Galanin microinjection into rostral ventrolateral medulla of the rat is hypotensive and attenuates sympathetic chemoreflex

Stephen B. G. Abbott and Paul M. Pilowsky
Australian School of Advanced Medicine, Macquarie University, Sydney, Australia
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Abbott SBG, Pilowsky PM. Galanin microinjection into rostral ventrolateral medulla of the rat is hypotensive and attenuates sympathetic chemoreflex. Am J Physiol Regul Integr Comp Physiol 296: R1019–R1026, 2009. First published January 21, 2009; doi:10.1152/ajpregu.90885.2008.—Galanin is present in neurons in the brain that are important in the control of arterial pressure, and intracisternal administration of galanin evokes hypotension, but the site of action is unknown. In urethane-anesthetized, vagotomized mechanically ventilated Sprague-Dawley rats (n = 34), we investigated the effects of microinjecting galanin (1 mM, 50 nL, 50 pmol) into the rostral ventrolateral medulla on resting splanchnic sympathetic nerve activity, arterial pressure, heart rate, and phrenic nerve activity. Second, we determined the effect of microinjecting galanin into the rostral ventrolateral medulla on the cardiovascular response to stimulation of central and peripheral chemoreceptors, arterial baroreceptors, and the somatosympathetic reflex. Galanin caused a prolonged reduction in resting splanchnic sympathetic nerve activity (−37.0 ± 7.2% of baseline), mean arterial pressure (−17.0 ± 3.5 mmHg), and heart rate (−25.0 ± 9.1 beats/min). Galanin increased the sympathoinhibitory response to aortic depressor nerve stimulation by 51.8%, had no effect on the somatosympathetic reflex, and markedly attenuated the effect of hypercapnia and hypoxia on arterial pressure (by 65% and 92.4% of control, respectively). These results suggest a role for galanin neurotransmission in the integration of the cardiovascular responses to hypoxia, hypercapnia, and the sympathetic baroreflex in the rostral ventrolateral medulla. The data suggest that galanin may be an important peptide in the homeostatic regulation of chemosensory reflexes.

baroreflex; somatosympathetic reflex; hypercapnia; hypoxia

THE ROSTRAL VENTROLATERAL medulla (RVLM) contains a heterogeneous population of presympathetic and propriobulbar respiratory neurons that are crucial in central cardiorespiratory control (2, 34); destruction or inhibition of neurons in the RVLM causes severe sympathoinhibition and hypotension and eliminates most adaptive cardiovascular responses such as the baro- and chemoreceptor reflexes (34). Neuronal activity of the RVLM is determined in the short term by the action of fast neurotransmitters, such as GABA and glutamate, but is influenced in the longer term by the pre- and postsynaptic effects of metabotropic neurotransmitters, including neuropeptides, whose roles in cardiovascular control are still not fully understood (33). Here we investigated the effects of microinjection of galanin, a 29-amino acid peptide that is implicated in feeding behavior, antinociception, and mood disorders (16), into the RVLM. Galanin binds to at least three G protein-coupled receptor subtypes that differentially activate or inhibit multiple intracellular signaling cascades, including inhibiting adenylate cyclase, opening G protein-coupled inwardly rectifying potassium channels, closing N-type calcium channels, and phospholipase C activation (16). Galanin fiber networks are found in the RVLM of the rat with immunofluorescence (22), and many galanin-expressing neurons are found in regions of the medulla, pons, and hypothalamus that regulate the activity of RVLM neurons (15, 22, 37). Most notably, galanin is expressed in neurons of the nucleus of the solitary tract (22), a region that integrates information from peripheral sensory neurons, including arterial chemoreceptor and baroreceptor afferent neurons (12, 34), and in putative central chemoreceptor neurons in the retrotrapezoid nucleus (RTN) (30, 37). Intracisternal administration of galanin evokes a depressor response (7), while intracerebroventricular administration induces a pressor response (10). Galanin also enhances the hypotensive effects of serotonin (9). Clearly, galanin acts on central sites that control arterial pressure, but the exact sites and mechanism of action are unknown.

We hypothesized that the effects of galanin on arterial pressure observed in other studies are due to direct action on neurons in the RVLM. To test this hypothesis, we microinjected galanin into the RVLM while recording splanchnic sympathetic nerve activity (sSNA), arterial blood pressure, and heart rate (HR). We also evaluated the effect of galanin on the cardiovascular response to stimulation of central and peripheral chemoreceptors, arterial baroreceptors, and the somatosympathetic reflex.

METHODS

General procedures. Procedures were approved by the Macquarie University Animal Ethics Committee under the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Male Sprague-Dawley rats (n = 34, 350–600 g) from the Animal Resources Centre (Perth, Australia) were initially anesthetized with a bolus of urethane (10%) diluted in saline (ethyl carbamate, 1.3 g/kg ip; Sigma-Aldrich, St. Louis, MO). Additional doses of urethane (30 mg in a 10% solution) were delivered intravenously as required to maintain adequate levels of anesthesia. Depth of anesthesia was assessed by checking for an absence of the withdrawal reflex and/or arterial blood pressure changes after a hind paw pinch. The left jugular vein and right carotid artery were cannulated for administration of drugs and fluids and for measurement of arterial blood pressure, respectively. The trachea was cannulated, and animals were paralyzed (pancuronium; 0.8 mg initially, then 0.4 mg/h) and mechanically ventilated (Ugo Basile) with room air enriched with 100% oxygen. End-tidal CO₂ was monitored and maintained between 4.0% and 4.5%.

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by adjusting the rate and depth of ventilation. Animals were infused with 5% glucose in saline (1.8 ml/h) to ensure hydration. The left greater splanchnic nerve and phrenic nerve were isolated, and the distal ends were cut to permit recording of efferent sSNA and phrenic nerve activity (PNA). In an additional subset of animals, the aortic depressor nerve (ADN) and sciatic nerve were isolated. Nerve recordings and stimulations were made with bipolar platinum wire electrodes. Nerve signals were amplified (500–2,000 Hz), filtered (100–3,000 Hz), and recorded with a CED 1401 data capture system and Spike2 software (CED, Cambridge, UK). The medulla was exposed after an occipital craniotomy. Microinjections of glutamate (100 mM, 50 nl, 5 nmol; Sigma-Aldrich), galanin (1 mM, 50 –100 pmol; Sigma-Aldrich), and vehicle [phosphate-buffered saline (PBS), pH 7.4] were made with single-barrel or multibarrel glass pipettes and were delivered bilaterally in the RVLM in all rats. The RVLM was identified with microinjections of glutamate (100 mM, 50 nl, 5 nmol), with a prerequisite mean arterial pressure (MAP) increase of >30 mmHg when microinjected unilaterally, as determined in previous studies (19). At the completion of experiments the brain stem was removed and fixed by immersion in 4% formaldehyde in 0.1 M PBS (pH 7.4), and histology was performed to identify injection sites marked with Pontamine blue (1% in saline).

**Experimental design.** Reflexes were evoked as described previously (18, 24) and were performed after MAP and sSNA stabilized after bilateral microinjections of galanin. In some cases, multiple reflexes were conducted in a single rat; in such cases, recorded parameters were allowed to return to prestimulus levels. The sympathetic baroreflex function was assessed with two methods. Sympathetic baroreflex function curves were generated by sequential intravenous injection of sodium nitroprusside (10 µg/kg) and phenylephrine (10 µg/kg). The heart rate baroreflex was not assessed because the rats were vagotomized and methylethramine (2 mg/kg) was administered. Changes in sSNA were plotted against systolic blood pressure to generate function curves with a four-parameter sigmoidal dose-response curve (31). Intermittent electrical stimulation of the ADN with bipolar electrodes was also used to estimate baroreflex function. Stimulus threshold was determined by reducing the stimulus voltage until no response was observed. In the experimental protocol, the ADN was stimulated at 4 times threshold (5–30 V, 0.2-ms pulse width, 100–200 cycles at 1 Hz across 100–200 s) and the average sSNA response was analyzed off-line (Spike2 version 6). Activation of the somatosympathetic reflex was achieved by stimulating the left sciatic nerve. Stimulus threshold was determined as described above, the nerve was stimulated at 4 times threshold (20–30 V, 0.2-ms pulse width, 100 pulses at 0.4 Hz across 250 s), and the average response of sSNA was analyzed off-line. Central chemoreceptors were stimulated by ventilating animals with CO2 balanced in O2 mixture (5% CO2 in O2) for 3 min. Because oxygenation of the blood was maintained throughout the challenge, the observed effects are the result of the action of CO2 on the peripheral circulation combined with the activation of central chemoreceptors. Conversely, peripheral chemoreceptors were stimulated by ventilating the animal with 10% O2 in N2 for 1 min.

**Data analysis.** For averaging purposes, sSNA was rectified and smoothed (τ = 2 s); sSNA was normalized against preinjection baseline, and the effect of microinjections on resting sSNA was measured as the change from baseline. Peak sSNA changes to reflex challenges were normalized against prestimulus values and measured as the change from baseline. Zero sSNA was taken as the minimum background activity after death, and this value was subtracted from sSNA before analysis with off-line software (Spike2 version 6). To evaluate cardiorespiratory coupling, cycle-triggered averages of rectified, but unsmoothed, sSNA were triggered from the end of the inspiration of the phrenic cycle. The phrenic cycle, and corresponding sSNA, were divided into three phases: inspiratory, postinspiratory, and expiratory. The area under the curve of each phase, less baseline, was normalized against vehicle under normocapnic conditions; this was used to compare the respiration-related peaks in sSNA. Peak amplitude (peak PNA) of phrenic discharge and frequency of discharge (PNF; cycles/min) were used as a measure of inspiratory drive. Time course and peak changes in MAP (mmHg), HR (beats per min (bpm)), and sSNA (% of baseline) and respiratory variables are expressed as means ± SE. Comparative results are presented as control versus galanin. Student’s t-test or one-way ANOVA was used to analyze peak effects or area under the curve, and a two-way ANOVA with multiple t-tests and Bonferroni’s correction was used to compare time course changes; P < 0.05 was considered significant.

**RESULTS**

**RVLM microinjection of galanin decreases MAP, HR, and sSNA and reduces cardiorespiratory coupling.** In six animals (baseline MAP 105 ± 7 mmHg and HR 458 ± 7 bpm), bilateral microinjections of galanin (1 mM, 50 nl, 50 pmol) evoked a long-lasting reduction in MAP (−17 ± 3 mmHg from baseline; Fig. 1B), sSNA (−37 ± 7% of baseline; Fig. 1C), and HR (−25 ± 9 bpm from baseline; Fig. 1D), with the maximal effect occurring between the 20th and 40th minutes and recovery to prestimulus values between the 60th and 80th minutes. The magnitude of the reduction in MAP, sSNA, and HR was significantly greater than that caused by microinjections of vehicle (P < 0.01, P < 0.001, P < 0.01, respectively; Figs. 1, B–D). In some cases, individual microinjections of galanin or vehicle caused a small pressor response which returned to baseline within 1 min. This was attributed to volume effects of microinjections on sympathoexcitatory RVLM (Fig. 1A).

In six animals, galanin transiently reduced mean PNF (101.0 ± 5.0% vs. 44.6 ± 12.1% of baseline; P < 0.001) but did not effect peak PNA compared with vehicle at this time point (105.0 ± 3.0% vs. 88.2 ± 18.9% of baseline). A typical example is shown in Fig. 2A. It is worth noting that the effect of galanin on PNA was manifest after a brief lag compared with the cardiovascular effects of galanin; this may be attributed to the time required for the peptide to diffuse into the adjacent respiratory cell groups.

To confirm that the cardiovascular effects produced by galanin were specific to the RVLM, we microinjected galanin (50 nl, 1 mM, 50 pmol) ~0.8 mm caudal and 1 mm dorsal to the RVLM pressor region in a subset of animals (n = 6); this region corresponds to the respiratory central pattern generator, the Pre-Bötzinger Complex. In these animals, galanin microinjection caused no change in MAP (6.4 ± 5.0 mmHg from baseline; P = 0.26), sSNA (6.0 ± 7.7% of baseline; P = 0.35), or HR (6.4 ± 5.0 bpm from baseline; P = 0.60) compared with vehicle (Fig. 1F). On the other hand, we observed pronounced apnea (i.e., cessation of PNA) in five of six rats and severe respiratory depression in the remaining rat (data not shown). The onset of these effects was rapid, supporting the hypothesis that the respiratory effects evoked in the RVLM were due to the diffusion of microinjections of galanin into adjacent respiratory cell groups.

Under normal conditions, the postinspiratory (P-I) peak present in sSNA was increased to 206 ± 42% of normocapnic control (P < 0.05) during hypercapnia (5% inspired CO2). The CO2-related increase in the P-I peak was attenuated by galanin (206 ± 42% vs. 96 ± 31% of normocapnic control, P < 0.05; Fig. 2C). There was no change in peak PNA in rats treated with galanin under normocapnic or hypercapnic conditions compared with the challenge, the observed effects are the result of the action of CO2 on the peripheral circulation combined with the activation of central chemoreceptors. Conversely, peripheral chemoreceptors were stimulated by ventilating the animal with 10% O2 in N2 for 1 min.
pared with vehicle (153 ± 15% vs. 140 ± 12% of normocapnic control; Fig. 2C).

Post hoc analysis of microinjection sites, marked with Pontamine blue, confirmed that RVLM sites were contained within the region containing bulbospinal cardiovascular neurons defined by other studies (4, 35), limited to within 500 μm of the caudal end of the facial nucleus (Fig. 1E).

Galanin enhances effect of ADN stimulation on sSNA and increases gain of sympathetic baroreflex. In six animals (baseline MAP 97 ± 5 mmHg and HR 437 ± 13 bpm), ADN stimulation inhibited sSNA by −37 ± 5% of prestimulus baseline, with the trough occurring, on average, 171 ± 22 ms after the stimulus and return to baseline 367 ± 33 ms after onset (Fig. 3C). After galanin microinjections (1 mM, 50 nl, 50 pmol), ADN-evoked inhibition of sSNA averaged −58 ± 8% of prestimulus baseline, occurring 156 ± 17 ms after the stimulus and returning to baseline 393 ± 37 ms after onset (Fig. 3C). The spatial and temporal characteristics of the grouped data were analyzed by comparing the area under the curve of the sympathoinhibitory response with a paired t-test (Fig. 3D), which revealed that bilateral microinjection of galanin significantly increased ADN-evoked inhibition of sSNA by 52% (P < 0.05).

In five animals (baseline MAP 97 ± 2 mmHg and HR 460 ± 8 bpm), galanin (1 mM, 100 nl, 100 pmol) modestly reduced the maximum plateau (117 ± 6% vs. 108 ± 5%; P < 0.05) and increased the maximum gain of the fitted sigmoid curve by 27% (1.7 ± 0.3 vs. 2.3 ± 0.4%/mmHg; P < 0.05) of the sympathetic baroreflex (Fig. 3, E–G).

Galanin has no effect on somatosympathetic reflex. In six animals (baseline MAP 103 ± 4 mmHg and HR 428 ± 9 bpm), sciatic stimulation evoked two distinct peaks in sSNA, consistent with previous observations (21), the first peak occurring 92 ± 4 ms after the stimulus and increasing averaged sSNA by 243 ± 29% of baseline, and the second occurring 174 ± 4 ms after the stimulus and increasing averaged sSNA by 192 ± 19% of baseline. After microinjections of galanin (1 mM, 50 nl, 50 pmol), neither the amplitude nor the latency of the peaks of averaged sSNA after sciatic stimulation were altered (1st peak: amplitude 261 ± 43% of baseline, latency 87 ± 3 ms; 2nd peak: amplitude 248 ± 46% latency 172 ± 5 ms).

Effects of galanin (1 mM, 50 nl, 50 pmol) on cardiovascular response to hypoxia and hypercapnia. In seven animals (baseline MAP 101 ± 6 mmHg and HR 458 ± 11 bpm), isocapnic hypoxia (10% O2 in nitrogen for 1 min) caused a reduction in MAP (from 104 ± 6 to 74 ± 4 mmHg; P < 0.001) and an increase in sSNA (48 ± 13% of baseline; P < 0.001) and HR (456 ± 10 to 491 ± 8 bpm; P < 0.001) (Fig. 4A). Peak effects occurred near the end of the stimulus and rapidly recovered to baseline. Galanin (1 mM, 50 nl, 50 pmol) increased the hypotension caused by isocapnic hypoxia on MAP by 26% (−29 ± 3 vs. −39 ± 5 mmHg; P < 0.05; Fig. 4B) and reduced the elevation of sSNA by 56% (48 ± 13% vs. 21 ± 7% of baseline; P < 0.05; Fig. 4B) but had no effect on evoked tachycardia (35 ± 4 vs. 35 ± 5 bpm; P = 0.94; Fig. 4B). Galanin increased peak phrenic discharge frequency by 41% but had no effect on peak amplitude of PNA during hypoxia (PNF: 69 ± 3 vs. 83 ± 6 cycles/min, P < 0.05;
DISCUSSION

The major findings of this study are that microinjection of galanin in the RVLM 1) produces sympathetically mediated hypotension and bradycardia, 2) increases sympathetic baroreflex sensitivity, 3) has no effect on the somatosympathetic reflex, and 4) attenuates the cardiovascular response to hypoxia and hypercapnia.

Galanin reduces sympathetic vasomotor tone. In this study we provide evidence that the hypotensive effect of centrally administered galanin reported in other studies (7) is due, at least in part, to a reduction in sympathetic vasomotor tone mediated by the RVLM. Other studies report that intracisternal administration of galanin results in weak hypotension and tachycardia (7). We observed a significant and sustained sympathetically mediated hypotension together with a sympathetically mediated bradycardia after microinjections of galanin into the RVLM. The tachycardia seen previously may be mediated via other brain sites activated by intracisternal injections and/or by actions on the cardiac vagal preganglionic neurons whose activity was blocked in the present study. It must be noted that we observed a significant reduction in PNF after microinjections in the RVLM, which may have contributed to the overall reduction in sympathetic discharge (32), although if this were the case, the apnea produced by galanin microinjections in the Pre-Bötzinger Complex would produce concomitant decreases in sSNA, which was not the case. The origin of the bradycardia and apnea caused by galanin is presumably due to direct actions on respiratory neurons in the ventral respiratory column, as indicated by our microinjections in the Pre-Bötzinger Complex that invariably caused apnea; this discovery is beyond the scope of this study and requires further investigation. Here we show that in the anesthetized and vagotomized rat, discrete application of exogenous galanin in the RVLM reduces sympathetically outflow regulating vascular smooth muscle tone and HR. The prolonged action of galanin is consistent with its binding to metabotropic receptors with subsequent activation of two messenger systems.

Galanin reduces sSNA respiration-related rhythm. Central respiratory activity is tightly coupled to the activity of many bulbospinal cardiovascular RVLM neurons (8, 27), and sSNA displays rhythmic fluctuations in activity correlated to PNA (25). We observed a reduction in the P-I peak of sSNA that differed from control only under hypercapnic conditions, where respiration-related rhythms are more pronounced. PNA did not differ between vehicle and galanin groups during hypercapnia; therefore, the effects of galanin on the P-I peak are unlikely to be caused by a reduction in neural respiratory drive influencing sympathoexcitatory RVLM neurons during hypercapnia. Galanin may decouple neural respiratory drive and the activity of sympathoexcitatory RVLM neurons by effect of hypercapnia on MAP by 67% (24 ± 3 vs. 8 ± 2 mmHg; \( P < 0.01 \); Fig. 4E). The sympathetic response did not differ from control (29 ± 5% vs. 20 ± 3% of baseline; \( P = 0.13 \); Fig. 4E), and there was no effect on response of HR (14 ± 3 vs. 12 ± 3 bpm; \( P = 0.56 \); Fig. 4E). Galanin did not affect the peak frequency or amplitude of PNA during hypercapnia (PNF: 52 ± 1 vs. 54 ± 2 cycles/min, \( P = 0.74 \); peak PNA: 153 ± 13% vs. 140 ± 11% of control baseline, \( P = 0.10 \); Fig. 4F).

peak PNA: 177 ± 12 vs. 162 ± 11% of control baseline, \( P = 0.16 \); Fig. 4C).

In eight animals (baseline MAP 98 ± 6 mmHg and HR 459 ± 14 bpm), hyperoxic hypercapnia (5% CO₂ in O₂ for 3 min) caused a progressive increase in MAP (from 108 ± 4 to 131 ± 3 mmHg; \( P < 0.0001 \); Fig. 4D), sSNA (28 ± 5% of baseline; Fig. 4D), and HR (from 458 ± 10 to 471 ± 8 bpm; \( P < 0.05 \); Fig. 4D) after an initial bradycardia at the onset of the stimulus. Galanin (1 mM, 50 nl, 50 pmol) reduced the
modulating fast neurotransmitter release (25, 26) or via a direct inhibition of neurons most active during postinspiration (8). Although galanin reduced the P-I peak of sSNA (with no smoothing applied) during hypercapnia, galanin did not reduce the change in total sSNA, averaged with a time constant of 2 s, to hypercapnia. The respiration-related fluctuations in sSNA become masked by application of a large time constant to sSNA for averaging purposes; as such, the effect of galanin on the P-I peak is not apparent in the averaged sSNA response to hypercapnia.

Galanin attenuates cardiovascular effects of central and peripheral chemoreceptor stimulation. Galanin dramatically attenuated the increase in sSNA and increased the hypotension evoked by isocapnic hypoxia. Hypoxia is known to elicit peripheral vasodilatation independent of neurogenic vasoconstriction mediated by the peripheral chemoreceptors (3, 14, 20). In this preparation, the local vasodilatory effect of hypoxia appears to be the predominant effect of hypoxia resulting in a reduction in arterial blood pressure. All things being equal, attenuating the sympathetic activation caused by hypoxia would further uncover its peripheral vasodilatory effect, resulting in a larger fall in arterial pressure, as observed in this study.

In addition to the effect of galanin on the sympathetic response to hypoxia, galanin dramatically reduced the hypertension caused by hypercapnia but did not change the increase in averaged sSNA associated with hypercapnia. It is surprising that galanin attenuated the rise in arterial pressure but not averaged sSNA. A recent study characterizing the sympathetic response to graded hypercapnia reported that, under urethane anesthesia, splanchnic nerve discharge doubled in response to changes in end-tidal CO₂ comparable to the present study (28). The aforementioned study was conducted in a barodenervated preparation, eliminating the buffering of arterial blood pressure and sSNA by the baroreflex, which is likely to counteract increases in synaptic drive from central chemoreceptors to the RVLM. It would be interesting to replicate the protocol used here in a barodenervated preparation to evaluate whether the disparity between MAP and sSNA observed in this study can be eliminated.

After microinjections of galanin, the hypotension evoked by isocapnic hypoxia was potentiated and the hypertension evoked by hyperoxic hypercapnia was blunted. We interpret these findings as being due to a reduction in neurogenic vasomotor response mediated by the RVLM. While we ob-
served a significant increase in peak PNF in response to hypoxia, there was no significant difference in peak amplitude response to either hypoxia or hypercapnia. Therefore it seems unlikely that the effect of galanin on the vasomotor response to hypoxia and hypercapnia is secondary to a reduction in neural respiratory drive. It is appealing to suggest that the depression of the central and peripheral chemoreflex by galanin is due to a single cause, such as the inhibition of a common neuronal substrate in the RVLM mediating both reflexes. Roughly half of the galanin-expressing neurons located in the RTN display the characteristics of putative central chemoreceptors (5, 37). The RTN defines a population of neurons that mediate the respiratory component of the central chemoreflex, and also the peripheral chemoreflex to some degree (38, 39), and may be involved in the cardiovascular component of these reflexes as well (6, 28). While it is speculative, the effects of galanin microinjections in the RVLM may be mimicking the endogenous release of galanin by galanin-expressing RTN neurons.

Galanin increased the sympathetic response to ADN stimulation and also increased the sympathetic gain and reduced the saturation point associated with acute changes in arterial pressure. Considering that there was only a small change in the operating point of sSNA, despite reduced sympathetic tone, it appears that the increased ADN-evoked inhibition of sSNA is probably a function of increased gain. On the basis of these results, we speculate that galanin increases GABA release or galanin increases the efficacy of postsynaptic GABAA receptors through intramembrane receptor interactions. This speculation conflicts with reports that galanin reduces the amplitude of evoked inhibitory postsynaptic potentials in isolated dorsal raphe neurons in the neonatal slice preparation (36). Apart from the many obvious differences in our preparations, we cannot easily account for this disparity, which needs further investigation.

Galanin has no effect on somatosympathetic reflex. Galanin modulates spinal processing of pain (17), but the role of galanin in supraspinal pain processing is underrepresented in the literature. Here we report that galanin does not modulate the somatosympathetic reflex at the level of the RVLM, the
primary site for coordinating the cardiovascular response to a painful stimulus (29). This suggests that galanin does not cause a generalized depression of RVLM neurons, which would result in a reduction in somatosympathetic reflex function.

Anatomic evidence for galanin receptors in RVLM. Currently there is a lack of receptor antibodies to galanin receptors, but this study suggests that galanin receptors exist in the RVLM region. Studies investigating the distribution of galanin receptors 1, 2, and 3 with receptor autoradiographic techniques have not identified galanin receptors in the RVLM (23). It is possible that this technique is not sensitive to sparse receptor populations, as may be the case in the RVLM. Immunofluorescent galanin-positive fiber networks are present within the RVLM in the rat (22) and around C1 neurons in the cat (1), suggesting a functional galaninergic network. Clearly, further investigation is needed to locate galanin receptors in the RVLM.

Perspectives

This study demonstrates that exogenous galanin applied to the RVLM reduces sympathetic cardiovascular tone, providing a site of action for the cardiovascular effects of galanin described in previous studies. Additionally, galanin enhanced the sympathetic baroreflex and reduced the cardiovascular effects of peripheral and central chemoreceptor stimulation, without affecting the somatosympathetic reflex. We also have presented preliminary evidence suggesting that galanin may also be involved in neural respiratory regulation in the medulla. We propose that the effect of galanin in the RVLM is related to a reduction in fast neurotransmission release onto RVLM vasomotor neurons. This hypothesis is functionally supported by in vitro studies that show that galanin reduces presynaptic glutamate release in the arcuate and supraoptic nuclei (11, 13), although the mechanism of action involved in the RVLM cannot be determined from this study. The use of galanin antagonists is required to determine whether galanin is released by neurons in response to the reflex challenges investigated here or whether it is released from neurons that modulate cardiovascular function at the level of the RVLM but are not directly involved in the expression of these challenges. These findings support the idea that the RVLM contains specific neuronal populations that are responsible for mediating distinct cardiovascular reflex pathways (33).

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