GALANIN MICROINJECTION INTO THE ROSTRAL VENTROLATERAL MEDULLA OF THE RAT IS HYPOTENSIVE AND ATTENUATES THE SYMPATHETIC CHEMOREFLEX

Stephen Bruce Gidley Abbott, Paul Martin Pilowsky.

Australian School of Advanced Medicine, Macquarie University

Running heading: Cardiovascular effect of galanin in the rat RVLM

Word Count: (not including figures) 6196

Abstract: 209

Contact Information
Paul M Pilowsky (paul.pilowsky@mq.edu.au)
Australian School of Advanced Medicine, Macquarie University 2109, AUSTRALIA
Ph: 61 2 9850 4015
Fx : 61 2 9850 4010
Abstract

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-anaesthetised, vagotomised mechanically ventilated Sprague Dawley rats (N=34), we investigated the effects of microinjecting galanin (1mM, 50nL, 50pmol) into the rostral ventrolateral medulla on resting splanchnic sympathetic nerve activity, arterial pressure, heart rate and phrenic nerve activity. Secondly, 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 ± 3.5mmHg) and heart rate (-25.0 ± 9.1bpm). 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.

Key Words: baroreflex, rats, somatosympathetic reflex, hypercapnia, hypoxia
**Introduction**

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 chemo- receptor 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 post-synaptic effects of metabotropic neurotransmitters, including neuropeptides, whose role in cardiovascular control are still not fully understood (33).

Here we investigate the effects of microinjection of galanin, a 29 amino acid peptide that is implicated in feeding behaviour, antinociception and mood disorders (16) into the RVLM. Galanin binds to at least 3 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 using 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 (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 is unknown.

We hypothesize that the effects of galanin on arterial pressure observed in other studies is 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. 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 anaesthetised with a bolus of urethane (10%) diluted in saline (ethyl carbamate 1.3 g/kg, i.p.; Sigma-Aldrich, St. Louis, MO). Additional doses of urethane (30mg in a 10% solution) were delivered intravenously as required to maintain adequate levels of anaesthesia. Depth of anaesthesia 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,
animals were paralyzed (pancuronium; 0.8 mg initially, then 0.4 mg/hr) and mechanically ventilated (Ugo Basile, Italy) with room air enriched with 100% oxygen. End-tidal CO₂ was monitored and maintained between 4.0-4.5% by adjusting the rate and depth of ventilation. Animals were infused with 5% glucose in saline (1.8ml/hr) to ensure hydration. The left greater splanchnic nerve and phrenic nerve were isolated, and the distal ends cut to permit recording of efferent sSNA and phrenic nerve activity (PNA). In an additional subset of the animals, the aortic depressor nerve (ADN) and sciatic nerve were isolated. Nerve recordings and stimulations were made using bipolar platinum wire electrodes. Nerve signals were amplified (500-2000Hz), filtered (100–3000 Hz), and recorded using a CED 1401 data capture system and Spike 2 software (CED, Cambridge, UK). The dorsal medulla was exposed after an occipital craniotomy. Microinjections of glutamate (100mM, 50nl; 5nmol; Sigma-Aldrich), rat galanin (1mM, 50-100nl; 50pmol-100pmol; Sigma-Aldrich) and vehicle (phosphate buffered solution; PBS, pH 7.4) were made using single- or multi- barrel glass pipettes and were delivered bilaterally in the RVLM in all rats. The RVLM was identified with microinjections of glutamate (100mM, 50nl, 5nmol), with a prerequisite MAP increase of >30mmHg when microinjected unilaterally, as determined by in previous studies (19). At the completion of experiments, the brainstem was removed and fixed by immersion in 4% formaldehyde in 0.1M phosphate buffered solution (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 mean arterial blood pressure (MAP) and sSNA stabilised following 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 pre-stimulus levels. The sympathetic baroreflex function was assessed using two methods. Sympathetic baroreflex function curves were generated by sequential i.v. injection of sodium nitroprusside (SNP; 10µg/kg) and phenylephrine (PE; 10µg/kg). The heart rate baroreflex was not assessed as the rats were vagotomised and methylatropine (2mg/kg) was administered. Changes in sSNA were plotted against systolic blood pressure (SBP) to generate function curves using a 4-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 (5-30V, 0.2ms pulse width, 100-200 cycles at 1 Hz across 100-200s), and the average sSNA response was analysed offline (Spike2 version 6). Activation of the somato-sympathetic reflex was achieved by stimulating the left sciatic nerve. Stimulus threshold was determined as described and the nerve was stimulated at 4 times threshold (20–30 V, 0.2ms pulse width, 100 pulses at 0.4Hz across 250s) and the average response of sSNA was analysed offline. Central chemoreceptors were stimulated by ventilating animals with CO₂ balanced in O₂ mixture (5% CO₂ in O₂) for 3 minutes. As 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 minute.

*Data Analysis*
For averaging purposes, sSNA was rectified and smoothed ($\tau = 2s$); sSNA was normalized against pre-injection baseline and the effects of microinjections on resting sSNA was measured as the change from baseline. Peak sSNA changes to reflex challenges were normalized against pre-stimulus 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 using offline software (Spike2, version 6). To evaluate cardio-respiratory coupling, cycle triggered averages of rectified, but unsmoothed, sSNA was triggered from the end of the inspiration of the phrenic cycle. The phrenic cycle, and corresponding sSNA, was divided into 3 phases; inspiratory, post inspiratory and expiratory. Area under the curve of each phase, less baseline, was normalized against vehicle under normocapnic conditions; this was used to compare the respiratory related peaks in sSNA. Peak amplitude (peakPNA) of phrenic discharge and frequency of discharge (PNF; cycles/min) was used as a measure of inspiratory drive. Time course and peak changes in mean arterial pressure (MAP; millimeters mercury; mmHg), heart rate (HR; beats per minute; bpm) and sSNA (% of baseline) and respiratory variables are expressed as mean ± SEM. Comparative results are presented as control vs. 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 microinjections of galanin decreases MAP, HR and sSNA and reduces cardiorespiratory coupling*
In 6 animals (baseline MAP 105 ± 7 and HR 458 ± 7), bilateral microinjections of galanin (1mM, 50nL, 50pmol) evoked a long-lasting reduction in MAP (-17 ± 3 mmHg from baseline; Figure 1B), sSNA (-37 ± 7 % of baseline; Figure 1C) and HR (-25 ± 9 bpm from baseline; Figure 1D), with the maximal effect occurring between the 20th and 40th minute recovering to pre-stimulus values between the 60th and 80th minute. 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) (Figure 1B, C, 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 (Figure 1A).

In 6 animals, galanin transiently reduced mean PNF (101.0 ± 5.0% of baseline vs. 44.6 ± 12.1% of baseline; P<0.001), but did not effect peak PNA compared to vehicle at this time point (105.0 ± 3.0% of baseline vs. 88.2 ± 18.9% of baseline). A typical example is shown in figure 2A. It is worth noting that the effect of galanin on PNA was manifest after a brief lag compared to 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 (50nl, 1mM, 50pmol) approximately 0.8mm caudal and 1mm dorsal to the RVLM pressor region in a subset of animals (N=6); this region corresponds to the respiratory central pattern generator, the PreBötzinger Complex. In these cases, galanin microinjection caused no change in MAP (6.4 ± 5.0mmHg
from baseline; P=0.26), sSNA (6.0 ± 7.7% of baseline; P=0.35) or HR (6.4 ± 5.0bpm from baseline; P=0.60) compared to vehicle (Figure 1F). On the other hand, we observed pronounced apnea (i.e. cessation of PNA) in 5 of 6 rats and severe respiratory depression in the remaining case (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 post inspiratory (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; Figure 2C). There was no change in peak PNA in rats treated with galanin under normocapnic or hypercapnic conditions compared to vehicle (153 ± 15 vs. 140 ± 12% of normocapnic control; Figure 2C).

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

Galanin enhances the effect of ADN stimulation on sSNA and increases the gain of the sympathetic baroreflex

In 6 animals (baseline MAP 97 ± 5mmHg and HR 437 ± 13bpm), ADN stimulation inhibited sSNA by -37 ± 5% of pre-stimulus baseline, with the trough occurring, on
average, 171 ± 22ms after the stimulus and returning to baseline 367 ± 33ms after onset (Figure 3C). Following galanin microinjections (1mM, 50nL, 50pmol), ADN evoked inhibition of sSNA averaged -58 ± 8 % of pre-stimulus baseline, occurring 156 ± 17ms after the stimulus and returning to baseline 393 ± 37ms after onset (Figure 3C). The spatial and temporal characteristics of the grouped data were analysed by comparing the area under the curve of the sympathoinhibitory response using a paired t-test (Figure 3D), revealing that bilateral microinjection of galanin significantly increases ADN evoked inhibition of sSNA by 52% (P<0.05).

In 5 animals (baseline MAP 97 ± 2 mmHg and HR 460 ± 8bpm), galanin (1mM, 100nL, 100pmol) 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%/mmHg vs. 2.3 ± 0.4%/mmHg; P<0.05) of the sympathetic baroreflex (Figure 3E, F, G).

Galanin has no effect on the somatosympathetic reflex

In 6 animals (baseline MAP 103 ± 4mmHg and HR 428 ± 9bpm), sciatic stimulation evoked two distinct peaks in sSNA, consistent with previous observations (21); the first peak occurring 92 ± 4ms after the stimulus and increased averaged sSNA by 243 ± 29% of baseline, and the second occurring 174 ± 4ms after the stimulus and increased averaged sSNA by 192 ± 19% of baseline. Following microinjections of galanin (1mM, 50nL, 50pmol), neither the amplitude nor the latency of the peaks of averaged sSNA following sciatic stimulation were altered (1st peak: amplitude; 261 ± 43% of baseline, latency; 87 ± 3ms. 2nd peak: amplitude; 248 ± 46, latency; 172 ± 5ms).
Effects of galanin (1mM, 50nL, 50pmol) on the cardiovascular response to hypoxia and hypercapnia

In 7 animals (baseline MAP 101 ± 6mmHg and HR 458 ± 11bpm), isocapnic hypoxia (10% O₂ in nitrogen for 1 min) caused a reduction in MAP (from 104 ± 6mmHg to 74 ± 4mmHg; P<0.001), and an increase in sSNA (48 ± 13% of baseline; P<0.001) and HR (456 ± 10bpm to 491 ± 8bpm; P<0.001; Figure 4A). Peak effects occurred near the end of the stimulus and rapidly recovered to baseline. Galanin (1mM, 50nL, 50pmol) increased the hypotension caused by isocapnic hypoxia on MAP by 26% (-29 ± 3mmHg vs. -39 ± 5mmHg; P<0.05; Figure 4B) and reduced the elevation of sSNA by 56% (48 ± 13% of baseline vs. 21 ± 7% of baseline; P<0.05; Figure 4B), but had no effect on evoked tachycardia (35 ± 4bpm vs. 35 ± 5bpm; P=0.94; Figure 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. peakPNA; 177 ± 12 vs. 162 ± 11% of control baseline; P=0.16; Figure 4C).

In 8 animals (baseline MAP 98 ± 6 mmHg and HR 459 ± 14bpm), hyperoxic hypercapnia (5% CO₂ in O₂ for 3 min) caused a progressive increase in MAP (from 108 ± 4mmHg to 131 ± 3mmHg; P<0.0001; Figure 4D), sSNA (28 ± 5% of baseline; Figure 4D), and HR (from 458 ± 10bpm to 471 ± 8bpm; P<0.05; Figure 4D) following an initial bradycardia at the onset of the stimulus. Galanin (1mM, 50nL, 50pmol) reduced the effect of hypercapnia on MAP by 67% (24 ± 3 mmHg vs. 8 ± 2mmHg; P<0.01; Figure 4E). The sympathetic response did not differ from control (29 ± 5% of baseline vs. 20 ± 3% of baseline; P=0.13; Figure 4E) and there was no effect on response of HR (14 ± 3bpm vs. 12 ± 3bpm; P=0.56; Figure 4E). Galanin did
not effect 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; Figure 4F)

Discussion

The major findings of this study are that microinjection of galanin in the RVLM: 1) produces a 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 following 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 following 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 PreBötzinger complex would produce concomitant decreases in sSNA, which was not the case. The origin of the bradypnea 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 PreBötzinger complex which invariably caused apnea; this discovery is beyond the scope of this study and requires further investigation. Here we show that in the anaesthetised and vagotomised rat, discrete application of exogenous galanin in the RVLM reduces sympathetic outflow regulating vascular smooth muscle tone and heart rate. The prolonged action of galanin is consistent with its binding to metabotropic receptors with subsequent activation of second messenger systems.

_Galanin reduces sSNA respiratory-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 differ from control only under hypercapnic conditions, where respiratory 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 de-couple neural respiratory drive and the activity of sympathoexcitatory RVLM neurons by modulating fast neurotransmitter release (25, 26), or via a direct inhibition of neurons most active during post-inspiration (8). Though 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 seconds, to hypercapnia. The respiratory related fluctuations in sSNA become masked by applying 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 the 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 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 anaesthesia, splanchnic nerve discharge doubled in response to changes in end-tidal CO$_2$ comparable to this 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 if the
disparity between MAP and sSNA observed in this study can be eliminated.

Following 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 observed 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 increases the gain of the sympathetic baroreflex_

This is the first study to investigate the effect of galanin on the sympathoinhibitory
effects of stimulating the sympathetic baroreflex. 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. Based on these result, we speculate that
galanin increases GABA release, or that galanin increases the efficacy of post-
synaptic GABA<sub>A</sub> receptors through intramembrane receptor interactions. This
speculation conflicts with reports that galanin reduces the amplitude of evoked
inhibitory post-synaptic 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 the somatosympathetic reflex*

Galanin modulates spinal processing of pain (17), but the role of galanin in supra-
spinal pain processing is under-represented 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 generalised depression of RVLM neurons,
which would result in a reduction in somatosympathetic reflex function.

*Anatomical evidence for galanin receptors in the 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 receptor 1, 2 and 3 using 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.

**Perspective**

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 galanin reduces pre-synaptic glutamate release in the arcuate and supraoptic nucleus (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 if galanin is released by neurons in response to the reflex challenges investigated here, or if 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).
ACKNOWLEDGEMENTS

Work in the Authors laboratory is supported by grants from the National Health and Medical Research Council of Australia (211023, 211196, 457068, 457080, 457069) and the Garnett Passe and Rodney Williams Memorial Foundation. SGBA is supported by a Macquarie Research Excellence Scholarship.

References


26. **Miyawaki T, Minson J, Arnolda L, Chalmers J, Llewellyn-Smith I, and Pilowsky P.** Role of excitatory amino acid receptors in cardiorespiratory coupling in


Figure 1. The effect on MAP, HR and sSNA of bilateral galanin microinjections in the RVLM. A) The effects of bilateral galanin microinjections (denoted by arrows). Traces from top to bottom represent arterial blood pressure (AP), heart rate (HR) and integrated splanchnic sympathetic nerve activity (sSNA) (arbitrary units). Integrated sSNA (grey) is superimposed over rectified sSNA. Lower panels represent the grouped time course effects of galanin (closed circles) and vehicle (open circles) on MAP (B); HR (C); and sSNA (D); (N=6). * P<0.05; **P<0.01; ***P<0.001 compared with multiple comparisons. ### P<0.001; ## P<0.01 interaction effect (drug*time) as measured by two-way repeated measures ANOVA. E) Microinjection sites from rats used in the grouped data for figure 1 and a section showing an injection site counterstained with cresyl violet. Nucleus ambiguus (NA), spinal trigeminal tract (sp5), pyramidal tract (py). Circles-vehicle and galanin microinjection sites in the RVLM (N=6); squares-galanin microinjection sites in the PreBötzingger region (N=6). Open symbols-right hand side (RHS); closed symbols-left hand side (LHS). F) Comparison of the peak cardiovascular effects produced by microinjections in the RVLM and PreBötzingger region with either galanin or vehicle. *** P<0.001; * P<0.05 compared with vehicle.

Figure 2. The effect of galanin on sSNA respiratory related rhythm. A) An experimental recording of a period where phrenic cycle triggered averages (CTA) of sSNA were obtained (shaded areas). Periods of increased inspired CO₂ (5% CO₂ in O₂) indicated by black bars below trace. Increasing inspired CO₂ raised end tidal CO₂ to greater than 7.5% B) Cycle triggered averages of PNA and sSNA in (A) under four conditions (from left to right; normocapnia; hypercapnia (5% inspired CO₂ in O₂); normocapnia following galanin; hypercapnia following galanin). Phrenic cycle is
divided into three phases; inspiration (I), post-inspiration (P-I) and late expiration (E). C) Grouped change in area under the curve (% normocapnic control) of sSNA for each division of the respiratory cycle and peak PNA (N=6). * P<0.05 compared to normocapnic control; ** P<0.01 compared to normocapnic control; † P<0.05 compared to hypercapnic control; †† P<0.01 compared to hypercapnic control.

**Figure 3. The effect of galanin on the sympathetic baroreflex.** A) An experimental recording demonstrating the protocol used to identify changes in baroreflex sensitivity. ADN stimulation (StimADN) displays periods where the ADN was electrically stimulated; each series (1-4) consisted of 100 sub-maximal stimuli. Arrows denote microinjections of galanin. B) Cycle triggered averages of sSNA from (A) displaying the effect of galanin on the ADN evoked inhibition of sSNA in the corresponding series. C) Grouped effect of ADN evoked inhibition of sSNA before and after galanin microinjections from 6 rats. D) Comparison of area under the curve of (C). E) Experimental recording of the effect of changes in arterial pressure on sSNA before (left panel) and after (right panel) galanin microinjection. F) Average 4 parameter sympathetic response curves generated for data (N=5) before (left panel) and after (right panel) galanin microinjection, superimposed over contributing replicates. G) Comparison of parameters defined by response curves; control (white bars) galanin (black bars) (F). Galanin reduces the saturation level and increases the maximum gain of the sympathoinhibitory to SBP changes. *P<0.05 compared to control.

**Figure 4. The effect of galanin on the cardiovascular response to normocapnic hypoxia and hyperoxic hypercapnia.** A) An experimental recording. The left panel
represents control hypoxic episodes and the right panels represent hypoxic episodes after microinjections of galanin. Bars below trace indicate periods of hypoxia (10% O₂ in N₂). Smoothed sSNA (grey) is superimposed over rectified sSNA. Hypoxia was associated with minor changes in end-tidal CO₂ probably due to changes in perfusion of the lungs or metabolism. B) The peak change from baseline in MAP, HR and sSNA caused by hypoxia (N=7). C) Grouped absolute values of peak phrenic nerve discharge frequency (PNF) and peak PNA during hypoxia (N=7). D) An experimental recording. As in (A), except bars below trace indicates periods of hyperoxic hypercapnia, where inspired CO₂ was increased to 5%. E) The peak change from baseline in MAP, HR and sSNA caused by hypercapnia (N=8). F) Grouped absolute values of peak PNF and peak PNA during hypercapnia (N=8). * P<0.05, **P<0.05 vs. control
Figure 1: Abbott and Pilowsky
Figure 2: Abbott and Pilowsky
Figure 3: Abbott and Pilowsky
Figure 4: Abbott and Pilowsky