used. We found that this was the best way to separate the pressor effects from depressor effects (respectively), which are also known to be evoked on stimulation in these areas (50). The low and high frequencies were alternated with 5 min between each stimulus presentation. This stimulation protocol with the stimulus frequencies 2–4 and 20–40 Hz was also used to elicit pressor responses from the PPR.

Each cat was stimulated in not fewer than three consecutive trials of both the high- and low-frequency stimulation under control conditions to ensure the consistency. The stimulus was then repeated after infusion of an antagonist or antiserum.

Chemical stimulation. Chemical stimulation was used in a number of animals as a control to ensure that the results from electrical stimulation were not simply the result of stimulation of fibers en passage (15). Single-barrel micropipettes were made (1 mm OD, stock glass) with a pipette puller (Kopf 7000). The tips were broken back to a diameter of 15–20 µm. The micropipettes were filled with DL-homocysteic acid (DLH) (160 mM, pH 7.25–7.39), a glutamate agonist. Pontamine sky blue (1%) was also placed in the micropipettes for marking of stimulus sites. The DLH and the dye were separated from each other by a layer of mineral oil. A WPI PV830 pneumatic Picopump was used to administer small volumes of DLH (15–30 nl in 6–10 steps over 10 s). The amount injected was determined by measuring the fall of the meniscus formed at the oil-dye interface in the micropipette with a calibrated reticle in the eyepiece of a dissecting microscope.

Two or three repeatable control pressor responses evoked at 10- or 20-min intervals by DLH stimulation were recorded before intrathecal injection (both SP antagonists). The stimulation was continued after the intrathecal injection with 10 or 20 min separating each subsequent stimulus trial.

Intrathecal injection. An SP antagonist, [d-Pro2, d-Phe6, d-Trp9]-SP (Peninsula Laboratories 7477) at 500 µg or CP 96,345 (Pfizer) at 100 and 300 µg, an SP antiserum (Ab), or a control substance was infused (vol = 0.8–1.0 ml in all cases) through the cannula previously placed intrathecally at T1–3. The Peninsula Laboratories SP antagonist (SP-PL Atg) used in the present study is thought to be both an NK-1 (SP) and
NK-2 (neurokinin A) receptor antagonist (5). The action of the Pfizer CP 96,345 SP antagonist (SP-CP Atg) is thought to be restricted to NK-1 (23).

The SP Ab used in this study is thought to be specific for SP with very little reactivity with other potential neurotransmitters (30). The antiserum definitely does not cross react (~0.01%) to neuropeptide, Met5-enkephalinamide, or somatostatin. It does, however, cross react with physalaemin, an amphibian peptide, and with the peptide fragment SP4-11. The titer of the antiserum is 1:12,500, which represents the dilution that binds 50% of a standard amount of antigen (29).

In addition to the SP antagonists, SP-PL and CP 96,345, and the SP Ab, we used control substances to verify the alterations of the presumed SP-mediated responses. This included Locke-Ringer solution for the SP-PL Atg, inactive enantiomer (CP 96,344) (300 µg) for CP 96,345, and normal rabbit serum as per Dees et al. (13) for the SP Ab.

We used immunoabsorbed SP Ab (1 mg of SP to every ml of antiserum) to verify that the observed results were caused by an SP-like substance and not something unidentified, such as a nonspecific immunoglobulin effect. It is known that 50–150 µg of SP agonist is effective in immunoabsorption of 1 ml a 1:10 dilution of the primary antiserum (12, 30) as used in immunocytochemistry. When corrected for the amount of antiserum that we used in this experiment, this is a range of 0.35–1.05 mg/ml. Thus the immunoabsorb should have been adequate to immunoabsorb all the activity of this antiserum.

After infusion of the appropriate substance, the rVLM or the PPr was electrically stimulated intermittently as described previously for 60–90 min. In the case of the chemical stimulation, the stimulus was repeated only at the most probable times at which an effect would be observed on the basis of the electrical stimulation data, because it was likely that toxic effects of chemical stimulation would result with excessive repetition. Only one substance was infused intrathecally at a time, and MAP before and after intrathecal injection

Effects of SP antagonists and SP antiserum on resting HR and MAP before and after intrathecal injection

<table>
<thead>
<tr>
<th>Substrate</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
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<tbody>
<tr>
<td>PPr, SP-PL Atg (n = 5)</td>
<td></td>
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<tr>
<td>Preinjection</td>
<td>168.4 ± 10.8</td>
<td>97.2 ± 9.1</td>
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<tr>
<td>Postinjection</td>
<td>175.6 ± 9.6</td>
<td>99.2 ± 10.1</td>
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<tr>
<td>PPr, SP Ab (n = 4)</td>
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<tr>
<td>Preinjection</td>
<td>179.0 ± 25.5</td>
<td>100.3 ± 4.7</td>
</tr>
<tr>
<td>Postinjection</td>
<td>195.5 ± 29.5</td>
<td>100.5 ± 4.9</td>
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<tr>
<td>PPr, SP-CP Atg (n = 6)</td>
<td></td>
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<tr>
<td>Preinjection</td>
<td>203.0 ± 13.0</td>
<td>104.5 ± 5.0</td>
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<tr>
<td>Postinjection</td>
<td>202.5 ± 13.2</td>
<td>106.3 ± 4.4</td>
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<tr>
<td>rVLM, SP-PL Atg (n = 6)</td>
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<tr>
<td>Preinjection</td>
<td>163.1 ± 9.7</td>
<td>93.9 ± 5.4</td>
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<tr>
<td>Postinjection</td>
<td>167.3 ± 8.3</td>
<td>95.5 ± 6.9</td>
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<tr>
<td>rVLM, SP Ab (n = 6)</td>
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<tr>
<td>Preinjection</td>
<td>170.8 ± 16.2</td>
<td>111.9 ± 4.6</td>
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<tr>
<td>Postinjection</td>
<td>173.3 ± 15.6</td>
<td>111.8 ± 7.6</td>
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<tr>
<td>rVLM, SP-CP Atg (n = 6)</td>
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<tr>
<td>Preinjection</td>
<td>213.2 ± 17.6</td>
<td>99.7 ± 7.3</td>
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<tr>
<td>Postinjection</td>
<td>208.8 ± 17.1</td>
<td>96.6 ± 6.9</td>
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Values are means ± SE. Values are grouped according to area to be electrically stimulated, either parapyramidal region (PPr) or rostral ventrolateral medulla (rVLM). SP, Substance P; HR, heart rate; MAP, mean arterial blood pressure; SP-PL Atg, Peninsula Laboratories SP antagonist; SP-CP Atg, CP 96,345 SP antagonist; SP Ab, SP antiserum.
Low-frequency (2–5 Hz) stimulation of the rVLM consistently gave rise to depressor responses in all animals studied (n = 34, mean = −20.7 ± 2.3 mmHg). These responses were not significantly affected (P > 0.05) by the control substance, Ringer solution (n = 9, mean = −0.3 ± 1.7 mmHg). Changes in responses induced after intrathecal injections of antiserum (n = 6, mean = +4.0 ± 3.4 mmHg), rabbit serum (n = 7, mean = −2.7 ± 2.1 mmHg), SP-PL Atg (n = 6, mean = +0.3 ± 0.9 mmHg), and SP-CP Atg (n = 6, mean = +4.2 ± 2.8 mmHg) were not significantly different (P > 0.05) from those after Ringer solution infusion.

Effect of SP-PL Atg on high-frequency PPr-evoked responses. High-frequency (20–40 Hz) electrical stimulation of the PPr produced increases in MAP and HR (+22.0 ± 10.7 mmHg and +19.0 ± 2.3 beats/min, respectively; n = 5). These increases in MAP and HR were significantly attenuated (−13.6 ± 4.2 mmHg and −8.8 ± 2.3 beats/min, respectively) after 500 µg SP-PL Atg. An example recording is shown in Fig. 1. These data are summarized graphically in Fig. 2. Stimulus sites are shown in Fig. 6.

Effect of SP-CP Atg on high-frequency PPr-evoked responses. The responses to high-frequency stimulation of PPr were attenuated by intrathecal infusion of SP-CP Atg (100 or 300 µg; see RESULTS, Dose-response effects of SP-CP Atg). The MAP response was significantly reduced from +26.7 ± 4.3 to +11.3 ± 2.9 mmHg, whereas the HR response was also significantly reduced (P < 0.05) from +24.5 ± 7.4 to +4.3 ± 5.3 beats/min (n = 6). This result is shown graphically in Fig. 3. Stimulus sites are shown in Fig. 6.
Effect of SP-CP Atg on high-frequency rVLM-evoked responses. The responses to high-frequency stimulation of rVLM were augmented by intrathecal infusion of SP-CP Atg (300 µg). The MAP response was significantly increased from $+24.8 \pm 8.2$ to $+37.8 \pm 8.3$. The difference in HR responses was not significant. These results are expressed graphically in Fig. 3. Stimulus sites are shown in Fig. 6.

Dose-response effects of SP-CP Atg. Because we were granted access to sufficient quantities of CP 96,345 (courtesy of Charles Pfizer), the effects of SP-CP Atg on PPr- and rVLM-evoked responses were assessed at dosages of 100 and 300 µg. The 100 µg abolished the pressor response evoked by PPR stimulation in three of six animals. In the remaining three animals, 300 µg further reduced the response in only one of three animals. Although 100 µg altered response to rVLM stimulation in four of six animals, we found that 300 µg gave consistent responses in all cases, changing the evoked response from rVLM stimulation by 10% or greater.

Duration of SP-CP Atg effects. The blood pressure responses to electrical stimulations using SP-CP Atg varied in their ability to recover. For the rVLM high-frequency stimulations there was a sizable increase in the responses once CP 96,345 was intrathecally injected. This augmented response was seen between 10 and 20 min postinjection. At 30 min, four of six cats had responses smaller than their control responses. One out of six cats still had an elevated response at 1 h. For the PPr high-frequency electrical stimulations, the results were different. At 40 min after infusion of CP 96,345, responses of only two of six cats had increased above the experimentally attenuated responses. Two of six cats never recovered and actually developed depressor responses to high-frequency electrical stimulation by 60 min.

Effect of SP Ab on high-frequency PPr-evoked responses. The cardiovascular increases after PPr high-frequency electrical stimulation were attenuated 25–40 min after intrathecal injection of SP Ab. The increases in MAP and HR were significantly attenuated ($-9.5 \pm 2.3$ mmHg and $-10.8 \pm 3.4$ beats/min, respectively). The effect of the SP Ab is summarized graphically in Fig. 4. Stimulus sites are shown in Fig. 6.

Intrathecal SP Ab preabsorbed with purified SP had no apparent effect on the cardiovascular changes evoked from electrically stimulating the PPr of two animals. Stimulation sites for PPr stimulation during intrathecal SP Ab, SP-PL Atg, and SP-CP Atg administration are represented in Fig. 6.

Effect of SP Ab on high-frequency rVLM-evoked responses. The pressor response to high-frequency stimulation was significantly augmented (+48.8 ± 16.5 mmHg, n = 6) by intrathecal injection of SP Ab (Fig. 4). An example of these data are shown in Fig. 1. We again extensively tested for a vehicle control effect. Normal rabbit serum had no effect on the pressor responses to...
rVLM stimulation (n = 7). The difference between the effect of SP Ab and normal rabbit control serum was also significant (Fig. 7). In two animals an SP antiserum absorbed with purified SP was used as the intrathecal injection. This immunoabsorbed antiserum also had no effect on evoked cardiovascular responses. The sites of rVLM stimulation are also shown in Fig. 6.

DLH chemical stimulation. Intrathecal SP-PL Atg and SP-CP Atg deposited at T1–3 had effects on the responses evoked from chemical activation similar to those obtained from electrical stimulation of the rVLM and PPr. Unexpectedly, chemical stimulation of the PPr was found to be very problematic. In 35 animals a PPr response was obtained; however, in only 10 cases were the responses reproducible to the extent to allow experimentation to proceed.

Microinjection of DLH into the PPr produced an increase in blood pressure but a variable response in HR. These responses were attenuated (−15.0 ± 1.0 mmHg and −4.5 ± 3.5 beats/min; n = 2) after intrathecal SP-PL Atg. After intrathecal SP-CP Atg, the blood pressure response was significantly attenuated (−13.9 ± 3.3 mmHg; n = 4), whereas HR response was not significantly augmented (+14.3 ± 6.5 beats/min; n = 4). Results are shown in Fig. 5.

Microinjection of DLH into the rVLM produced large pressor responses accompanied by a decrease in HR, which may be attributable to baroreceptor activation. The pressor response increased (+40.45 ± 21.35 mmHg; n = 2) after intrathecal SP-PL Atg, whereas the HR depression was attenuated (−8.8 ± 0.5 beats/min). Blood pressure and HR responses to DLH were augmented (+18.3 ± 2.9 mmHg and +3.5 ± 6.5 beats/min; n = 4) after intrathecal SP-CP Atg injection (Fig. 5). Only the blood pressure responses were significantly altered by the microinjections. Control experiments for the rVLM region using only the vehicle did not significantly alter the responses (−12.3 ± 11.3 mmHg and −6.3 ± 4.7 beats/min; n = 4).

Effect of SP agonist. Intrathecal infusion of Sigma SP S-6883 in four cats produced several alterations in resting MAP and HR. Moments after intrathecal introduction of 100 and 300 µg of the SP agonist, there was a transient, significant (P < 0.05) depression in the MAP after 100 (−13.58 ± 2.28 mmHg) and 300 µg (−7 ± 2.42 mmHg) and a transient, significant rise in HR (5.25 ± 1.18 beats/min) after the 100-µg dose. This temporary decrease in MAP and increase in HR between 12 and 180 s after the injection were noted at all three dosages, but proved insignificant (P > 0.05) for the HR change at 300 µg (+7 ± 2.41 beats/min) and the MAP (12.98 ± 4.11 mmHg; n = 4) and HR (7.75 ± 4.07 beats/min) changes at 1,000 µg. MAP and HR were unchanged during the period from 5 to 25 min at all three concentrations. At 30 min after 100-µg infusion, significant (P < 0.05) MAP (5.25 ± 3.25 mmHg) and HR (+7.5 ± 4.37 beats/min) increases were noted, which remained significant for the remainder of the 45-min monitoring period. No significant alterations in resting MAP and HR were noted for the 5- to 40-min period.
after 300 and 1,000 µg infusions. See Fig. 8 for a plot of MAP and HR after intrathecal SP.

DISCUSSION

A previous study by Minson et al. (43) indicated that the rVLM and the PPr exert their pressor effects independently by separate chemical mechanisms. It was shown that serotonin (5-HT) mediated the PPr effect, whereas the rVLM effect was not altered by the serotonin neurotoxin 5,7-DHT. The present results provide further evidence for the separate identities of these cell groups. In addition, the current results provide the first evidence to indicate a differential role for SP in the cat spinal cord on the cardiovascular responses evoked from stimulation of the rVLM vs. the PPr. The pressor response obtained from stimulation of the rVLM was augmented by the intrathecal administration of SP Ab or SP Atgs, whereas the pressor response obtained from PPr stimulation was attenuated, seemingly diametrically opposed effects.

Of interest is that there were no significant differences in resting blood pressure and HR before and after infusion of the SP Atgs or SP Ab. This suggests that SP may not have a significant role in the tonic maintenance of MAP or HR in the cat. The current results differ somewhat from previous studies, which may be a result of species differences or variations in methodology. Other studies using rats have shown that intrathecal SP antagonists depress MAP to levels observed in spinal animals, suggesting that SP has a major role in tonic blood pressure maintenance (32, 33, 52). Some of the SP Atgs used in these studies, however, have been described as having nonspecific neurotoxic and vasoconstrictor effects in the rat (19, 21, 26). Keeler et al. (31) used the same antagonist as in the present study ([D-Pro2,D-Phe4,D-Trp9]-SP) and showed reversible depression of resting MAP when it was injected at T10–11 in the rat. They also used GABA inhibition (bicuculline) to excite the rVLM and evoke pressor responses. These responses were also attenuated.

It is possible that infusion of the substances at the T1-T3 area may have made a difference. In other studies the sites ranged from T4 to T10 levels in rats. We consider this unlikely for the following reasons. In our previous work in the cat (29), we found that similar volumes (1 ml) of dye injected into the lumbar subarachnoid space spread widely from the injection site, even to the cervical segments when the cat's head was level with the lumbar spine. This kind of spread was also seen on injection of Fast green dye through the cannula in the present study. However, the spread occurred...
caudally from the injection site, because the cat’s head was elevated well above the table to attempt to facilitate spread to the thoracic region.

More importantly, for the purposes of these experiments, the lack of effects by intrathecal SP Atgs and SP Ab on resting MAP in the present study would tend to suggest that general physiological conditions were not altered substantially by the injections.

Infusion of varying doses of SP did cause alterations in resting MAP and HR but did so inconsistently. The transitory depression in MAP and increase in HR was seen with significance only at the lower dosages. Persistent elevations in HR and MAP were only noted at the lowest dose and only after 30 min. These results are not consistent with observations after SP intrathecal infusion in rats in which increases in both HR and BP have been observed, with dose-dependent responses, within the time limits of our recordings (20, 31). Our observations suggest that SP does not play an important role in maintaining resting MAP and HR in the cat.

The most obvious interpretation of the current data is that the descending SP-containing projections from the PPr are antagonized or immunoneutralized at the IML by intrathecal injection of SP antagonist and SP antiserum, respectively. The general concept of modulation by SP of these pathways is not a new one (18, 31–33, 52). In the hands of several investigators, SP appeared to be responsible for mediating pressor activity evoked by stimulation of the ventral medulla at the level of the spinal cord (31–33, 52). This had never been confirmed in the cat.

The rVLM and PPr both participate in sympathoexcitation. If one assumes that SP has only one effect in the IML, sympathoexcitation, then it seems likely that the effects of SP antagonist or antiserum would be the same for responses evoked from both of these areas. Certainly, the effects of microiontophoretically applied SP would suggest that the only effect on sympathetic preganglionic cells is excitation (14, 40). Our results, which show diametrically opposed effects using high-frequency electrical and chemical stimulation of these areas, thus seem somewhat counterintuitive.

As electrical stimulation was used in these experiments, it is possible that fibers en passage were largely responsible for the effects observed. This seems unlikely because similar effects were observed with chemical activation of these areas.

The data gathered with the SP Atgs and also SP Ab, coupled with that obtained with immunoneutralized SP antiserum, would seem to suggest strongly that SP is responsible for the effects observed. However, it remains possible that the active substance involved was not SP but a related chemical. SP is one of the tachykinins and is designated as affecting NK-1 receptors. Other tachykinins, such as neurokinin A (NKA) or neurokinin B (NKB) (affecting primarily NK-2 and NK-3 receptors, respectively), may have been responsible for a portion of the effects. As mentioned, the SP-PL Atg is thought to affect both NK-1 and -2 receptors (5). It has been suggested that some SP antisera may cross react with other tachykinins (36), including NKA and NKB. However, the SP-CP Atg is specific for NK-1 receptors (23), and thus a portion of the responses observed are definitely attributable to SP.

Yashpal et al. (51) describe the locations of all three tachykinin receptors in the rat spinal cord. NK-1 receptors are the most numerous of the three and are heavily concentrated in the dorsal and ventromedial aspects of the dorsal horn and in the IML. The majority of NK-2 receptors in the thoracic spinal cord are located in the dorsal and ventromedial aspects of the dorsal horn and in a small area connecting the two lateral horns. The NK-3 sites are most concentrated in the dorsal border of the dorsal horn, with moderate numbers located in the IML. We have described extensively the bulbospinal projection to the NK-1 receptors; however, the remaining possibilities of an NK-2- or NK-3-
mediated response are supported by recent discoveries. SP has been shown to stimulate NK-2 receptors (10). NKB has been identified in cell bodies scattered in and near the rat ventrolateral medulla (38), which could possibly have a spinal projection. Against these possibilities is the view that NK-3 receptors do not appear to have a well-defined physiological effect at the spinal level, as evidence is conflicting (23). Thus the potential role of these other tachykinin receptors in PPr or rVLM stimulations cannot be ruled out.

Low-frequency (2–5 Hz) electrical stimulation of the rVLM consistently caused depressor responses. Low-frequency depressor responses were unaffected by intrathecal SP Atgs or SP Ab. This finding suggests that SP is not involved in these responses. Similar depressor effects have been observed previously (50), but they are difficult to explain on the basis of known connections from the rVLM, perhaps suggesting fibers on passage are responsible. This may be reflective of the inhibitory action of epinephrine microiontophoretically applied on sympathetic preganglionic neurons (9, 11, 40). A bradycardia has been seen with higher doses of microinjected epinephrine known to be mediated by α2-receptors (37).

Although the limitations of electrical stimulation are well known to stimulate fibers as well as cells, even localized chemical stimulation of either the rVLM or PPr may activate other medullary sites because of spread of the stimulus or via a synaptic projection. These factors, combined with the multiple nature of the receptor that could have been stimulated, suggest, as emphasized by Couture et al. (10), that we cannot rule out the possibility that other neuronal projections to the IML known to participate in cardiovascular regulation could have been affected by the intrathecal injections.

Despite these drawbacks, the present study has shown that SP or a related substance is likely to play a role in the modulation of both rVLM and PPr stimulus-evoked responses at spinal levels. However, this role may be more complex than a simple role as an excitatory transmitter. Dekfelt and colleagues (24) have presented at least two alternative mechanisms. In addition to having a general postsynaptic effect, one possibility they present has the release of SP dependent on impulse traffic. The second mechanism has SP blocking an inhibitory 5-HT autoreceptor, resulting in accentuated 5-HT release on stimulation. A third possibility has been suggested by Dampney (11). In this model the monoamines and/or peptides may be coreleased with another transmitter, such as an excitatory or inhibitory amino acid neurotransmitter. The presence or absence of one of the transmitters determines the nature of the other transmitter’s effects.

Our own previous studies (2, 28) demonstrated that the two cell areas also appear to differ in responses to stimulation by static muscular contraction. Cells of the rVLM typically exhibit a sustained discharge during muscular contraction of several seconds duration, with varying degrees of adaptation in their firing. In contrast, cells of the PPr are likely to exhibit a short burst of activity during the onset and cessation of static muscular contraction (28). Thus the two systems may contribute different components to a sympathoexcitatory response.

In conclusion, these data appear to demonstrate that the projections from the PPr and rVLM are affected by SP agonists in different ways. These antagonists block effects of PPr stimulation but conversely appear to augment responses to rVLM stimulation. These data further support the prior contention of Minson et al. (43) that the PPr and rVLM are areas with clearly separate chemical identities in terms of their action at the IML.

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