Intrathecal bombesin is sympathoexcitatory and pressor in rat

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Zogovic B, Pilowsky PM. Intrathecal bombesin is sympathoexcitatory and pressor in rat. Am J Physiol Regul Integr Comp Physiol 301: R1486–R1494, 2011. First published August 17, 2011; doi:10.1152/ajpregu.00297.2011.—Bombesin, a 14-amino-acid peptide, is pressor when administered intrathecally in rat and pressor and sympathoexcitatory when applied intracerebroventricularly. To determine the spinal effects of bombesin, the peptide was administered acutely in the intrathecal space at around thoracic spinal cord level six of urethane-anesthetized, paralyzed, and bilaterally vagotomized rats. Blood pressure, heart rate, splanchnic sympathetic nerve activity (sSNA), phrenic nerve activity, and end-tidal CO2 were monitored to evaluate changes in the cardiorespiratory systems. Bombesin elicited a long-lasting excitation of sSNA associated with an increase in blood pressure and tachycardia. There was a mean increase in arterial blood pressure of 52 ± 5 mmHg (300 μM; P < 0.01). Heart rate and sSNA also increased by 40 ± 4 beats/min (P < 0.01) and 162 ± 33% (P < 0.01), respectively. Phrenic nerve amplitude (PNamp, 73 ± 8%, P < 0.01) and phrenic expiratory period (+0.16 ± 0.02 s, P < 0.05) increased following 300 μM bombesin. The gain of the sympathetic baroreflex increased from −2.8 ± 0.7 to −5.4 ± 0.9% (P < 0.01), whereas the sSNA range was increased by 99 ± 26% (P < 0.01). During hyperoxic hypercapnia (10% CO2 in O2, 90 s), bombesin potentiated the responses in heart rate (−25 ± 5 beats/min, P < 0.01) and sSNA (+136 ± 29%, P < 0.001) but reduced PNamp (from 58 ± 6 to 39 ± 7%, P < 0.05). Finally, ICI-216,140 (1 mM), an in vivo antagonist for the bombesin receptor 2, attenuated the effects of 300 μM bombesin on blood pressure (21 ± 7 mmHg, P < 0.01). We conclude that bombesin is sympathoexcitatory at thoracic spinal segments. The effect on phrenic nerve activity may be the result of spinobulbar pathways and activation of local motoneuronal pools.

blood pressure; heart rate; splanchnic sympathetic nerve activity; phrenic nerve activity; baroreflex

SYMPATHETIC PREGANGLIONIC neurons (SPN) located in the intermediolateral cell columns of the spinal cord are the final central output neurons in the autonomic control of sympathetic activity. Cell bodies within the pons and medulla oblongata play a vital role in the control of the cardiovascular system via regulating the activity of SPN (34, 40). The rostral ventrolateral medulla (RVLM) is the key brain stem structure that regulates tonic and reflex pathways that in turn control sympathetic activity (53, 58). Other key regulatory cardiovascular nuclei are found in the hypothalamus. In particular, the paraventricular nucleus (PVN) contains neurons that synthesize many peptides and project to the RVLM and the spinal cord where they are able to affect sympathetic outflow (56, 57, 72). Bombesin is a 14-amino-acid peptide originally isolated from the skin of the European discoglossid frog Bombina bombina (14). Bombesin is involved in many physiological actions, including gastric acid secretion, emotion, temperature, learning, and memory, actions that are presumably related to the widespread distribution of bombesin receptors in both the central nervous system and periphery (5, 23, 28, 43, 73). In mammals, bombesin acts at three receptors: bombesin receptor 1 (BB1), bombesin receptor 2 (BB2), and bombesin receptor 3 (BB3). Neuromedin B (NMB) preferentially activates the BB1 receptor, whereas gastrin-releasing peptide (GRP) activates the BB2 receptor (28). Currently, there is no known endogenous agonist for the BB3 receptor (28). Bombesin, and its three receptors (28, 46), are all present in the spinal cord of rat and other species, in particular, within the dorsal horn (71) and the autonomic nuclei (10). The effect of activating and inhibiting bombesin receptors in the thoracic spinal cord, on sympathetic nerve activity, phrenic nerve discharge, and adaptive reflexes remains unknown.

There is a growing body of evidence that different peptides, such as somatostatin, pituitary adenylate cyclase-activating polypeptide (PACAP), galanin, and orexin A, act as neurotransmitters/neuromodulators in the cardiorespiratory system (1, 7, 18, 63, 65). These peptides differentially affect sympathetic tone and adaptive reflexes. Previously, it was shown that bombesin has vasopressor actions and stimulates breathing when applied centrally or peripherally (15, 22, 25, 26, 30, 31). Here we aimed to determine whether or not intrathecal bombesin exerts cardiorespiratory effects. Second, we sought to determine which bombesin receptors are responsible for any observed actions. Finally, we investigated whether spinal bombesin receptors affect baroreceptor or chemoreceptor responses to hypercapnia.

MATERIALS AND METHODS

Experiments were performed on 24 anesthetized, paralyzed, vagotomized adult, male Sprague-Dawley rats (350–550 g; Animal Resources Centre, Perth, Australia). All animal handling and surgical preparations were approved by the Macquarie University Animal Ethics Committee and performed following the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (http://www.nhmrc.gov.au/files_nhmrc/publications/attachments/ea16.pdf).

General and surgical preparation. All drugs were purchased from Sigma-Aldrich (St. Louis, MO) unless stated otherwise. Animal preparation is as reported in detail elsewhere (1, 39). Briefly, rats were anesthetized (urethane, 1.3 g/kg, 10% in saline ip). Atropine (500 μg/1 ml; Astra) was given at induction of anesthesia to minimize airway secretion. Adequate depth of anesthesia (absent corneal or withdrawal reflexes, no change in blood pressure in response to noxious pinch) was maintained for the duration of the experiment. Supplemental urethane (30 mg in 10% saline iv) was given as required. Rats were placed on a homeothermic blanket with feedback regulation from a rectal thermometer. An external jugular vein and common carotid artery were cannulated, and the trachea was intubated to permit intravenous access, measurement of arterial blood pressure, and artificial ventilation. Ringer solution (1.5 ml/h) was administered intravenously to maintain fluid balance. The phrenic and greater splanchnic nerves were dissected from a dorsolateral approach and

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maintained under paraffin oil. Recordings of nerve activity were obtained with bipolar electrodes and an AC amplifier (CWE). Rats were positioned on a stereotaxic frame. The atlanto-occipital and atlanto-axial joints were exposed and separated from the occipital bone, the dura was incised, and an intrathecal catheter (PVC tubing; OD, 0.5 mm, ID, 0.2 mm; Critchley Electrical Products) was inserted in the subarachnoid space and positioned with the tip at around the sixth thoracic spinal cord segment (T6). Backflow of cerebrospinal fluid confirmed accurate catheter placement in the intrathecal space. ECG leads were attached to both fore limbs and the left hind limb for ECG monitoring. The signal was amplified (500–2,000 Hz) and filtered (100–3,000 Hz; CED Micro1401Mark II). Before neuromuscular blockade [pancuronium bromide iv (Astra); 0.4 mg initially, then 0.3 mg/h], the tracheal tube was connected to a rodent ventilator (Ugo, Basile, Italy), and mechanical ventilation with 100% O2 was initiated. Respiration volume was initially set up between 3.5 and 4 l/min at a frequency of 60–75 cycles/min (Capstar-100; CWE). At the completion of surgery, and after mechanical ventilation was commenced, volume was initially set up between 3.5 and 4 l/min at a frequency of 60–70 min. ICI-216,104 was then readministered followed by bombesin (300 μM) was injected, and data were recorded for a further 60–70 min. Finally, bombesin was injected, and data were recorded for 60–120 min. Chemoreflex responses to 10% CO2-90% O2 were examined at 20 min and then 30 min after administration of vehicle or bombesin as described above, the baroreflex was examined.

In the fourth group of animals (n = 6), the procedure was exactly the same as the third group, except that 300 μM bombesin instead of 30 μM bombesin was administered.

Cardiovascular timing. For cardiorespiratory modulation, splanchic sympathetic nerve activity (sSNA) was integrated at 0.05 s and plotted as ensemble averages triggered from the start of phrenic nerve discharge. Parameters measured were inspiratory sSNA peak and postinspiratory sSNA peak. The average activity over 5 min was acquired every 10 min, and the data were grouped for subsequent statistical analysis.

Adaptive reflexes. In the case of hypercapnic challenges, changes in mean arterial pressure (MAP), heart rate (HR), phrenic nerve amplitude (PNA), and sSNA before and after bombesin were analyzed. For baroreflex analysis, baroreflex sigmoid curves were generated. The baroreflex analysis was performed as previously described (9, 37). Briefly, using Prism (version 5.03) software (GraphPad), the baroreflex sigmoid curve was fitted using the equation \( Y = \frac{Y_{\text{upper}} - Y_{\text{lower}}}{1 + 10^{(X-X_{\text{midpoint}})} \times \text{Hill Slope}} \). “Lower” is the value of sSNA on the ordinate at the lower plateau, “upper” is the sSNA value on the ordinate at the upper plateau, and “Midpoint” is the value on the curve halfway between “lower and upper.” sSNA range was calculated as the difference between upper and lower. Maximum gain is the steepest part of the curve, quantified by the Hill slope, which is the gain coefficient. The operating range for blood pressure was calculated as the difference between the threshold and saturation levels.

Data analysis. MAP was derived from the pulsatile arterial pressure (\( \tau = 10 \) s). ΔMAP values were calculated as the difference between MAP values taken during drug treatment (values acquired every 5 min in a period of 40 min) and compared with a control period 5 min before drug (vehicle, bombesin, or ICI-216,140) delivery. HR was derived from the ECG. Recordings of sSNA were rectified and integrated (\( \tau = 1 \) s). For the purposes of statistical comparison, integrated sSNA was measured as a percentage change in activity from baseline. Baseline activity at the start of sSNA recording was designated as 100%. The baseline level of activity 5 min after death was designated as 0% activity. Phrenic nerve activity (PNA) was rectified and smoothed at \( \tau = 0.05 \) s. As with sSNA, integrated PNA values at the start of recording were used for calibration; a phrenic burst was calibrated as the area under the curve and then normalized as a percentage; mean values of phrenic nondischarge periods were calibrated as zero. Phrenic nerve frequency (PNF) was derived from PNA as the number of phrenic bursts. PNA was integrated over the period during which the point was designated as 100%. Thus, the peak value of each normalized PNA burst. etCO2 was made from the data waveform as peak values in each cycle. ΔSNA, ΔHR, ΔPNamp, and ΔetCO2 were calculated in the same manner as ΔMAP. All data were analyzed using a one-way ANOVA followed by Student’s t-test with Bonferroni’s correction. Grouped data are presented as means ± SE. Where P < 0.05, the difference between means, the comparison was considered to be significant.

RESULTS

Intrathecal bombesin increases MAP, sSNA, and HR. Intrathecal bombesin caused gradual increases in sSNA and MAP within 5 min (Fig. 1). Increases in MAP were noted following administration of two bombesin doses, 30 and 300 μM; mean responses were 35 ± 4 and 52 ± 5 mmHg in the case of 30 and 300 μM, respectively (Fig. 2). Thirty-five minutes after bombesin treatment, MAP values started to fall, returning to control levels by 1 h (Fig. 1). The overall bombesin effect on MAP was statistically significant (P < 0.01, 30 and 300 μM; Fig. 2). sSNA rose dose-dependently, reaching a maximum at ~35 min following bombesin delivery, measuring 123 ± 25% (P < 0.05) and 162 ± 33% (P < 0.01) for the 30 and 300 μM, respectively (Figs. 1 and 2). sSNA did not return to control levels by 1 h (Fig. 1). The overall bombesin effect on sSNA was statistically significant (P < 0.01, 30 and 300 μM; Fig. 2).
values during the 2-h observation period (Fig. 1). Finally, bombesin (30 μM) elicited a tachycardia (Figs. 1 and 2) where the average increase reached 32 ± 3 (P < 0.05) and 40 ± 4 (P < 0.01) beats/min, following 30 and 300 μM bombesin, respectively.

Intrathecal bombesin increases PNamp and etCO2. Bombesin dose-dependently increased the amplitude of PNA. The observed increase was greater within the first 20 min, after which the amplitude continued to rise, but with a lesser amplitude (Fig. 1). The responses in PNamp reached a mean of 40 ± 3% following 30 μM bombesin (P < 0.05) and 73 ± 8% following 300 μM bombesin (P < 0.01; Fig. 3). PNamp remained elevated for up to 1 h after bombesin injection. Changes in PNamp were accompanied by a neural “bradypnoea.” PNf was significantly and dose dependently decreased following 30 μM bombesin, reaching a mean of 14 ± 2 bursts/min (P < 0.01), as well as following 300 μM, where the average drop measured was 14 ± 3 bursts/min (P < 0.01; Fig. 3). Further analysis of the duration of the phrenic nerve bursts (phrenic inspiratory period) as well as the silent period between bursts [phrenic expiratory period (Te)] showed a marked increase in Te of +0.14 ± 0.03 and +0.17 ± 0.02 s (30 and 300 μM, respectively; P < 0.05 in both cases) following intrathecal bombesin (Fig. 4). Finally, only 300 μM bombesin was associated with a significant increase in etCO2 (1.4 ± 0.1%; P < 0.01; Figs. 1 and 3).

Effects of ICI-216,140, a BB2 antagonist. ICI-216,140 is a selective antagonist at the BB2 receptor (8, 60). The effects of ICI-216,140 (1 mM) were tested against two bombesin doses. In the presence of ICI-216,140, some of the typical bombesin responses were no longer seen. The response in MAP observed following 30 μM bombesin was blocked when ICI-216,140 preceded bombesin injection and reached a mean of 11 ± 5 mmHg (Fig. 2). The overall effect of ICI-216,140 (1 mM) was a significant attenuation of the MAP response to bombesin (P < 0.01). Similarly, the effect of ICI-216,140 on 300

![Fig. 1](http://ajpregu.physiology.org/)

![Fig. 2](http://ajpregu.physiology.org/)
μM bombesin was attenuated and measured 21 ± 7 mmHg (Fig. 2; P < 0.01).

ICI-216,140 administered alone did not affect MAP (P = 0.3; Fig. 2). sSNA responses to 30 μM bombesin (average of 62 ± 10%) and 300 μM (average of 93 ± 20%) were attenuated (Fig. 2; P < 0.05 in both cases). The changes in HR induced by bombesin were not affected by ICI-216,140 (P = 0.7 and P = 0.1 in the case of 30 and 300 μM bombesin; Fig. 2). A tachycardia was observed following ICI-216,140 (33 ± 8 beats/min, P < 0.01; Fig. 2). Changes in PNamp and etCO2 to bombesin were not modified by ICI-216,140: PNamp, P = 0.8 (30 μM, PNamp), P = 0.8 (300 μM, PNamp), and P = 0.5 (300 μM, etCO2) (Fig. 3). In contrast, ICI-216,140 alone caused an increase in PNamp (responses peak at 38 ± 4%, P < 0.05). Finally, the reduction in PNf following 30 and 300 μM bombesin was abolished and reversed by ICI-216,140: 5 ± 1 (P < 0.05) and 7 ± 1 bursts/min (P < 0.01), respectively (Fig. 3).

Bombesin in cardiorespiratory synchronization. Inspiratory-triggered averaging of sSNA demonstrated an increase in the inspiratory peak for +100 ± 17 and +148 ± 21% (1 peak, 30 and 300 μM, respectively), as well as the postinspiratory peak for +105 ± 19 and +168 ± 21% (post-I peak, 30 and 300 μM, respectively) compared with control values (Fig. 5, P < 0.01 in all cases).

**Intrathecal bombesin potentiates the changes in HR, sSNA, and PNamp during hypercapnia.** Bombesin (300 μM) enhanced the bradycardic response to hypercapnia (10% CO2 in O2 for 90 s) (−25 ± 5). The maximum effect caused by bombesin was much stronger than the one caused by the vehicle, −23 ± 2 vs. −14 ± 3 beats/min (P < 0.01; Fig. 6). Bombesin increased the amplitude of sSNA following hypercapnia (254 ± 51 vs. 118 ± 22% baseline, P < 0.001; Fig. 6) (P < 0.05 and P < 0.001, respectively; Fig. 6). Last, the PNamp response was reduced following bombesin (39 ± 7%) compared with vehicle (58 ± 6%, P < 0.05; Fig. 6). In contrast, the changes in MAP were not altered markedly by hypercapnia (P = 0.1).

**Intrathecal bombesin improves barosensitivity.** Bombesin (30 and 300 μM) shifted the baroreflex curve upward by increasing both the lower and upper plateau, for +75 ± 26% (30 μM bombesin; P < 0.05) and +72 ± 21% (300 μM bombesin; P < 0.05; Fig. 7) and +174 ± 43% (30 μM bombesin; P < 0.01) and +187 ± 52% (30 μM bombesin; P <

\[\text{Fig. 3. Grouped data summarizing the average effects of it applied vehicle, bombesin (30 and 300 μM), and ICI-216,140 (1 mM) on PNamp (PNampl) and PNf and etCO2.} \]
0.01; Fig. 7) accordingly. Thus the range of the sympathetic baroreflex was also increased for +99 ± 26% (30 μM bombesin) and +114 ± 34% (300 μM bombesin; P < 0.01; Fig. 7). Bombesin increased the gain of the baroreflex for −2.6 ± 0.4 (30 μM bombesin; P < 0.01) and −3.1 ± 0.6% baseline/mmHg (300 μM bombesin; P < 0.01; Fig. 7). Blood pressure range was not affected by bombesin.

DISCUSSION

The key findings of the present study are: first, that intrathecal application of bombesin at around T6 causes a significant increase in MAP, HR, and sSNA. Second, bombesin enhances the inspiratory and postinspiratory peaks of sSNA. Third, bombesin facilitates the gain and range of the sympathetic baroreceptor reflex. Fourth, the tachycardic and sympathoexcitatory responses to hyperoxic hypercapnia were enhanced. Fifth, the BB2 antagonist ICI-216,140 abolished some, but not all, of the effects of bombesin, suggesting involvement of the BB2 receptor in the cardiovascular actions of bombesin.

Cardiovascular neurons in the ventrolateral medulla are present as paired longitudinal columns of cells immediately ventral to the ventral respiratory column. Bulbospinal RVLM neurons are found in a column 600 μm in length and 200 μm in diameter from the caudal pole of the facial nucleus to the level of the inferior olive (48–52, 54, 64, 67). Neurons in the RVLM integrate inputs from the periphery, and the center, to generate a tonic output to SPN (53, 55). Activation of peripheral baroreceptors stimulates a reflex involving the NTS, RVLM, and caudal ventrolateral medulla (CVLM) that employs both ionotropic amino-acidergic and neuropeptidegic transmission. Recently, an important modulatory role in central cardiorespiratory regulation for many different neuropeptides has been reported, including: substance P (36), somatostatin (7), PACAP (18), galanin (1, 66), and orexin A (63). These different neuropeptides all act via multiple G protein-coupled receptors (GPCR). In mammals, bombesin activates three GPCRs: the BB1, BB2 (28), and, with a very low affinity, BB3 (33) receptors. NMB and GRP preferen-
BOMBESIN IN RAT THORACIC SPINAL CORD

Fig. 7. A: sigmoid curve showing the parameters of the sympathetic baroreflex that were assessed. B–D: group data show the effects of it vehicle and bombesin (30 and 300 μM) on blood pressure (BP) operating range (B), sympathetic range (C), and the gain of the baroreflex (D). **P < 0.01.

tially bind to BB1, and BB2, respectively (28). BB1 and BB2 receptors activate protein kinase C via G1α (29), tyrosine kinases (59, 68), phospholipase A2 (44), and phospholipase D (3, 12). BB2, but not BB1, is coupled to adenylyl cyclase (21, 28).

BB1, BB2, and BB3 are expressed in the central nervous system and the periphery in both human and rat (28). Bombesin-like peptides (bombesin, NMB, and GRP) and bombesin receptors (BB1, BB2, and BB3) are reported to be present in the spinal cord in studies using both electrophysiological and molecular approaches. NMB is present in both porcine and rat spinal cord (41, 42). The BB2 receptor is abundantly expressed by NOS-positive preganglionic neurons in the autonomic nuclei of the lumbar spinal cord of Long-Evans rats as determined by GRP activation of the BB2 receptor, RT-PCR, and immunohistochemistry (46). BB2 was predominantly expressed in the dorsal horn of the spinal cord in mice (38). Intrathecal bombesin administration in conscious Sprague-Dawley rats caused a dose-dependent prolongation in the tail flick reflex response to noxious stimuli (heat) while NMB had a biphasic effect (32). Extensive GRP-like immunoreactivity was found in the marginal layer and substantia gelatinosa (layers I and II) of the spinal cord in Sprague-Dawley rats (71). The dorsal horns of cats express high concentrations of bombesin-like and bombesin-like receptor-like immunoreactivity throughout all segments of the spinal cord, including autonomic nuclei (10). In rabbit (16), rat, cat, and mouse, bombesin-like immunoreactivity is found in the dorsal horns of cervical spinal cord segments (45). In situ hybridization studies of rat spinal cord demonstrated that GRP and NMB are present in the substantia gelatinosa (70). An in vitro study that used cervical and thoracic spinal cord sections of Sprague-Dawley rats reported release of bombesin-like peptides following depolarizing stimuli (13).

In rat spinal cord, BB3 mRNA is undetectable using RT-PCR (27). BB3 receptor-like immunoreactivity was found in the lumbar ventral and dorsal horns (27). Taken together, the findings support the idea that bombesin can activate somatosensory pathways (dorsal horn) that may lead to changes in phrenic nerve discharge. The finding that bombesin is present in autonomic nuclei in addition to the dorsal horn suggests that the sympathoexcitatory responses may be due to a combination of direct effects of bombesin on preganglionic neurons and activation of dorsal horn neurons that are part of a somatosensory pathway.

In the periphery, intravenous bombesin causes a pressor and tachycardic response (15). Centrally, moderate, to high bombesin immunoreactivity is found in neurons and fibers in the nucleus tractus solitarius (NTS), RVLM, CVLM, PVN, and spinal cord (11, 35). NTS microinjection or intracerebroventricular bombesin potentiates respiration (22, 26) and increases plasma catecholamines (6). Intracerebroventricular bombesin causes a reduction in sympathetic outflow to brown fat that is associated with hypothermia (4). Intracerebroventricular bombesin also decreases HR, elevates blood pressure (6), and increases FOS expression in adrenal chromaffin cells. The increase in Fos expression in adrenal chromaffin cells is reported to involve a thromboxane A2 mechanism (69).

The blood pressure increase observed in our experiments was abolished following BB2 receptor blockade. This finding is consistent with earlier studies in which the combined BB1–BB2 antagonist [d-Phe]{12}-bombesin attenuated the cardiorespiratory effects of intravenous bombesin in spontaneously breathing male adult Wistar rats (30), an effect not seen with the BB1 antagonist BIM-23127. In the present study, we noted a sustained tachycardia that was not affected by the BB2 antagonist ICI-216,140. Moreover, ICI-216,140 alone caused tachycardia, suggesting that it may act as a partial agonist. The tachycardia observed contrasts with a previously reported bradycardia that may be explained by the different species used in the experiments (rat vs. dog) or different experimental paradigms (it vs. iv route, vagotomy vs. intact vagi) (20).

Intrathecal bombesin potently, and dose-dependently, increased sSNA via BB2 activation. The BB2 receptor is expressed in the dorsal horn and autonomic nuclei. Therefore, activation of intraspinal autonomic and ascending sensory pathways may play a role in the changes observed in sSNA. A study in urethane-anesthetised, nonvagotomized adult male Wistar rats also describes dose-dependent sSNA increase following intracerebroventricular bombesin (47). Key differences between this study and others that evaluated the effects of bombesin on breathing (22, 25, 26) are the route of administration and the use of direct recording of the phrenic nerve to determine central inspiratory activity. A common finding, here
and in earlier studies, is the increase in respiratory amplitude and decrease in respiratory rate. In our case, bombesin strongly increased PNamp and was associated with prolongation of the Te, resulting in a net decrease in phrenic frequency.

Given that bombesin is likely to be released as a neurotransmitter from primary afferent neurons and act on receptors in the dorsal horn, it is possible that the changes observed in phrenic nerve discharge are the result of activation of spinobulbar pathways. Nevertheless, although the catheter tip was localized to T6, this does not imply that the drug administered is restricted to this segment. In fact, this would be extremely unlikely. It was for this reason that dye was injected at the end of each experiment. In general, the dye spread for at least two segments above and below the site of injection. Such findings correspond to the data published previously where drugs administered intrathecally were distributed only few segments above and below the tip of the intrathecal catheter (62). In theory, drug may have gradually spread rostrally to cervical segments 3–5, where phrenic motoneurons are located. If this had occurred, it might explain effects on PNamp but not on phrenic nerve frequency, which is under the control of respiratory pattern generators in the brain stem. Because, in a separate study, we have demonstrated that application of local anesthetic at C8 entirely abrogates the effects on phrenic nerve discharge (63), we believe that the likeliest explanation for the responses observed in phrenic nerve discharge is activation of spinobulbar pathways.

Compared with the effects of other peptides in identical experimental preparations, intrathecal orexin A, neuromedin U, and PACAP all cause sympathoexcitation but have distinct blood pressure effects: orexin A causes a rise in blood pressure, neuromedin U has a biphasic effect, while PACAP has no effect on blood pressure (2, 17, 63). Bombesin, by contrast, increased blood pressure and sSNA.

This is the first study to show that intrathecal bombesin markedly potentiates the sympathetic baroreflex. sSNA was reset to a higher level, but the gain and range of the sympathethic baroreflex were increased significantly in spite of the fact that the level of blood pressure was unchanged at the time of testing. This response contrasts with our earlier work where intrathecal PACAP did not alter the baroreflex (17). During hyperoxic hypercapnia, bombesin enhanced the HR and sSNA responses but attenuated the effect on PNamp. MAP was unaffected.

In conclusion, we report that bombesin in the spinal cord, acting at least in part through BB2, causes potent sympathoexcitatory actions and increases in central respiratory activity associated with increases in etCO2. Bombesin increases the sensitivity of central chemoreceptors, resulting in a potentiated bradycardia and sSNA, and enhances the sympathetic baroreceptor reflex. Together, these data suggest a significant role for bombesin in the regulation of SPN in the spinal cord that regulate the cardiovascular system.

Perspectives and Significance

The findings of this study complement the many previous studies from our laboratory, and others, which demonstrate a differential effect of peptide neurotransmitters on central cardiorespiratory regulation. Bombesin and its receptors are found throughout the brain stem and spinal cord, suggesting an anatomic substrate for the results reported here. Although the exact molecular and intracellular mechanisms underlying the actions of bombesin in this study are unknown, it is clear, at least from a pharmacological perspective, that activation of bombesin receptors in the spinal cord results affects cardiorespiratory functions. The effects are presumably the result of activation of SPN in the spinal cord as well as spinobulbar pathways that activate autonomic sites in the brain stem. Other gut neuropeptides are also known to be involved in central autonomic functions, including chemoreception (galanin) (65) and postprandial sympathoinhibition and splanchnic hyperemia (cholecystokinin) (61). It is likely, considering the results shown in this work, that bombesin and the presence of bombesin receptors in the brain stem may suggest involvement in “higher integrative autonomic processes.” Given the demonstration here that bombesin receptor activation affects cardiorespiratory regulation, future studies may have implications for the therapy of disorders such as hypertension, stroke, and heart disease.

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DISCLOSURES

No conflict of interest is declared by the authors.

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