Catestatin has an unexpected effect on the intrathecal actions of PACAP dramatically reducing blood pressure

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Gaede AH, Inglott MA, Farnham MM, Pilowsky PM. Catestatin has an unexpected effect on the intrathecal actions of PACAP dramatically reducing blood pressure. *Am J Physiol Regul Integr Comp Physiol* 303: R719–R726, 2012. First published August 8, 2012; doi:10.1152/ajpregu.00202.2012.—This study focuses on presympathetic neurons of the rostral ventrolateral medulla (RVLM) that regulate sympathetic vasomotor tone. Many neurotransmitters are colocalized in RVLM neurons and are released under specific conditions to modulate efferent homeostatic responses. Of particular interest here are two peptides colocalized in catecholaminergic RVLM neurons: catestatin and pituitary adenylate cyclase-activating polypeptide (PACAP). Chromogranin A-derived catestatin is a potent endogenous noncompetitive nicotinic and adrenoreceptor antagonist. Catestatin impairs adenylate cyclase and phospholipase C action: mechanisms engaged by PACAP. Although PACAP and catestatin are likely coreleased, the possible effects of this are unknown. We aimed to determine whether catestatin affects the normal sympathoexcitatory but isostensive responses to intrathecal PACAP. Urethane-anesthetized, vagotomized, ventilated Sprague-Dawley rats (n = 22) were given an intrathecal injection of catestatin at different times prior to intrathecal administration of PACAP-38. Arterial pressure, splanchnic sympathetic nerve activity, heart rate, and reflex responses to baroreceptor and chemoreceptor activation were recorded. The key findings of this study are that pretreatment with catestatin time dependently enhances the PACAP-38 effect on mean arterial pressure and enhances sympathetic barosensitivity and chemosensitivity. The timescale of the effect of catestatin on the response to PACAP-38 strongly suggests that catestatin is either causing changes in gene expression to exert its effects, or modifying intracellular mechanisms normally engaged by PAC1 receptors. The ability of catestatin pretreatment to enhance barosensitivity and chemosensitivity after PACAP-38 injection supports the hypothesis that catestatin manipulates the intracellular environment within sympathetic neurons in a way that increases responses to PACAP.

chromogranin a; sympathetic; baroreflex; chemoreflex; epinephrine

HYPERTENSION is a major human health problem that is due, in large part, to increased sympathetic drive that emanates from the rostral ventrolateral medulla (RVLM) (42, 44, 45). Central neural mechanisms maintain arterial blood pressure by integrating information from reflexes and central behavioral and emotional states to alter efferent sympathetic and parasympathetic activity, according to need (18, 44, 45). The RVLM plays a critical role in this system as the final integrative pathway in the regulation of sympathetic tone and cardiovascular adaptive reflexes (e.g., the baroreceptor and chemoreceptor reflexes) (45). The RVLM contains a functionally heterogeneous cell population that includes barosensitive, presynaptic (C1) neurons, which project to sympathetic preganglionic neurons (SPN) in the intermediolateral cell column of the spinal cord. SPN regulate sympathetic outflow through innervation of the adrenal medulla and sympathetic postganglionic neurons that project to the heart, kidneys, and blood vessels. Increased excitability of RVLM neurons, SPN, and, by extension, sympathetic drive targeting organs and blood vessels in the periphery, is critically implicated in the pathophysiology of essential hypertension, a major underlying cause of morbidity and mortality.

Many neurotransmitters are colocalized within adrenergic (C1) and nonadrenergic RVLM bulbospinal neurons, suggesting that interactions occur when these neurotransmitters are released together. Such interactions will affect the excitability of SPN and the sympathetic outflow reaching the target organs in the periphery. However, the nature and importance of such interactions remain unclear. Glutamate is the major excitatory transmitter released by these neurons, but many metabotropic transmitter receptors are also present and modulate SPN activity, including enkephalin (31), neuropeptide Y (38, 46), and substance P (27). We hypothesize that dysfunction of, or interaction between, colocalized or temporally coreleased neurotransmitters arising from the RVLM, raphé, hypothalamus, or other sites, may contribute to the increased excitability of the RVLM and SPN in essential hypertension. Two vesicular peptides of interest in the current study are catestatin (human chromogranin A352-372), a vasoactive chromogranin A (CgA) cleavage product (32), and pituitary adenylate cyclase-activating polypeptide (PACAP) (39). Catestatin is contained within 88% of C1 RVLM neurons, and PACAP-38 is expressed in 85% of bulbospinal C1 RVLM neurons, indicating extensive colocalization of these neurotransmitters in this nucleus (9, 13). The separate effects of these peptides have been studied following injection into the intrathecal space and into the RVLM, (9, 12, 49, 50), but the physiological significance of this extensive colocalization is unclear.

Catestatin and PACAP are both vasodilator peptides in the periphery (11, 54). Intrathecal PACAP increases splanchnic sympathetic nerve activity (sSNA) and heart rate (HR) but does not affect mean arterial blood pressure (MAP) (9). Intrathecal catestatin on its own has no effect on MAP, sSNA, or HR; however, it is pressor and sympathoexcitatory in the RVLM and significantly enhances the sympathetic baroreflex, although it blunts the hypoxic chemoreflex and somatosympathetic reflexes (13). Catestatin is cardioprotective and blunts the sympathoexcitatory response to spinal stimulation in the pithed rat (23, 35, 43). In the spinal cord, catestatin stabilizes cardiovascular parameters by attenuating the responses to nicotine and isoproterenol (12). Catestatin may attenuate the ability of adenylate cyclase and phospholipase C to act intra-
cellularly. Because adenylate cyclase and phospholipase C are two systems engaged by PACAP through the PAC1 receptor, we hypothesized that coapplication of catestatin prior to PACAP would alter the ability of PACAP to influence sympathetic nerve activity, blood pressure, and heart rate.

Here, we investigate two different catestatin pretreatment regimes to examine the temporal effects of catestatin on the response to PACAP. The principal objective of this study was to determine the role of catestatin in central cardiovascular modulation of the intrathecal PACAP-38 response and to determine whether corelease of PACAP and catestatin within the circuitry controlling sympathetic vasomotor tone may be critical in mediating essential hypertension. Secondly, we aimed to determine the separate effects of intrathecal catestatin and PACAP-38 on basal cardiovascular parameters and reflex control of the cardiovascular system.

METHODS

All procedures and protocols were approved by the Macquarie University Animal Ethics Committee in accordance with the guidelines set forth by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Experiments were conducted on adult male Sprague-Dawley rats (400–550 g; Animal Resource Centre, Western Australia) in accordance with these guidelines.

Surgical Procedures

General surgical methods were carried out as previously described (12, 19). Briefly, anesthesia was induced in male Sprague-Dawley rats (n = 22) with a bolus of urethane (ethyl carbamate, 10%, 1.3 g/kg ip in 0.9% saline wt/vol; Sigma-Aldrich). Additional doses of urethane (30–40 mg iv in 10% solution) were given as needed to maintain a stable degree of anesthesia. Absence of the withdrawal reflex or lack of arterial pressure changes (>10 mmHg) in response to a hind paw pinch was used to assess the depth of anesthesia. The right jugular vein and common carotid artery were cannulated for administration of fluids and measurement of arterial blood pressure, respectively. The trachea was cannulated to allow for artificial ventilation (rodent ventilator; UGO Basile, Biological Research Apparatus). Rats were bilaterally vagotomized, paralyzed (pancuronium bromide; 0.8 mg kg−1 body wt; Astra Pharmaceuticals), and ventilated with 100% oxygen-supplemented room air. End-tidal CO2 was maintained between 4.0% and 5.0% by altering the stroke volume and frequency of ventilation as needed (Capstar-100 CO2 analyzer; CWE). Core temperature was maintained at 37 ± 0.5°C. The left splanchic sympathetic nerve was isolated, dissected, and prepared for recording on a bipolar platinum electrode immersed in paraffin oil. Nerve activity was amplified (1,000–100,000 × gain), filtered (30–3,000 Hz), and sampled at 5,000 Hz using an ADC system (model no. 1401; Cambridge Electronic Design) and Spike 2 analysis software (version 7.07).

An intrathecal catheter (polyvinylchloride tubing; ID, 0.2 mm; OD, 0.5 mm; Critchley Electrical Products), with a dead space of ~6 μl was inserted through an incision in the dura mater at the atlanto-occipital junction and passed caudally in the subarachnoid space to the level of T5/T6. Intrathecal injections (PACAP-38, 1 mM; Auspep, Australia; catestatin, 0.1 mM; Phoenix Pharmaceuticals) were given in volumes of 10 μl, followed by 6 μl of vehicle (10 mM PBS (pH 7.4) using a 25-μl Hamilton syringe.

Experimental Protocol

All intrathecal injections (10 μl of drug wash in with 6 μl of 10 mM 0.9% PBS) were made using a Hamilton syringe. All rats received control injections of the vehicle, PBS (10 μl of PBS washed in with 6 μl PBS), 30 min prior to treatment as described below. Cardiovascular reflexes, namely, the baroreflex and peripheral chemoreflex, were each evoked twice at specified times both pre- and post-treatment, and during each of the conditions described below. The sympathetic baroreflex was tested using intravenous injections of phenylephrine (PE; 10 μg/kg). Carotid chemoreceptors were activated by ventilating with N2 for 12 s (isocapnic anoxia).

Catestatin pretreatment (15 min) followed by PACAP. Intrathecal catestatin was administered (n = 8), and responses were recorded for 15 min. At 5 min postcatestatin, the baroreflexes and chemoreflexes were evoked. At the end of the pretreatment period, PACAP-38 was injected into the intrathecal space [referred to herein as catestatin (15)+PACAP]; responses were recorded for 90 min. Cardiovascular reflexes were elicited at 10 min and 60 min post-PACAP-38 injection. The 15-min pretreatment period was chosen, because previously, intrathecal catestatin demonstrated its maximal effect on the nicotine response at 15 min (12).

Catestatin pretreatment (90 min) followed by PACAP. In a separate group of rats (n = 4), intrathecal injections of catestatin were given, and the responses were recorded for 90 min. During this pretreatment period, cardiovascular reflexes were elicited at 0, 20, and 75 min postcatestatin injection. Subsequently, PACAP-38 was injected into the intrathecal space [referred to as catestatin(90)+PACAP]; responses were recorded for a further 90 min. Cardiovascular reflexes were tested at 10 and 60 min post-PACAP. The 90-min pretreatment period was used to determine whether catestatin would exert long-term effects.

Intrathecal PACAP-38 and cardiovascular reflexes. Rats (n = 5) were given intrathecal injections of PACAP-38, and responses were recorded for 90 min. During this period, the baroreflexes and chemoreflexes were tested at 10 and 60 min following PACAP-38 injection.

Data analysis. The recording system for nerve activity was calibrated by introducing a known voltage at the amplifier headstage, and rectified and smoothed for analysis (sSNA time constant = 1 s). Postmortem background activity was subtracted from the nerve activity. MAP, HR, and sSNA were analyzed from 1-min averages taken 10 and 5 min prior to, and 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min after intrathecal injections of PACAP-38 (up to 30 min), catestatin (up to 90 min), and PACAP-38 (up to 120 min). Sympathetic barosensitivity was determined by plotting the sSNA vs. MAP responses to intrathecal phenylephrine. A first-order polynomial was fitted to the steepest portion of these curves, and the slopes were used to compare barosensitivity. The sympathetic response to activation of the peripheral chemoreceptors was determined by fitting a Boltzmann sigmoid curve to the rectified, smoothed sSNA response, and the MAP response, to hypoxia. Slope and range of the fitted curve were then analyzed. Statistical analysis was carried out using Prism 5 (version 5.03; GraphPad Software). Statistical significance was determined using one- or two-way ANOVA with Bonferroni’s correction. P < 0.05 was considered significant.

RESULTS

In the Presence of Catestatin, PACAP-38 Is Hypotensive and Increases HR and sSNA

Catestatin(90)+PACAP-38 has a significant depressor effect on MAP compared with baseline (~30 ± 1 mmHg from a baseline of 115 ± 9 mmHg; P < 0.01; n = 4) and the untreated PACAP-38 response (P < 0.05). The depressor response reached a maximum ~10 min after the PACAP-38 injection and remained below baseline for the duration of the experimental period (60 min; Figs. 1B and 2A). The decrease observed following the vehicle-treated PACAP-38 injection was amplified by pretreatment with catestatin (Fig. 2A).
Catestatin(15)+PACAP-38 does not significantly affect MAP when compared with vehicle (-12 ± 7 mmHg from a baseline of 110 ± 7 mmHg; 60-min time course; n = 8; Figs. 1A and 2A) or when compared with the vehicle-treated intrathecal PACAP-38 response (Fig. 2A).

Catestatin(15)+PACAP-38 increased HR [31 ± 11 beats per minute (bpm) from a baseline of 437 ± 11 bpm; P < 0.01; n = 8; Figs. 1A and 2B], as did catestatin(90)+PACAP-38 (45 ± 14 bpm from a baseline of 428 ± 12 bpm; P < 0.01; n = 4; Figs. 1B and 2B). Both responses reached a maximum ~20 min post-PACAP-38 injection (Figs. 1, A and B, and 2B). The HR response to intrathecal PACAP-38 after 15- or 90-min catestatin pretreatment periods was not significantly different from the HR response to PACAP-38 after vehicle pretreatment (Fig. 2B).

Catestatin(15)+PACAP-38 augmented sSNA (73 ± 27% from a baseline of 3 ± 0.6 µV; P < 0.001; n = 7; Figs. 1A and 2C). Furthermore, catestatin(90)+PACAP-38 significantly increased sSNA (78 ± 32% from a baseline of 3 ± 0.4 µV; P < 0.01; n = 4; Figs. 1B and 2C). The sSNA responses in both treatment groups increased gradually before reaching a maximum at ~50 min post-PACAP-38 injection (Figs. 1, A and B, and 2C). The sSNA response to intrathecal PACAP-38 15 or 90 min after catestatin pretreatment was not significantly altered compared with the vehicle-treated intrathecal PACAP-38 response (Fig. 2C).

**Pretreatment with Catestatin Increases Barosensitivity and Chemosensitivity After Intrathecal Injection of PACAP**

PACAP-38 administered after pretreatment with catestatin significantly increases barosensitivity and is more effective than either PACAP-38 or catestatin individually (P < 0.001; Fig. 3). The slopes of the baroreflex curves generated after
catestatin(90)+PACAP-38 were significantly increased at both 10- (P < 0.01; n = 5) and 60-min (P < 0.01; n = 5) time points post-PACAP-38 injection (Fig. 3).

The response to hypoxia following PACAP-38 administration, 90 min after pretreatment with catestatin, is a significant increase in the range of the MAP response (P < 0.01; Fig. 4A) and the slope of the sSNA response (P < 0.01; Fig. 4C). Specifically, the range of the MAP response to hypoxia is increased after catestatin(90)+PACAP, at both 10- (P < 0.05; Fig. 4A) and 60-min (P < 0.05; Fig. 4A) post-PACAP-38 time points, when compared with the responses to hypoxia after catestatin(15)+PACAP-38. The slope of the sSNA response to hypoxic stimuli after catestatin(90)+PACAP-38 was significantly augmented at 60 min post-PACAP-38 (P < 0.05). The slope of the sSNA response to hypoxia 60 min after catestatin(90)+PACAP-38 was significantly greater than the response observed after catestatin(15)+PACAP-38 (P < 0.05; Fig. 4C). The sSNA range was not affected following catestatin(90)+PACAP-38 (Fig. 4B); however, the sSNA range was attenuated following catestatin(15)+PACAP-38 compared with PACAP-38 alone (P < 0.05; Fig. 4B).

**Intrathecal Catestatin Does not Significantly Affect MAP, HR, and sSNA**

Intrathecal catestatin did not significantly affect MAP, HR, or sSNA (MAP = Δ5 ± 2 mmHg; HR = Δ12 ± 8 bpm; sSNA = Δ9 ± 6%; n = 8; Figs. 1 and 2) for up to 90 min after administration compared with the vehicle response (MAP = Δ12 ± 3 mmHg; HR = Δ5 ± 10 bpm; sSNA = Δ22 ± 12%; n = 4; Figs. 1, and 2). There was no significant difference between the peak response to catestatin and the vehicle response at both time points for all measured parameters (Figs. 1 and 2).

**Intrathecal Catestatin Does not Significantly Affect Baroreflex or Chemoreflex Sensitivity**

The sympathetic baroreflex was measured at three time points (0 min; n = 13; 20, and 75 min; n = 5) postcatestatin and postvehicle. Compared with the postvehicle responses, the slope of the sympathetic baroreflex was not significantly affected by intrathecal catestatin (Fig. 3). The MAP and sSNA responses to hypoxic chemoreflex stimulation were not affected by intrathecal administration of catestatin (Fig. 4).

**Intrathecal PACAP-38 Increases HR and sSNA, but Does not Affect MAP**

Intrathecal injection of PACAP-38 caused a significant increase in HR (Δ59 ± 8 bpm; P < 0.0001; n = 6) and sSNA (Δ209 ± 90%; P < 0.05; n = 5), but no change in MAP (Δ−3 ± 9 mmHg; P > 0.05; n = 6) throughout the 90-min recording period compared with the vehicle response (Fig. 2).

**Intrathecal PACAP-38 Does not Affect Baroreflex and Chemoreflex Sensitivity**

In five animals, intrathecal PACAP-38 did not significantly change the slope of the sympathetic baroreflex compared with postvehicle baroreflex responses (Fig. 3). Data are presented as a percentage change from the postvehicle (PBS) response to baroreflex stimulation.
Intrathecal injection of PACAP-38 did not significantly alter the MAP range or sSNA slope responses to hypoxia compared with postvehicle responses to hypoxic chemoreflex stimulation (Fig. 4). However, the sSNA range is increased following PACAP-38 (P < 0.05; Fig. 4B). Data are presented as percentage change from the postvehicle (PBS) response to chemoreflex stimulation.

Effects of Catestatin vs. PACAP-38 on Basal MAP, sSNA, and HR

Catestatin and PACAP-38 caused significantly different cardiovascular changes in HR (P < 0.05) and sSNA (P < 0.01) during the 60-min recording period (Fig. 2).

DISCUSSION

The data demonstrate a potent synergistic effect of catestatin and PACAP-38 in the spinal cord. Both peptides are present in nerve terminals that surround SPN, and both peptides exert potent cardiovascular effects elsewhere in the brain but have no effect on blood pressure or reflexes on their own in the spinal cord (9, 12, 13). A key difference, however, is that catestatin delivered intrathecally does not affect MAP, HR, sSNA, or adaptive reflexes (12). PACAP on the other hand does not affect MAP but does cause increases in HR and sSNA (9).

The primary, novel findings of this study are first, that intrathecal catestatin, followed by intrathecal PACAP, significantly decreases MAP, but has no effect on the PACAP-38-induced sympathoexcitation. Second, pretreatment with catestatin (90 min), before intrathecal administration of PACAP-38, enhances baroreflex and chemoreflex sensitivity. Third, intrathecal catestatin alone does not significantly affect reflex control of blood pressure. Fourth, intrathecal administration of PACAP-38 alone minimally effects reflex control of the cardiovascular system.

Catestatin Amplifies Responses to PACAP-38

Intrathecal microinjection of PACAP-38 90 min after catestatin injection [catestatin(90)+PACAP] significantly decreased MAP without affecting the increase in sSNA. Catestatin pretreatment 15 min prior to PACAP-38 injection [catestatin(15)+PACAP] did not affect the MAP response. The finding that the enhanced depressor effect occurred after a 90-min pretreatment with catestatin strongly suggests a role for gene expression. Alternatively, or additionally, catestatin may alter the activity of intracellular mechanisms that are coupled to Gαs (1, 29), including adenylate cyclase and phospholipase C (10).

PACAP-38 activates three G protein-coupled receptors: PAC1, VPAC1, and VPAC2. In human adrenal chromaffin cells, blockade of VPAC receptors does not block PACAP-mediated catecholamine release, suggesting that PAC1 receptors are crucial for PACAP-induced catecholamine secretion (36). The results reported here may be due to direct actions by catestatin that alter PAC1 receptor function, or interactions between catestatin and other G protein-coupled receptors, either directly, or by translocating across the cell membrane (55), so that the activity of VPAC1 and VPAC2 receptors is enhanced (54). In effect, there may be a shift in the balance of PAC1, compared with VPAC1 and VPAC2, function. Furthermore, the chemical phenotype of SPN that project to epinephrine-releasing chromaffin cells is different to that of SPN, regulating norepinephrine-releasing chromaffin cells (24). Therefore, the results observed here may be due to partial blockade of PAC1 receptors by catestatin, with a change in the balance toward excitation of epinephrine-releasing pathways that cause an increase in heart rate, a large decrease in peripheral resistance, and a subsequent fall in blood pressure (14, 16). However, further study, such as catecholamine measurement, is required to support this hypothesis.

Catestatin may increase the excitability of SPN or other spinal interneurons, resulting in augmented activation of adrenal chromaffin cells and other sympathetic targets. PACAP-38-induced activation of SPN projecting to the adrenal gland may increase the release of catestatin, catecholamines, PACAP-38, and other costored substances. In particular, the hypotensive response to catestatin(90)+PACAP-38 may be a result of increased epinephrine release that, in turn, enhances peripheral vasodilation (3, 23, 40).

Intravenous catestatin (to reach an extracellular concentration of 6 mmol/l; 0.3 μmol·l⁻¹·rat⁻¹) caused histamine release-mediated hypotension following sympathoexcitation by electrical stimulation. Catestatin significantly increased plasma epinephrine by 11-fold, while having no effect on plasma norepinephrine (23); this contradicts in vitro studies that demonstrated the ability of catestatin to inhibit catecholamine release. Therefore, the endocrine effect of intravenous catestatin in the rat appears to be that of indirect vasodilation. In general,
Catestatin acts to stabilize baseline conditions in several in vivo and in vitro models (2, 5, 15, 30, 34, 48). As such, the role of catestatin may not be apparent until an appropriate stimulus is applied. Here, catestatin appears to prime the system so that PACAP-38 may induce hypotension and increase barosensitivity and chemosensitivity. As noted above, we speculate that this occurs as a result of activation of SPN that project to epinephrine-secreting chromaffin cells.

Baroreceptor-induced sympathoinhibition is mediated by inhibition of tonically active RVLM neurons (17, 44, 45). The present study demonstrates that pretreatment with catestatin primes this pathway so that PACAP-38 significantly increases sympathetic baroreflex sensitivity. Catestatin(90)+PACAP-38 may result in either disfacilitation or direct inhibition at the spinal level to augment baroreflex sensitivity.

Hypoxia causes a rapid and reversible excitation of bulbospinal sympathoexcitatory RVLM neurons that monosynaptically project to SPN. Sympathetic excitation elicited by hypoxia requires glutamatergic transmission (52). Intrathecal pretreatment of catestatin before administration of PACAP-38 increases the range of the pressor response and markedly augments the slope and range of the sSNA response to the chemoreflex.

Catestatin Alone Does not Alter the Tonic or Reflex Control of the Cardiovascular System

Intrathecal injection of catestatin did not affect basal MAP, sSNA, and HR (12) (Figs. 1 and 2), or the sympathetic baroreflex, or the hypoxic chemoreflex (Figs. 3 and 4).

The mechanism by which catestatin acts in the spinal cord is unknown. Catestatin exerts divergent actions depending on the tissue being investigated (23, 30, 34). In the periphery, catestatin causes vasodilation (11) and has antimicrobial actions in the human epidermis, suggesting a role for catestatin in the cutaneous defense system (47). In the brain stem, it is sympathoexcitatory and enhances sympathetic barosensitivity (13).

The first characterized reported action of catestatin was as a nAChR antagonist that blocks nicotine-induced catecholamine secretion from PC12 cells (31), bovine adrenal chromaffin cells (32), and rat hippocampal neurons (4). Recent studies reported that catestatin affects many intracellular signaling pathways (7, 34, 55). For example, catestatin inhibits the actions of nicotine and isoproterenol in the intrathecal space, most likely through interactions with nAChR and β-adrenoceptors (12). The finding that intrathecal catestatin does not exert any effects on its own may be because constitutively active nAChR are not present on SPN, the effects of catestatin are not visible under basal conditions, or that catestatin causes many opposing effects that are balanced under normotensive conditions, resulting in no net change.

Intrathecal administration of catestatin does not affect the baroreflex or the cardiovascular responses to hypoxic chemoreflex stimulation at any time point. This indicates that intrathecal catestatin on its own is not involved in the reflex control of the cardiovascular system. Previously, we reported that microinjection of catestatin into the RVLM increased barosensitivity and attenuated chemosensitivity (13). Differences in injection method, site, and dose may explain this discrepancy.

Many of the known actions exerted by catestatin in other systems are modulatory. For example, catestatin attenuates the pressor response to electrical stimulation in the pithed rat (23), and levels of circulating catestatin are diminished in hypertensive and prehypertensive individuals (6, 41). Furthermore, CgA knockout mice (Chga<sup>?/–</sup>) have elevated blood pressure levels compared with their wild-type counterparts. Exogenous catestatin reduces the blood pressure of Chga<sup>?/–</sup> mice by 30 mmHg, suggesting that the elevation in blood pressure is due to the absence of the CgA cleavage product catestatin (28). These modulatory properties likely explain why catestatin alone does not elicit any visible changes in the spinal cord and forms the basis for our investigation into the effect of catestatin in enhancing the response to PACAP-38.

PACAP-38 Alone Alters Tonic, but not Reflex, Control of the Cardiovascular System

PACAP-38 is colocalized with catestatin in bulbospinal C1-tyrosine-hydroxylase immunoreactive neurons in the RVLM, a region of the brain stem critical in the central control of blood pressure (9, 13). Presympathetic RVLM neurons regulate sympathetic vasomotor tone and blood pressure (44, 45). The excitatory effects of PACAP-38 are well characterized in the cardiovascular system (10, 54). PACAP-38 excites SPN in spinal cord slices from juvenile rats (25) and is localized in at least 82% of C1 presynaptic bulbospinal RVLM neurons (9). Functional evidence supports these findings; intrathecal PACAP-38 causes a prolonged tachycardia and sympathoexcitation in multiple sympathetic beds through activation of SPN, but no change in MAP (9, 19). Previous studies report conflicting findings regarding the MAP response to PACAP-38; Lai et al. (25) found a significant increase in MAP (25), while others saw no change in MAP (9). This study supports the earlier findings that PACAP-38 does not affect MAP in vivo (9, 19). Despite the well-described effects of intrathecal PACAP-38 on the cardiovascular system, the role of PACAP-38 in the reflex control of the cardiovascular system is relatively unstudied.

Intrathecal PACAP-38 does not affect the slope of the baroreflex or the hypoxic chemoreflex. The results demonstrate that while spinal PACAP-38 appears to have little effect on adaptive reflexes under basal conditions. However, following pretreatment with catestatin, effects are apparent. To date, this is the only study to examine the role that PACAP-38 plays in the chemoreflex. The finding that the sympathetic baroreflex is unaffected is in contrast to a previous study examining HR barosensitivity in the anesthetized rainbow trout (26). Apart from the obvious differences in dose, species, and injection site, the baroreflex sensitivity was calculated using HR rather than sSNA. PACAP-38 did not change HR in the study by Lancien et al. (26), likely explaining the observed depression in barosensitivity. Here, we used direct recordings from barosensitive, pulse-modulated, sympathetic nerve fibers to obtain direct, real-time measures of nerve activity during stimulation of the baroreflex, enabling continuous measurement of changes in sympathetic baroreflex sensitivity.

In conclusion, we report for the first time that catestatin, a peptide found throughout the nervous system, including efferent cardiovascular pathways, acts in the spinal cord as a potent negative modulator of the sympathoexcitatory effects of the neuropeptide PACAP-38. The finding that PACAP-38 augments adaptive reflexes and causes hypotension in the presence
of catestatin, suggests that catestatin may interact with adenylate cyclase or phospholipase C, two intracellular mechanisms through which both peptides are thought to act. Furthermore, this suggests that catestatin may be a useful target for the future development of therapeutic agents.

**Perspectives and Significance**

There is increasing evidence that elevated sympathetic tone contributes to the development of most forms of hypertension (8, 18, 33, 44). This may be due to increased tonic RVLM activity (20–22, 37, 42). Therefore, it is reasonable to propose that the increase in vasomotor tone seen in some cases of hypertension (42) may be caused by altered neurochemistry and activity of RVLM neurons and a resultant increase in firing of presympathetic bulbospinal RVLM neurons, SPN, and, therefore, sympathetic outflow to effector sites, such as the blood vessels (53). Given the role that catestatin has in inhibiting catecholamine release and its effects on the actions of PACAP, the use of catestatin in the treatment of neurogenic hypertension may prove effective in reducing the significant burden of disease that essential hypertension places on society.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

Author contributions: A.H.G. and M.A.I. conceived and designed research; A.H.G. and M.A.I. performed experiments; A.H.G., M.A.I., and P.M.P. interpreted results of experiments; A.H.G., M.A.I., and P.M.P. prepared figures; A.H.G., M.A.I., and P.M.P. drafted manuscript; A.H.G., M.A.I., M.M.-J.F., and P.M.P. edited and revised manuscript; A.H.G., M.A.I., M.M.-J.F., and P.M.P. approved final version of manuscript.

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