Intrathecal PACAP-38 causes increases in sympathetic nerve activity and heart rate but not blood pressure in the spontaneously hypertensive rat

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Intrathecal PACAP-38 causes increases in sympathetic nerve activity and heart rate but not blood pressure in the spontaneously hypertensive rat. Am J Physiol Heart Circ Physiol 300: H214–H222, 2011. First published October 15, 2010; doi:10.1152/ajpheart.00662.2010.—The rostral ventrolateral medulla contains presynaptic neurons that project monosynaptically to sympathetic preganglionic neurons (SPN) in the spinal cord and are essential for the tonic and reflex control of the cardiovascular system. SPN directly innervate the adrenal medulla and, via postganglionic axons, affect the heart, kidneys, and blood vessels to alter sympathetic outflow and hence blood pressure. Over 80% of bulbospinal, catecholaminergic (C1) neurons contain pituitary adenylate cyclase-activating polypeptide (PACAP) mRNA. Activation of PACAP receptors with intrathecal infusion of PACAP-38 causes a robust, prolonged elevation in sympathetic tone. Given that a common feature of most forms of hypertension is elevated sympathetic tone, this study aimed to determine in the spontaneously hypertensive rat (SHR) and the Wistar Kyoto rat (normotensive control) 1) the proportion of C1 neurons containing PACAP mRNA and 2) responsiveness to intrathecal PACAP-38. We further investigated whether intrathecal infusion of the PACAP antagonist, PACAP(6–38), reduces the hypertension in the SHR. The principal findings are that 1) the proportion of PACAP mRNA-containing C1 neurons is not different between normotensive and hypertensive rats, 2) intrathecal PACAP-38 causes a strain-dependent, sustained sympathoexcitatory and tachycardia with variable effects on mean arterial pressure in normotensive and hypertensive rats, and 3) PACAP(6–38) effectively attenuated the effects of intrathecal PACAP-38, but had no effect alone, on any baseline variables. This finding indicates that PACAP-38 is not tonically released in the spinal cord of rats. A role for PACAP in hypertension in conscious rats remains to be determined.

sympathetic activity; spinal cord; pituitary adenylate cyclase-activating polypeptide

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THE ROSTRAL VENTROLATERAL MEDulla (RVLM) is crucial for the tonic and reflex control of the cardiovascular system (see Refs. 15 and 43 for reviews). RVLM presynaptic neurons are commonly defined as being inhibited following baroreceptor activation and having a spinal axon (2, 28, 29). Neurochemically, 60–80% of presynaptic neurons are C1 neurons (29, 42, 48, 49), having all of the enzymes required for adrenaline synthesis. Presynaptic RVLM neurons project monosynaptically to sympathetic preganglionic neurons (SPN) in the intermediolateral (IML) cell column of the spinal cord (34, 38). SPN, in turn, regulate the activity of the heart, kidneys, blood vessels, and adrenal chromaffin cells, thereby determining sympathetic outflow and ultimately blood pressure.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is an excitatory 38-amino-acid peptide originally identified in ovine hypothalamus (32) that also exists as a truncated 27-amino-acid form (33). The distributions of PACAP mRNA and PACAP peptide in the central nervous system have been mapped using in situ hybridization (ISH) and immunohistochemistry (9, 17–19, 41). In particular, PACAP mRNA is present in over 80% of catecholaminergic RVLM neurons that project to the thoracic IML (10). PACAP elicits its effects via three G protein-coupled receptors, PAC1, VPAC1, and VPAC2, which primarily act to increase adenylate cyclase activity (see Refs. 8, 50, and 51 for reviews). The PAC1 receptor is specific for PACAP (3) and is the primary form in the central nervous system (4, 25). Infusion of PACAP-38 into the intrathecal space has sympathoexcitatory effects (10, 26) although the pressor effects reported by Lai et al. (26) contrast with the findings of Farnham et al. (10), who reported no change in blood pressure.

A common feature associated with essential hypertension in humans is elevated sympathetic tone (46). The spontaneously hypertensive rat (SHR), which was developed in 1963 (37), is a model of neurogenic (23) or essential hypertension, similar to essential hypertension in humans (22, 31). Sympathetic tone is elevated in young SHR well before the onset of hypertension. Both the SHR and its genetic normotensive control, the Wistar Kyoto (WKY) rat, were derived from the normotensive Wistar strain (37). The WKY was the primary normotensive control for this study, with Sprague-Dawley (SD) rats having been studied previously (10). Where a significant difference in responses existed between the two inbred normotensive rat models (SD and WKY) in the present study, the outbred Wistar was also tested to determine whether the observed differences had a genetic basis.

Here we test the hypothesis that increased spinal PACAP activity contributes to sympathoexcitation and hypertension in the SHR.

The first aim of this study was to determine any difference in the proportion of RVLM catecholaminergic neurons containing PACAP mRNA in SHR and WKY rats. The second aim tested whether the spinal PACAP receptors differed in number or activity between hypertensive and normotensive rat models by investigating responsiveness to intrathecal infusion of PACAP-38. Intrathecal infusion of the PACAP antagonist, PACAP(6–38), was used to determine whether the effects seen on blood pressure, sympathetic nerve activity, and heart rate (HR) with PACAP-38 were attributable to effects at the PAC1R and the VPAC2R. Finally, infusion of PACAP(6–38) was used to investigate a possible role for tonic activation of the PAC1R and VPAC2R.
MATERIALS AND METHODS

Animals

All procedures and protocols were approved by the Animal Care and Ethics Committee of Macquarie University. Experiments were conducted on adult male SD, WKY, Wistar, and SHR (350–500 g; Animal Resource Center, Perth, Australia) in accordance with the Australian code of practice for the care and use of animals for scientific purposes.

Tail-cuff Blood Pressure

The blood pressure phenotype of hypertensive and normotensive rats was confirmed at >18 wk of age by tail-cuff phymgomonometer. Hypertension was defined as a tail-cuff systolic pressure ≥150 mmHg and normotension as ≤140 mmHg.

Briefly, an inflatable cuff was placed around the base of the tail, and a pressure transducer was secured over the tail artery immediately caudal to the cuff. The signal from the pressure transducer was amplified, digitized, and recorded on a computer (Spike 2 v6.09; CED, Cambridge, UK). The cuff was inflated to a pressure sufficient to occlude the artery (~160 mmHg for WKY rats and ~200 mmHg for SHRs) then released. The highest tail-cuff pressure at which a pulse could be detected was recorded as the systolic blood pressure. Measurements were repeated at least five times and averaged. All SHR in this study had a systolic blood pressure ≥150 mmHg (mean: 183 ± 2 mmHg; range: 171–195 mmHg), and the WKY had a systolic blood pressure ≤140 mmHg (mean: 124 ± 2 mmHg; range: 113–136 mmHg).

Combined ISH and Fluorescence Immunohistochemistry

Experiments were conducted as described previously (10, 27, 40). Three SHR and three WKY rats were examined to identify neurons in the RVLM that expressed PACAP mRNA and were also immunoreactive (ir) for tyrosine hydroxylase (TH), a marker for catecholaminergic neurons.

Rats were deeply anaesthetized with sodium pentobarbital (80 mg/kg ip) and perfused through the left ventricle/ascending aorta with heparinized 0.9% saline followed by 4% paraformaldehyde in 0.1 M PBS (pH 7.4). The brain was postfixed overnight in the same fixative. Brainstems were sectioned coronally (40 μm) and collected sequentially into four containers containing PBS with 0.1% Tween-20.

Sense and antisense probes were synthesized using an amplified DNA fragment of the PACAP gene from rat brain cDNA using forward and reverse primers with SP6 and T7 promoters attached at the 5′ and 3′ ends, respectively (PACAP-ISH f: GGATCCATTTAGGTGACAC-TATAGAATGGTACG-ATCAGGACGGAAACC; PACAP-ISH r: GAAT-TCTAATACGACTCACTATAGGGAGATGC-ACGCTTATGAATT-MGTC). Sense and anti-sense riboprobes were transcribed in vitro using digoxigenin-11-UTP and an Sp6 or T7 RiboMAX large scale RNA production system (Promega, Alexandria, New South Wales, Australia). The specificity of the riboprobe was confirmed with a dot blot and by running it on a 1.2% agarose gel, which showed a single band.

For combined ISH (for PACAP mRNA) and fluorescence immunohistochemistry (for TH), free-floating sections of rat brain were hybridized overnight before addition of the primary antibodies, alkaline phosphatase-conjugated sheep anti-digoxigenin (1:1,000; Roche, Dee Why, New South Wales, Australia), and mouse anti-TH (1:2,000; Sigma Aldrich, Castle Hill, New South Wales, Australia). After 48 h, TH immunoreactivity was subsequently revealed by incubation overnight with Cy3-conjugated donkey anti-mouse IgG (1:500, Jackson Laboratories, Bar Harbor, ME). A colorimetric reaction using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate salts in a solution of 0.1 M NaCl, 0.1 M Tris·HCl, pH 9.5, 0.1 M MgCl2, 0.1% Tween-20, and 2 mM levamisole revealed digoxigenin-labeled neurons as those containing dark purple precipitants. Sections were mounted sequentially on glass slides and cover slipped with Vectashield (Vector Laboratories, East Brisbane, Queensland, Australia).

Imaging and analysis. All image capture and cell counts were conducted on an epifluorescence microscope (AxioImager Z1; Zeiss, Jena, Germany). The RVLM was defined as a triangular area ventral to the nucleus ambiguus, medial to the spinal trigeminal tract, and lateral to the inferior olive or the pyramidal tracts. Bilateral cell counts were taken from the section with the rostral pole of the inferior olive (bregma ~11.96 mm). The proportion of PACAP mRNA-containing TH neurons within the RVLM was compared between SHR, WKY, and SD with a one-way ANOVA.

Intrathecal Administration of PACAP-38 and PACAP(6–38)

PACAP-38 was administered intrathecally to SHR, WKY, and Wistar rats to investigate any differences in responsiveness to PACAP-38 between normotensive and hypertensive strains. The PACAP antagonist, PACAP(6–38), was administered intrathecally to investigate whether PACAP is tonically released in the spinal cord and whether it contributes to the hypertension of the SHR. PACAP-38 was also administered intrathecally to SD rats 15 min after intrathecal PACAP(6–38) to determine the extent to which the antagonist was effective in attenuating the PACAP response. A PACAP-38 dose-response curve was constructed by infusing 10 μl of 100 and 300 μmol/l (n = 3) concentrations in SD rats and comparing the results with the previously reported responses to a 1,000 μmol/l solution (10). An antagonist dose-response curve was constructed in a similar manner by administering 10 μl of 100 (n = 4), 300 (n = 3), and 1,000 μmol/l (n = 6) concentrations of PACAP(6–38) 15 min before administering 10 μl of 1,000 μmol/l (1 μmol/l) PACAP-38.

Anesthesia and surgical preparation. All rats (n = 12 SHR, n = 9 WKY, n = 7 Wistar, n = 16 SD) were anesthetized with 10% urethane (1.0–1.5 g/kg ip). Atropine sulphate (100 μg/kg ip) was administered in the same injection to reduce bronchial secretions before vagotomy. Surgical level of anesthesia was defined as the absence of any withdrawal reflex to any nociceptive or tactile stimuli such as a tail pinch or corneal touch. While under neuromuscular blockade, a rise in blood pressure >10 mmHg in response to a tail or paw pinch indicated a need for an anesthetic boost. Additional anesthetic (30–40 mg urethane iv) was administered as required. Complete details of surgical preparation and data acquisition methods are as described previously (10).

Briefly, rats were secured in a stereotaxic frame, and temperature was maintained at 37 ± 0.5°C using a rectal probe connected to a homeothermic heating blanket (Harvard Apparatus, Holliston, MA). The right carotid artery and jugular vein were cannulated for the measurement of arterial blood pressure and administration of drugs and fluids, respectively. A tracheotomy was performed and the vagi cut to permit artificial ventilation. ECG leads were placed in forepaws to record HR. The left splanchnic sympathetic nerve was dissected, cut at the celiac ganglion, and prepared for recording (2 kHz sampling rate, 1–100 k gain, 0.1–2.0 kHz filtering). The rat was artificially ventilated with oxygen-enriched room air and paralysed with pancuronium bromide (0.4 mg given as a 0.2 ml bolus iv, then an infusion of 20% pancuronium in 0.9% saline at a rate of 1 ml/h).

The atlanto-occipital junction was exposed, and a catheter (polyvinylchloride tubing, ID 0.2 mm; OD 0.5 mm) with a dead space of ~6 μl was inserted into the intrathecal space through the slit in the dura and advanced caudally to the level of T5/T6.

Intrathecal drug administration protocol. A vehicle infusion of 10 μl of 10 mmol/l PBS was washed in with 6 μl of 10 mmol/l PBS and mean arterial pressure (MAP), HR, and splanchnic sympathetic nerve activity (sSNA) were recorded for 35 min. Ten microliters of 1 mmol/l PACAP-38 or PACAP(6–38) was then administered and flushed in with 6 μl of PBS. In the SD rats, 10 μl of 1 mmol/l PACAP-38 was infused 15 min after infusion of 10 μl of 1 mmol/l PACAP(6–38). All infusions were made over a 10–15-s period. MAP, HR, and sSNA
responses were recorded for up to 125 min following the final drug infusion. At the conclusion of the experiments, the rats were euthanized with 0.5 ml of 3 M potassium chloride (KCl iv). Postmortem verification of the location of the catheter tip was achieved by injecting 10 μl of India ink and recording the vertebral segments where both the tip of the catheter and the ink stain were observed.

Data Acquisition and Analysis

Before analysis, raw sSNA [2 kHz sampling rate, 1–100 k gain, 0.1–2.0 kHz filtering; a 50/60 Hz line frequency filter (Humbug: Quest Scientific; North Vancouver, British Columbia, Canada) was also used] was rectified and a 2-s smoothing function applied. This resulted in a trace of mean sympathetic activity that was analyzed in the same manner as HR and blood pressure with an additional step of normalizing the signal to 0 by subtracting residual activity 5 min after death.

Five-minute time periods of MAP, HR, and sSNA were analyzed before and following intrathecal infusions of PBS (up to 35 min) or PACAP-38/PACAP(6–38) (up to 125 min).

Analysis was conducted with GraphPad Prism (v. 5.0). Two-way ANOVA with repeated measures for time was used to examine the effects of drug over time. Two-way ANOVA with Bonferroni’s correction was used to compare the drug responses between the strains for both PACAP-38 and PACAP(6–38) compared with PBS unless otherwise stated. A P value <0.05 was taken as a significant difference.

RESULTS

PACAP mRNA Colocalization with TH in the RVLM of Normotensive and Hypertensive Models

In the SHR (n = 3), 85.1 ± 1.5% of TH-ir neurons within the RVLM (bregma −11.96 mm) contained PACAP mRNA, compared with 89.3 ± 1.5% in the WKY (n = 3). Similarly, an 84.4 ± 4.2% colocalization of TH-ir and PACAP mRNA neurons over the entire RVLM was reported in the SD rat (10) (Fig. 1). There was no difference in the proportion of PACAP mRNA-containing TH-ir neurons between the three strains (P > 0.05) or in the number of TH-ir neurons between the SHR (81 ± 13 neurons) compared with the WKY (73 ± 7 neurons) (P > 0.05).

Dose-Response Curves for PACAP-38 and PACAP(6–38)

Dose-response curves were generated for both intrathecal PACAP-38 and PACAP(6–38) followed by intrathecal PACAP-38 to determine the most effective dose for use in this study. The PACAP-38 dose-response curve was generated at 100 (n = 3), 300 (n = 3), and 1,000 μmol/l (n = 6) solutions (Fig. 2). One-way ANOVAs of the peak responses revealed that the 1,000 μmol/l concentration to be effective in significantly elevating HR (66 ± 9 beats/min; P < 0.0001) and sSNA (93.2 ± 26.5%; P < 0.0001), but none of the concentrations tested significantly changed MAP. To test the effectiveness of the antagonist in vivo a PACAP(6–38) dose-response curve was generated by administering 1 mmol/l PACAP-38 15 min after 100 (n = 3), 300 (n = 3), and 1,000 μmol/l (n = 6) solutions of PACAP(6–38). The results were compared with SD rats that were only treated with intrathecal 1,000 μmol/l PACAP-38 (10). The 1,000 μmol/l dose of PACAP(6–38) was the only dose that attenuated the sSNA (Δ−72%; P < 0.05) and HR (Δ−73%; P < 0.05) responses to intrathecal 1,000 μmol/l PACAP-38 significantly (Fig. 2). Administration of PACAP(6–38) had no effect on MAP (Fig. 2). PACAP-38 and PACAP(6–38) were therefore used at the 1,000 μmol/l (1 mmol/l) concentration for the remainder of this study.

Fig. 1. Proportion of pituitary adenylate cyclase-activating polypeptide (PACAP) mRNA-containing tyrosine hydroxylase-immunoreactive (TH-ir) neurons in the rostral ventrolateral medulla (RVLM) of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY). A: mean ± SE proportion of PACAP mRNA-containing TH-ir neurons in the RVLM at bregma −11.96 mm is plotted for SHR (n = 3) and WKY (n = 3). The mean ± SE proportion of PACAP mRNA-positive and TH-ir neurons over the entire RVLM (bregma −11.6 to −12.6 mm) is plotted for the Sprague-Dawley rats (SD) (n = 3; Ref. 10) for comparison. There was no difference in the proportion of PACAP mRNA-containing TH-ir neurons between the three strains (P > 0.05). B: diagram of a coronal section of rat brainstem. The small box within the RVLM represents the area from which the photos in C and D were taken. C: photo taken from the RVLM of an SHR. D: photo taken from the RVLM of a WKY. The red label is for TH-ir neurons, whereas the black neurons are positive for PACAP mRNA. The scale bars in both C and D represent 50 μm. NTS, nucleus of solitary tract; nAmb, nucleus ambiguous; Sp5, spinal trigeminal tract; Py, pyramidal tracts; IO, inferior olive.
Effects of Intrathecal PACAP-38 on HR in Normotensive and Hypertensive Models

Baseline MAP under anesthesia was significantly higher in SHR (116 ± 5 mmHg; n = 6) compared with WKY (90 ± 2 mmHg; n = 5) and Wistar (96 ± 4 mmHg; n = 7) (P < 0.005; 1-way ANOVA with a post hoc Bonferroni’s correction). Intrathecal infusion of PACAP-38 had a significant effect on MAP over time in all strains (P < 0.0001), and, although strain itself was not a significant factor, there was a significant interaction of strain over time (P < 0.05). In all strains MAP decreased (P < 0.05) 5 min after intrathecal infusion of PACAP-38. Following this decrease, MAP in the Wistar and WKY remained depressed, whereas the SHR returned to control levels within 10–20 min before steadily decreasing again to a level similar to that of the Wistar (Figs. 3 and 4A). Whereas the time-course response to PACAP-38 was not strain dependent, the peak response of MAP was. Figure 5A compares the peak responses to PACAP-38 in all three strains. PACAP-38 significantly decreases MAP compared with PBS in all strains (P < 0.01) (Fig. 5A). This decrease was significantly lower in the Wistar compared with both the SHR and the WKY (P < 0.001) (Fig. 5A).

Effects of Intrathecal PACAP-38 on HR in Normotensive and Hypertensive Models

The baseline HR was 433 ± 4 beats/min in the WKY (n = 5), 395 ± 9 in the SHR (n = 6), and 438 ± 11 in the Wistar (n = 7). The baseline HR of the SHR was significantly lower than that of the Wistar (P < 0.05; 1-way ANOVA with a post hoc Bonferroni’s correction). Intrathecal infusion of PACAP-38 significantly increased HR over time in all strains (P < 0.0001) compared with PBS (Figs. 3 and 4B). This effect was strain dependent (P < 0.05), and the tachycardic response to PACAP-38 persisted until the end of the experiment (125 min postinfusion). The peak HR response after PACAP-38 was significantly increased compared with PBS in the SHR (P < 0.001) and the Wistar (P < 0.05; Fig. 5B). The peak PACAP-38 HR response of the SHR (Δ54 ± 11 beats/min) was significantly greater than that of the WKY (Δ16 ± 13 beats/min) and Wistar (Δ30 ± 9 beats/min) responses (P < 0.01; Fig. 5B).

Effect of Intrathecal PACAP-38 on sSNA in Normotension and Hypertension

Not all strains responded to PACAP-38 in the same way because the time by strain effect was highly significant. Intrathecal infusion of PACAP-38 significantly and differentially altered sSNA over time in the three strains (P < 0.0001) (Figs. 3A, 3B, and 3C). The peak sSNA response to PACAP-38 was significantly greater than the PBS response only in the SHR (P < 0.001; Fig. 5C). The peak sSNA response to PACAP-38 of the SHR was significantly greater than that of the WKY (P < 0.05) and the Wistar (P < 0.001).

Effect of Intrathecal PACAP(6–38) on MAP, HR, and sSNA in Normotension and Hypertension

The PACAP antagonist, PACAP(6–38), was administered intrathecally to test the hypothesis that PACAP has greater tonic activity in the SHR. The effects of the PACAP(6–38) on cardiovascular parameters were tested in the SHR (n = 6) and WKY (n = 4) but not the Wistar because there was no difference in effect between the SHR and WKY.

Intrathecal PACAP(6–38) had no effect on HR or sSNA in either strain compared with PBS (P > 0.05; Fig. 5, B and C). MAP, on the other hand, was significantly increased 5 min after PACAP(6–38) administration in the SHR (Δ11 ± 3 mmHg; P < 0.01; Fig. 5A). The response returned to baseline within 60 min but did not fall below the baseline level at any time during the test period.

DISCUSSION

The novel findings of this study are first that the proportion of TH-ir neurons within the RVL that also contain PACAP mRNA is not different between SD, WKY, and SHR strains. Second, intrathecal PACAP-38 causes sympathoexcitation and tachycardia in Wistar, WKY, and SHR strains, as well as in SD rats (10). The sympathoexcitation and tachycardia observed varies in magnitude and is unrelated to the resting MAP before
PACAP infusion in the four strains examined. Finally, intrathecal administration of PACAP(6–38), an antagonist at PAC1R and VPAC2R, did not affect HR or SNA, suggesting that PACAP-38 is not tonically released in the spinal cord of SHR or WKY, at least in the anesthetized preparation used here. Thus we conclude that PACAP is unlikely to be the underlying cause of hypertension in SHR.

To date, the extent of PACAP mRNA expression in the RVLM of hypertensive rats is unknown. In the normotensive SD, PACAP mRNA is present in ~82% of TH-ir RVLM neurons that project to the spinal cord (10) and also in phenylethanolamine N-methyltransferase-ir RVLM neurons that project to the paraventricular nucleus (6). The present study, together with our earlier work, enables a comparison of brainstem PACAP mRNA expression in hypertensive and normotensive strains. Although the proportion of PACAP mRNA-containing TH-ir neurons within the RVLM is not different between the strains, a caveat to our findings is that the approach does not measure the total amount of mRNA, only its presence or absence. ISH is not quantitative and does not accurately measure PACAP RNA content of RVLM sympathetic premotor neurons. Furthermore, mRNA detection may not reflect its translation into, and thus the expression of, PACAP peptide.

To investigate the role of spinal cord PACAP in hypertension, PACAP-38 and its antagonist, PACAP(6–38), were infused intrathecally in SHR and WKY rats; clear differences were observed between the two strains in the MAP, HR, and sSNA responses. The responses to PACAP-38 were compared with those seen in SD in our previous study (10). Interestingly the responses observed in SD and SHR were of a similar magnitude (e.g., SNA increased >100%) whereas the WKY responses appeared blunted (SNA increased by 53%) (Figs. 2, 3, and 4). The unexpected difference between the SD and WKY prompted an investigation of the Wistar, the outbred normotensive parent strain of the WKY and SHR, with the intention of revealing which normotensive strain, SD or WKY, was atypical in its responses. Instead, the Wistar responses to intrathecal PACAP-38 were intermediate in magnitude between the SHR and WKY for MAP and HR. On the other hand,
the sSNA response of the Wistar was less than that of the WKY. These conflicting results have complicated a straightforward answer to the aforementioned question. One important factor may be that the Wistar is an outbred rat strain compared with the inbred SD and WKY strains. The Wistar also showed greater variation in responses, particularly in the sSNA responses. In five animals, the sSNA increased in response to intrathecal PACAP, but, in two animals, there was a decrease of a similar magnitude. This heterogeneity in response was not observed in any of the other three strains studied. Our results have highlighted the need for investigation of the complexity of cardiovascular differences between strains. The significance of these differences in responsiveness between strains is also difficult to interpret because the cellular distribution of the PACAP receptors in the spinal cord is presently unknown. One possible explanation is that a presently unknown difference in spinal cord PACAP receptor expression exists between the strains. It is known that splice variants of PAC1 receptors exist, but their expression and functional significance in the spinal sympathetic column remains unexplored at this stage (8, 47). Another important caveat to note is that the observed differences in effects between the strains may not be strictly cardiovascular in nature but may be attributable to confounding effects of PACAP receptor differences in other systems activated by intrathecal PACAP such as the dorsal horn. Questions could also be raised about the accessibility of PACAP to the SPN. First, there is evidence that some dendrites of SPN reach the surface of the spinal cord (44). Second, although the SPN may indeed be less accessible to intrathecal infusion of PACAP, the time course of the responses following PACAP administration is consistent with this fact. The responses are not immediate, and nerve activity starts to rise 5–10 min after PACAP administration. We suggest that this time reflects the amount of time that it takes for PACAP infusion to fully occupy the available receptors on SPN.

The final aim of this study was to determine whether endogenous PACAP-38 contributes to the hypertension observed in SHR. PACAP(6–38) is an antagonist specific for the PAC1 receptor, which is the predominant form in the central nervous system (5, 25) but also acts at the VPAC2 receptor, albeit with a 10-fold lesser affinity (7, 20, 45). PACAP(6–38)
and sSNA responses to intrathecal administration of PACAP did not reduce any measured parameter from baseline in either the SHR or the WKY. Unexpectedly, PACAP(6–38) did cause an initial small increase in MAP (70 mmHg, P < 0.05) in the SHR. The results from this study indicate that PACAP-38 is probably not tonically released in the spinal cord and is not contributing to the hypertension of SHR in this preparation. It is possible that the VPAC1 receptors may compensate for the PAC1/VPAC2 antagonist but is unlikely because the results show absolutely no reduction in activity. The question of SPN accessibility also arises for PACAP(6–38), but we believe the suggestion given for PACAP-38 is also applicable here. The dose-response curve in Fig. 2 provides evidence that the antagonist is able to access much the same receptor sites as PACAP-38 itself.

The decreases in MAP following intrathecal infusion of PACAP-38 are paradoxical given the sympathoexcitatory effects observed but are not baroreflex mediated as determined previously in the SD (10). One possible explanation is that PACAP differentially affects sympathetic outflows to various vascular beds. PACAP activating the splanchnic sympathetic bed may cause vasoconstriction in the mesenteric vascular bed, but inhibition of the lumbar sympathetic bed may cause a large vasodilatation in the tail vascular bed. Whereas the responses of vasomotor nerves other than the splanchnic sympathetic nerve are yet to be studied in rat, PACAP-induced differential regional blood flow was observed previously in the hindquarters and pulmonary circulation of the cat (30).

The contradictory MAP response observed in this study may also be driven by differential actions of the three PACAP receptors. The different PACAP receptors have had specific actions demonstrated in several systems. The PAC1 receptor is important in renin secretion (21) and PACAP-induced depolarizations in superior cervical ganglion neurons (1). The VPAC1 receptor, on the other hand, is necessary for pressure-induced vasodilatation (11), and evidence from our present study suggests that it may be primarily involved in the MAP response observed after intrathecal infusion of PACAP-38.

PACAP(6–38) is an antagonist of the PAC1 and VPAC2 receptors and attenuated the HR and sSNA effects of intrathecal PACAP-38 in the SD by over 70%, but the MAP response remained unaffected. These results provide evidence that PACAP(6–38) is an effective antagonist and also suggests a differential role for the receptors in the spinal cord. PACAP(6–38) was reported to have slight agonistic properties at high doses (39), which could explain the small significant increase in MAP and small insignificant increases in sSNA and HR of the SHR following intrathecal PACAP(6–38). If the hypothesis that PACAP receptors have differing actions in the spinal cord is true, it could mean that the VPAC1 receptor has opposing vasodilatory effects on blood pressure and predominates over the vasoconstrictor actions of the PAC1/VPAC2 receptors. The sSNA and HR responses, on the other hand, are dependent on all three receptors for full expression of the PACAP response.

It is still unknown which stimuli cause the release of PACAP-38 physiologically from presympathetic neurons. PACAP is implicated in glucose metabolism (14, 16, 36) and thermoregulation (13) (see Refs. 8, 50, and 51 for reviews) and is frequently implicated in responses to other stressors such as adrenaline release and hemorrhage (24, 35). The increased HR and sSNA responses to intrathecal administration of PACAP are consistent with an enhanced metabolic response and may also contribute to the “fight or flight” response.

In summary, three major findings are reported. First, the proportion of PACAP mRNA-containing TH-ir neurons is similar within the RVLM of normotensive and hypertensive rats. Second, intrathecal PACAP-38 causes strain-dependent, sustained sympathoexcitation and tachycardia with variable effects on MAP in both normotensive and hypertensive rats. The MAP effects may be primarily mediated via the VPAC1 receptor. Last, intrathecal administration of PACAP(6–38), an agonist at PAC1R and VPAC2R, did not affect HR or SNA, suggesting that PACAP-38 is not tonically released in the spinal cord of SHR or WKY. We conclude that PACAP in the spinal cord is not an underlying cause of the hypertension in SHR rats, at least in our urethane-anesthetized, vagotomized, paralyzed, and artificially ventilated preparation.

**Perspectives**

The potent excitatory peptide PACAP is present in catecholaminergic bulbospinal neurons in the RVLM (10), in SPN in the spinal cord (12), in the adrenal medulla (24), and in chromaffin cell carcinomas (12). PACAP receptors are also present on blood vessels and in many other sites throughout the body (50, 51). This widespread distribution suggests a crucial role for this pleiotropic polypeptide in regulating the cardiovascular system. Although the precise roles played by PACAP in RVLM neurons in regulating SPN still remain topics for future study, the findings here suggest that PACAP can differentially regulate blood pressure via PAC1, VPAC1, and VPAC2 receptors. Additional specific agonists and antagonists for each of the receptors are needed to further explore this hypothesis.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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