Intrathecal PACAP-38 causes prolonged widespread sympathoexcitation via a spinally mediated mechanism and increases in basal metabolic rate in anesthetized rat

Melissa A. Inglott, Melissa M. J. Farnham, and Paul M. Pilowsky
Australian School of Advanced Medicine, Macquarie University, Sydney, Australia
Submitted 19 October 2010; accepted in final form 25 March 2011

Inglott MA, Farnham MM, Pilowsky PM. Intrathecal PACAP-38 causes prolonged widespread sympathoexcitation via a spinally mediated mechanism and increases in basal metabolic rate in anesthetized rat. Am J Physiol Heart Circ Physiol 300:H2300–H2307, 2011. First published April 1, 2011; doi:10.1152/ajpheart.01052.2010.—The rostral ventrolateral medulla (RVLM) is critical for the tonic and reflex maintenance of the cardiovascular system (15, 17, 44) and possibly in the genesis of hypertension (21, 34, 37). The RVLM generates sympathetic outflow to the cardiovascular system via sympathetic preganglionic neurons (SPNs) in the intermediolateral column of the spinal cord (SC) (18, 40). Stimulation of distinct subregions within the RVLM causes differential changes in sympathetic nerve activity (SNA) (4, 6, 7, 30, 39). Some areas in the RVLM, when activated, increase sympathetic nerve activity and heart rate, but not mean arterial blood pressure. The mechanism behind this response is unknown but may be due to a differential control of sympathetic outflows. In this study we sought 1) to investigate whether intrathecal PACAP differentially affects sympathetic outflow, 2) to determine whether the intrathecal responses to PACAP are solely due to a spinally mediated mechanism, and 3) to determine whether intrathecal PACAP affects metabolic function. Experiments using urethane-anesthetized, vagotomized, ventilated, and paralyzed adult male Sprague-Dawley rats were conducted in this study. Intrathecal injections of PACAP-38 were given, and mean arterial pressure, heart rate, the activity of regional sympathetic nerves, end-tidal CO2, and core temperature were recorded. The novel findings of this study are that 1) intrathecal PACAP-38 causes a prolonged widespread sympathoexcitation in multiple sympathetic beds, 2) this widespread sympathoexcitation is mediated within the spinal cord itself since spinal transection does not abrogate the response, and 3) that intrathecal PACAP-38 increases basal metabolic rate. Therefore, we conclude that intrathecal PACAP acts in the spinal cord to cause a prolonged widespread sympathoexcitation and that PACAP also causes an increase in basal metabolic rate that includes an increase in brown adipose tissue thermogenesis in our rat preparation.

blood pressure; spinal cord; sympathetic beds; metabolism; spinal transection; pituitary adenylate cyclase-activating polypeptide

Intrathelcal PACAP-38 causes prolonged widespread sympathoexcitation via a spinally mediated mechanism and increases in basal metabolic rate in anesthetized rat. Am J Physiol Heart Circ Physiol 300:H2300–H2307, 2011. First published April 1, 2011; doi:10.1152/ajpheart.01052.2010.—The rostral ventrolateral medulla (RVLM) is critical for the tonic and reflex maintenance of the cardiovascular system (15, 17, 44) and possibly in the genesis of hypertension (21, 34, 37). The RVLM generates sympathetic outflow to the cardiovascular system via sympathetic preganglionic neurons (SPNs) in the intermediolateral column of the spinal cord (SC) (18, 40). Stimulation of distinct subregions within the RVLM causes differential changes in sympathetic nerve activity (SNA) (4, 6, 7, 30, 39). Some areas in the RVLM, when activated, increase sympathetic nerve activity and heart rate, but not mean arterial blood pressure. The mechanism behind this response is unknown but may be due to a differential control of sympathetic outflows. In this study we sought 1) to investigate whether intrathecal PACAP differentially affects sympathetic outflow, 2) to determine whether the intrathecal responses to PACAP are solely due to a spinally mediated mechanism, and 3) to determine whether intrathecal PACAP affects metabolic function. Experiments using urethane-anesthetized, vagotomized, ventilated, and paralyzed adult male Sprague-Dawley rats were conducted in this study. Intrathecal injections of PACAP-38 were given, and mean arterial pressure, heart rate, the activity of regional sympathetic nerves, end-tidal CO2, and core temperature were recorded. The novel findings of this study are that 1) intrathecal PACAP-38 causes a prolonged widespread sympathoexcitation in multiple sympathetic beds, 2) this widespread sympathoexcitation is mediated within the spinal cord itself since spinal transection does not abrogate the response, and 3) that intrathecal PACAP-38 increases basal metabolic rate. Therefore, we conclude that intrathecal PACAP acts in the spinal cord to cause a prolonged widespread sympathoexcitation and that PACAP also causes an increase in basal metabolic rate that includes an increase in brown adipose tissue thermogenesis in our rat preparation.

blood pressure; spinal cord; sympathetic beds; metabolism; spinal transection; pituitary adenylate cyclase-activating polypeptide

The rostral ventrolateral medulla (RVLM) is critical for the tonic and reflex maintenance of the cardiovascular system (15, 17, 44) and possibly in the genesis of hypertension (21, 34, 37). The RVLM generates sympathetic outflow to the cardiovascular system via sympathetic preganglionic neurons (SPNs) in the intermediolateral column of the spinal cord (SC) (18, 40). Stimulation of distinct subregions within the RVLM causes differential changes in sympathetic nerve activity (SNA) (4, 6, 7, 30, 39). Some areas in the RVLM, when activated, increase SNA to more than one target tissue (5, 29, 30, 35, 43). The cause of this differential control remains unknown. The neurochemistry of RVLM neurons is heterogeneous, with all neurons containing at least one amino acid neurotransmitter and at least one other neurotransmitter. Generally, there are many other colocalized metabotropic neurotransmitters including enkephalin (42), neuropeptide Y (32), and pituitary adenylate cyclase-activating polypeptide (PACAP) (14) that act with glutamate and GABA to excite or inhibit RVLM neurons (40). This broad spectrum of colocalized neurotransmitters in RVLM neurons may explain its ability to differentially control SNA (11, 22, 35, 41). Previous work from our laboratory and others indicates that PACAP may be involved in this process (13, 14, 31).

PACAP is a 38-amino acid, excitatory neuropeptide (33) that exerts its effects on three receptors: the PACAP-specific receptor (PAC1 receptor) and two other receptors that have an equal affinity for vasoactive intestinal polypeptide and PACAP (VPAC1 and VPAC2 receptors) (8, 45). PACAP mRNA and PACAP peptide are present within the central nervous system (9, 10, 20, 26). In the brain stem, PACAP mRNA is found in 82% of C1 (epinephrine synthesizing) presympathetic bulbospinal neurons within the RVLM (14). An intrathecal administration of PACAP to examine the cardiovascular effects of activation of these SPN results in tachycardia and sympathoexcitation in normotensive (14) and hypertensive (13) rats. Paradoxically, the sympathoexcitation that is observed following the administration of PACAP intrathecally is not accompanied by an increase in mean arterial blood pressure (MAP) (14). This response is not altered in Sprague-Dawley (SD) rats that have been surgically barodenervated (15).

PACAP is also implicated in the maintenance of normal energy homeostasis, including brown adipose tissue (BAT) thermogenesis and glucose metabolism (1, 45), processes that the RVLM has a role in and are known to affect MAP (28). Therefore, the aims of this study are 1) to investigate whether or not intrathecal PACAP, differentially or globally, affects sympathetic outflows by recording from multiple sympathetic nerves; 2) to determine whether the previously reported (15) responses to intrathecal PACAP are due to a spinally mediated mechanism by intrathecal injection of PACAP-38 in C1 spinal transected rats; and 3) to determine whether intrathecal PACAP affects metabolic function through the measurement of end-tidal CO2, pH, arterial partial pressure of CO2 (PaCO2), arterial partial pressure of O2, core temperature, and glucose levels.

METHODS

Ethics approval. All experiments were conducted on adult male SD rats (350–500 g; Animal Resources Centre, Perth, Australia) in accordance with the guidelines set out by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and
were approved by the Animal Care and Ethics Committee and Bio-
safety Committee of Macquarie University.

Surgical preparation. General surgical methods were carried out as
previously described (14). All rats (n = 21) were anesthetized with
urethane (ethyl carbamate; 1.3–1.5 g/kg ip; Sigma-Aldrich). Anes-
thetic depth was monitored by observing reflex responses to nocice-
ptive and tactile stimuli (periodic tail/paw pinches) and the corneal
touch reflex.

Additional anesthetic was administered (30–40 mg urethane iv), as
required to maintain adequate anesthesia. Atropine sulfate (100 µg/
kg, ip; Astra Pharmaceuticals) was administered to reduce bronchial
secretions. All rats were secured in a stereotaxic frame. A rectal probe,
connected to a heating blanket (Harvard Apparatus), allowed core
body temperature to be measured and maintained between 36.5 and
37.5°C over the experimental period.

General surgery. In all rats, the right carotid artery and jugular
veins were cannulated for the recording of blood pressure and the
administration of drugs and fluids, respectively. A tracheal cannula
was inserted to permit artificial ventilation (rodent ventilator; UGO
Basile, Biological Research Apparatus) and CO₂ monitoring (Capstar-
100 CO₂ analyzer; CWE). Heart rate (HR) was derived from the ECG
recording. The rats were bilaterally vagotomized, artificially venti-
lated, and paralyzed with pancuronium bromide (0.4 mg given as a 0.2
ml bolus iv, followed by an infusion of 10% pancuronium in 0.9% saline at a rate of 2 ml/h; Astra Pharmaceuticals). The left cervical,
splanchnic, and/or lumbar sympathetic nerves were isolated, dis-

---

Fig. 1. In vivo effects of intrathecal pituitary adenylate cyclase-activating polypeptide (PACAP)-38 administration on sympathetic nerve activity (SNA). Examples of traces from 2 Sprague-Dawley (SD) rats showing the effects of intrathecal vehicle (PBS) and PACAP-38 injection on cervical SNA (cSNA; A) and lumbar SNA (lSNA; B). Arrows indicate times of PBS and PACAP-38 administration. C: grouped data showing the time course of responses in lSNA (n = 6; i) and cSNA (n = 4; ii) following PBS and PACAP-38 injection. Arrow indicates time of PACAP-38 injection. PBS denotes the period after intrathecal injection of PBS, and PACAP-38 denotes the period after intrathecal administration of PACAP-38. D: 30-min peak responses in lSNA (Δ 105 ± 10%; P < 0.0001; n = 4), cSNA (Δ 96 ± 18%; P < 0.0001; n = 6), and splanchnic SNA (sSNA; Δ 105 ± 10%; P < 0.0001; n = 6) following administration of PBS and PACAP-38. There was no difference between the responses of the nerves (P > 0.05) after PACAP-38 injection. AU, arbitrary units. Scale bars represent 30 min. *P < 0.05; **P < 0.001; ns, not significant.
Intrathecal administration of PACAP-38. A catheter (polyvinylchloride; outer diameter, 0.61 mm; inner diameter, 0.28 mm; Critchley Electrical Products) with a dead space of ~6 μl was inserted into the intrathecal space of all rats through a slit in the dura at the atlanto-occipital junction and advanced caudally to the level of T5/6. A control injection of 10 μl of 0.9% saline (PBS) was washed in with 6 μl PBS. Next, 10 μl of 1 mM PACAP-38 (Auspep) was administered and flushed in with 6 μl PBS. Injections were done over a 10- to 15-s period, as previously described (14). Responses were recorded for 30 (PBS) and 90 (PACAP-38) min. Six rats also had respiratory blood gas (O2 and CO2), pH (electrolyte and blood gas analyzer; IDEXX Laboratories), and blood glucose analysis (blood glucose meter; Roche Diagnostics) conducted during the recording periods. At the conclusion of the experiments, all rats were euthanized with 0.5 ml of 3 M potassium chloride (KCl; iv). Post-mortem verification of the location of the intrathecal catheter was achieved by injecting 10 μl of India ink washed in with 6 μl of PBS into the intrathecal catheter and exposing the SC. The spinal segment level of the catheter was recorded as the level where the tip was observed or where the black spot appeared most intensely on the SC. If the catheter was not found at T5–6, the results were not included in the present study.

Intrathecal administration of PACAP-38 with surgical spinal transection. In a separate group of five rats (instrumented as described above), C1 surgical spinal transection was completed by exposure and laminectomy of the of the C1 vertebra to expose the corresponding spinal segment. The dura was removed and the SC was carefully transected. To ensure complete transection, sympathetic baroreflex tests [phenylephrine (PE), 10 μg/kg iv, 0.1-ml bolus; ICN Biomedicals] were conducted before and after transection, confirming the elimination of neural transmission. The lesion was closely inspected at the time of transection and, following death, to ensure no tracts remained intact. Data collection was only conducted after a successful verification of spinal transection (baroreflex abolition). Following the baroreflex tests, all parameters were recorded until readings returned to baseline. PBS and PACAP-38 were then injected intrathecally as described in the above protocol. After the responses of MAP, HR, CSNA, and arterial blood glucose levels were measured 10 and 5 min before and 5, 10, 20, 30, 40, 50, 60, and 90 min after intrathecal injections of PBS (up to 30 min) or PACAP-38 (up to 90 min). Arterial blood gas (O2, CO2, and pH) and arterial blood glucose levels were measured 10 and 5 min before and 30 min after intrathecal injections of both PBS and PACAP-38. Statistical analysis was carried out in GraphPad Prism software (version 4.0). Statistical significance was determined using one- or two-way ANOVA with Bonferroni’s correction, unless otherwise stated. P < 0.05 was considered to indicate a significant difference between the means.

RESULTS

In vivo effects of intrathecal PACAP-38 on SNA. Intrathecal PACAP-38 significantly increased the activity of cervical, splanchnic, and lumbar sympathetic nerves over time (P < 0.0001; Fig. 1). All three nerve activities doubled (~100% increase from baseline; P > 0.05) ~30 min post-PACAP-38 injection (Fig. 1D). Intrathecal PACAP-38 increased cSNA by zero by subtracting residual activity 5–10 min after death (SSNA data were rectified and smoothed/averaged to 2 s and normalized to baseline). PBS and PACAP-38 were then injected intrathecally as described in the above protocol. After the responses of MAP, HR and splanchnic SNA (sSNA) to PBS (30 min) and PACAP-38 (90 min) were recorded, the animal was euthanized, and the location of the catheter tip was verified as above.

Data acquisition and analysis. Data were acquired using a CED 1401 ADC system (Cambridge Electronic Design) and Spike 2 acquisition and analysis software (version 7.02; Cambridge Electronic Design). Cervical SNA (cSNA), lumbar SNA (lSNA), and sSNA raw data were rectified and smoothed/averaged to 2 s and normalized to zero by subtracting residual activity 5–10 min after death (SSNA data from spinally transected animals were not normalized, as baseline activity before data collection was equal to 0). MAP, HR, cSNA, sSNA, lSNA, end-tidal CO2, and core temperature were analyzed from 5-min blocks taken 10 and 5 min before and 5, 10, 20, 30, 40, 50, 60, and 90 min after intrathecal injections of PBS (up to 30 min) or PACAP-38 (up to 90 min). Arterial blood gas (O2, CO2, and pH) and arterial blood glucose levels were measured 10 and 5 min before and 30 min after intrathecal injections of both PBS and PACAP-38. Statistical analysis was carried out in GraphPad Prism software (version 4.0). Statistical significance was determined using one- or two-way ANOVA with Bonferroni’s correction, unless otherwise stated. P < 0.05 was considered to indicate a significant difference between the means.

Fig. 2, In vivo effects of intrathecal PACAP-38 administration in spinally transected and spinally intact SD rats. Grouped data showing the changes in mean arterial blood pressure (MAP; A), heart rate (HR; B), and sSNA (C) following PBS and PACAP-38 injection in spinally transected rats (●; n = 5) and rats with an intact spinal cord (○; n = 6). PACAP-38 caused an increase in HR [△48 ± 5 beats/min (bpm); n = 6; P < 0.0001] and sSNA (△105 ± 10%; n = 6; P < 0.0001) but did not significantly affect MAP (△12 ± 4 mmHg; n = 6; P > 0.05) in rats with an intact spinal cord. *P < 0.05; **P < 0.001. The MAP and HR responses were unaffected by spinal transection (P > 0.05); however, the peak of the HR response was significantly higher in the transected animals compared with the intact rats for the first 5 and 10 min of the response (P < 0.05). In spinally transected rats, the sSNA response to PACAP-38 was similar to that of the intact rats for 20 min (P > 0.05), after which activity declined compared with spinally intact animals (P < 0.05) but still remained elevated compared with baseline. *P < 0.05; **P < 0.001. Arrow indicates time of PACAP-38 injection. Scale bar represents 30 min. Cervical and lumbar sympathetic nerve activities were not recorded in spinally transected rats.
Intrathecal PACAP and Sympathetic Activity

100 ± 35% (n = 4; Fig. 1, A and C), tSNA by 96 ± 18% (n = 6; Fig. 1, B and C), and sSNA by 105 ± 10% (n = 6; Fig. 2C). L-, S- and cSNA remained markedly increased for the remainder of the experimental period (90 min) (Figs. 1 and 2C).

As was previously reported (13), we found that MAP was not affected (Δ -12 ± 4 mmHg; n = 6; P > 0.05) and HR was increased significantly from baseline (Δ 48 ± 5 beats/min; n = 6; P < 0.0001) following an intrathecal injection of PACAP-38 (Fig. 2, A and B).

Effect of spinal transection on the intrathecal PACAP-38 response. As expected, following spinal transection, the baseline values of MAP (109 ± 12 mmHg; n = 5), HR (438 ± 11 beats/min; n = 5), and sSNA (3.5 ± 0.2 μV; n = 5) were significantly reduced (P < 0.05) to 59 ± 7 mmHg, 358 ± 15 beats/min, and 1.6 ± 0.2 μV, respectively (Figs. 3 and 4). Following spinal transection, the sSNA response to PE is eliminated (Fig. 4, A and B), as is the pulse modulation (Fig. 4C) and the 8-Hz HR peak within the sSNA power spectrum (Fig. 4D). The abolition of all three of these indexes of supraspinal sympathetic activity confirms the effectiveness of the transection. Following surgical spinal transection, sSNA fell to a level that was no different to that observed 5–10 min after death (1.2 ± 0.2 μV; n = 5) (P > 0.05; Fig. 4, B and C).

An intrathecal injection of PACAP-38, following spinal transection, significantly increased HR (Δ 86 ± 16 beats/min; P < 0.0001; n = 5) and sSNA (Δ 84 ± 20%; P < 0.0001; n = 5) over time compared with PBS (Figs. 2, B and C, and 3). The HR and sSNA responses peaked ~10 min post-PACAP-38 injection and then slowly declined, but both remained above baseline for the duration of the experimental period (90 min; Figs. 2, B and C, and 3). On average, no significant effect on MAP was observed over time (Δ 11 ± 14 mmHg; P > 0.05; n = 5) (Figs. 2A and 3). When compared with the six spinally intact rats treated with intrathecal PACAP-38 (Fig. 2), the MAP and HR responses were unaffected by spinal transection (P > 0.05). However, the peak of the HR response was significantly higher in the transected animals compared with the intact rats for the first 5 and 10 min of the response (P < 0.05). In spinally transected rats, the sSNA response to PACAP-38 was similar to that of the intact rats for 20 min (P > 0.05), after which the activity declined compared with spinally intact animals (P < 0.05) but still remained elevated compared with baseline (Fig. 2).

In vivo effects of intrathecal PACAP-38 on end-tidal CO2, pH, core temperature, PaCO2, and glucose levels. The metabolic effects of PACAP-38 were assessed by recording changes in end-tidal CO2, pH, core temperature, PaCO2, and blood glucose levels (Fig. 5). Intrathecal PACAP-38 significantly increased end-tidal CO2 (Δ 0.67 ± 0.05% end tidal; n = 6; P < 0.0001). The end-tidal CO2 response peaked ~30 min post-PACAP-38 injection and remained elevated for the rest of the recording period (90 min) (Fig. 5A). PACAP-38 also significantly increased PaCO2 (Δ 7 ± 1 mmHg; n = 6; P < 0.05) and significantly decreased pH (Δ -0.9 ± 0.1; n = 6; P < 0.05) 30 min post-PACAP-38 administration compared with control (period before injection of vehicle) and vehicle (PBS; Fig. 5B). Blood glucose decreased significantly (Δ -1.7 ± 0.6 mmol/l; n = 6; P < 0.05) 30 min after PACAP-38 administration compared with control and vehicle (Fig. 5B). Core temperature was significantly increased 30, 40, 50, 60, and 90 min after PACAP-38 injection compared with PBS (Δ 0.4 ± 0.2°C; n = 6; P < 0.0001; unpaired t-test; Fig. 5A).

Discussion

This is the first study to investigate the possible mechanisms by which intrathecal PACAP exerts potent sympathoexcitatory effects, but with no corresponding change in MAP (14). The original findings of Farnham et al. (14) are confirmed in this study. In addition, the novel findings of the present study are as follows. First, despite the lack of MAP response, intrathecal PACAP causes a prolonged sympathoexcitation in all measured sympathetic beds. Second, the response to intrathecal PACAP is mediated within the SC since spinal transection does not abrogate the response. Finally, intrathecal PACAP causes an increase in metabolic rate as suggested by the effects on end-tidal CO2, pH, core temperature, PaCO2, and glucose levels. Therefore, we conclude that intrathecal PACAP acts via a spinally mediated mechanism to cause a prolonged widespread...
sympathoexcitation and that intrathecal PACAP causes an increase in metabolic rate (12, 27, 36).

To date, it is known that PACAP excites SPNs in SC slices from juvenile rats (25) and that an intrathecal administration of PACAP results in a prolonged tachycardia and a 100% increase in SSNA (14). However, contradictory effects on MAP have been reported. Lai et al. (25) described a pressor response, whereas Farnham et al. (14) reported no MAP effect following intrathecal PACAP-38. This present study confirms the findings observed by Farnham et al. (14), showing increases in SSNA and HR, but no significant change in MAP.

In this study we measured the outflow from multiple sympathetic nerves in response to intrathecal PACAP-38 to determine whether the lack of MAP response is due to a differential activation of sympathetic beds. Our data reveal that intrathecal PACAP-38 injection at the level of T6 increased cSNA, tSNA, and gSNA by ~100%, all of which are known to contain vasoconstrictor fibers. The results indicate that intrathecal PACAP causes a widespread prolonged excitation of sympathetic vasoconstrictor pathways and that the unaltered MAP following intrathecal PACAP is not due to a differential sympathetic outflow to these vascular beds. These findings contradict a previous study that found that intra-arterial injections of PACAP produced differential effects in the perfusion pressures of the pulmonary and hindquarter vascular beds of the cat (31). Perhaps the major reason for this difference is that in cat there are substantial intrathoracic pulmonary vascular noradrenergic innervations, whereas in rat there is none (3). Apart from the differences in dose, species, and injection method, the measures used to infer centrally mediated changes to sympathetic vasomotor tone may explain the differences observed. Perfusion pressure is an indirect measurement of changes in vasomotor tone. Our study used recordings from barosensitive, pulse-modulated sympathetic nerves to obtain direct, more reliable measures of changes in vasomotor tone. The vascular isolation of the pulmonary bed and sympathetic denervation of the hindquarter vascular bed could also account for the difference observed between this and the study of Minkes et al. (31).

C1 spinal transection allowed us to confirm that the responses to intrathecal PACAP-38 observed in this and the previous study (14) are due to the activation of SPNs in the intermediolateral of the SC and not due to the activation of MAP.
presympathetic neurons in the brain stem. C1 spinal transection did not prevent the response to intrathecal PACAP-38 from occurring, confirming that intrathecal PACAP-38 acts via a spinally mediated mechanism to cause the increases in HR and sSNA observed in this and previous studies (15). The peak HR response to intrathecal PACAP-38 in the spinalized rats was significantly larger than that seen in the spinally intact rats. A technical caveat to our study and this preparation is that the SC and the sympathetic nervous system in the spinally transected rats are greatly damaged, and, therefore, the responses seen may be influenced by this. The difference seen between the HR responses in the spinalized and spinally intact rats may be due to this. The HR and sSNA responses to intrathecal PACAP-38 observed in spinalized animals were not as long lasting as those seen in spinally intact rats. In spinalized rats, the blood pressure is reduced to around 50 mmHg, and the resting level of SNA is greatly reduced. The lack of normal resting sympathetic tone present in the spinalized rats may account for the discrepancy seen in the HR and sSNA responses between the spinalized and spinally intact rats; the normally prolonged response may not be maintainable in this preparation.

Previous work in knockout mice (with no prepro-PACAP gene present) suggests that PACAP plays a role in normal energy homeostasis, in glucose metabolism, and in thermogenesis (1, 8, 45). However, this is the first study to show that an intrathecal injection of PACAP-38 significantly increases end-tidal CO₂, Paₐₐ₉, and core temperature and decreases pH and blood glucose levels. The results indicate that intrathecal PACAP causes an increase in metabolic rate. The increase in end-tidal CO₂ may be representative of a decrease in SNA (vasodilation) to the pulmonary vascular bed (31). This is unlikely since, as noted above, rats lack an intrathoracic sympathetic innervation (3). The concurrent increase in Paₐ₉, decrease in pH, and decrease in blood glucose suggest that the increase in expired CO₂ occurs as a result of increased metabolic rate. Increased metabolism is commonly associated with an increase in BAT thermogenesis. Increased SNA to BAT causes an increase in BAT thermogenesis and increases BAT temperature, end-tidal CO₂, and core body temperature. Although changes in BAT temperature are mirrored by changes in core body temperature, the response observed in core body temperature is always smaller than in BAT temperature (12, 27, 36). The coupled increases observed in end-tidal CO₂ and core body temperature point to a possible role for PACAP in BAT thermogenesis. In one experiment (data not shown), bupivacaine, a local anesthetic, was injected into the intrascapular brown fat after PACAP was injected, resulting in a fall in core body temperature.

The initiation of BAT thermogenesis may amplify or cause a decrease in blood glucose levels following intrathecal PACAP. Nakata and Yada (38) suggest that PACAP potentiates many metabolic processes. It is suggested that PACAP is involved in energy storage after feeding through insulin release and action, adipogenesis, adipocyte differentiation, and by stimulating feeding (1). However, in the fasting state, PACAP is involved in energy utilization through catecholamine- and
glucagon-mediated lipolysis (19), through increased glucose output from the liver, and also by stimulating feeding. The significant decrease in blood glucose levels observed in this study indicates that energy utilization, and therefore metabolic rate, has increased in response to intrathecal PACAP. The work presented here suggests that the balance of glucose production versus glucose utilization tips toward a lower blood glucose level following intrathecal PACAP injection.

Taken together, the data illustrate an increase in SNA to the heart, splanchnic bed, hindlimbs, head and neck, brown adipose tissue, and epinephrine-secreting chromaffin cells. The release of epinephrine from the adrenal medulla would have the effect of increasing HR and mobilizing glucose from glucagon in the liver. In this preparation we have performed a cervical vagotomy bilaterally. This means that any effects observed can only be due to sympatoactivation, sympathoinhibition, or release of hormones from the adrenal gland or hypophysis. Plasma norepinephrine was not measured in these experiments, but the activation of baroreceptors always causes a complete inhibition of SNA in the splanchnic nerve, indicating the activation of fibers supplying noradrenergic chromaffin cells in the adrenal medulla. Epinephrine-secreting chromaffin cells are not innervated by barosensitive preganglionic neurons (39), but the fact that glucose and HR were increased support the idea that this pathway is also activated.

The results of the present study do not enable us to account for the lack of MAP response observed following intrathecal PACAP-38 injection (14). One possibility is that PACAP-38 activation of other output pathways determine the overall effect on MAP. For example, if there is a withdrawal of sympathetic activity to the capacitance system (veins), then the overall effect on blood pressure may be one of little or no change despite a marked increase in sympathetic activity to resistance arteries in the mesenteric or muscle beds.

Finally, PACAP is present in the preganglionic fibers that innervate the adrenal gland (2, 19, 23) and is a potent secretagogue of catecholamines from the adrenal medulla (24, 45). Future studies using adrenalectomy, plasma catecholamine measurement, and pharmacological investigations using specific receptor agonists/antagonists and inhibitors specific to different intracellular transduction mechanisms may help to clarify the paradoxical MAP response observed following intrathecal injection of PACAP-38. The results of the present study also indicate a role for PACAP in BAT thermogenesis, a suggestion supported by the finding that mice lacking PACAP-38 find it difficult to maintain normal core body temperature in a cold environment (16). Experiments that include the measurement of BAT temperature and BAT SNA in response to the central administration of PACAP-38 (27) and the effects of PACAP on cold- and chemically evoked BAT thermogenesis may help to further resolve the role of PACAP thermoregulation.

In summary, this study reports three major findings. First, the paradoxical MAP response seen after intrathecal PACAP injection (13) cannot be attributed to the differential control of sympathetic outflows at the level of the SC, as measured here. Second, the responses to intrathecal PACAP are spinally mediated and are not a result of activation of higher presympathetic areas such as the RVLM. Third, intrathecal PACAP causes an increase in metabolic rate. We conclude that intrathecal PACAP acts via a spinally mediated mechanism to cause a prolonged widespread sympathoexcitation and that PACAP causes an increase in metabolic rate that includes an increase in BAT thermogenesis in the urethane-anesthetized, vagotomized, paralyzed, and artificially ventilated male SD rat.

GRANTS
P. M. Pilowsky was supported by National Health and Medical Research Council of Australia Grants 457080, 457069, and 604002; Australian Research Council Grant DP110102110; Macquarie University; and the Garnett Passe and Rodney Williams Memorial Foundation. M. A. Inglott was supported by an Australian Postgraduate Award. M. M. J. Farnham was supported by a Macquarie Research Excellence Scholarship.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


