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VENTROLATERAL MEDULLARY NEURONES: EFFECTS ON MAGNITUDE AND RHYTHM OF DISCHARGE OF MESENTERIC AND RENAL NERVES IN CATS

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SUMMARY

1. Discharge of whole mesenteric and renal nerves was recorded in eighteen chloralose-anaesthetized, artificially respired cats.

2. Inhibition of tonic activity of neurones within the rostral ventrolateral medulla (RVLM blockade) by bilateral application of glycine caused significant reductions in discharge of renal and mesenteric nerves, arterial blood pressure and heart rate. The decrease in discharge of renal nerves was significantly greater than that of mesenteric nerves.

3. During the response to glycine application, the spinal cord was transected at the first cervical segment. The magnitude of renal nerve discharge after transection was not different from that during blockade of the RVLM. On the other hand, mesenteric nerve activity increased following spinal cord transection, returning to control levels.

4. Power spectral analysis revealed that mesenteric and renal nerves discharged with periodicities ranging from 1 to 6 Hz. Application of glycine to the RVLM reduced the slow rhythm in firing of mesenteric and renal nerves similarly. Transection of the spinal cord resulted in further reduction in the rhythmicity in discharge of both nerves.

5. The results indicate that excitatory drive from the RVLM is crucial for the maintenance of on-going discharge of renal, but not of mesenteric nerves. However, such inputs are apparently essential to maintain the slow rhythm in firing of both nerves.

INTRODUCTION

The rostral portion of the ventrolateral medulla (RVLM) contains neurones thought to provide tonic drive to vasomotor and cardiac sympathetic nerves.

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Destruction of these neurones or inhibition of their discharge causes decreases in on-going sympathetic nerve activity (Pilowsky, West & Chalmers, 1985; Dean & Coote, 1986; Yardley, Stein & Weaver, 1988) and hypotension, bradycardia and peripheral vasodilatation (Guertzenstein & Silver, 1974; Hilton, Marshall & Timms, 1983; Ross, Ruggiero, Park, Joh, Sved, Fernandez-Pardal, Saavedra & Reis, 1984; Willette, Punnen-Grandy, Krieger & Sapru, 1987). Histological and electrophysiological investigations have demonstrated projections from the RVLM to the intermediolateral cell column of the thoracic spinal cord (Amendt, Czachurski, Demowsky & Seller, 1979; Barman & Gebber, 1985; Caverson & Ciriello, 1987). All components of sympathetic outflow probably do not equally depend upon excitatory drive from the RVLM for the maintenance of on-going discharge. For example, our findings of sustained firing of mesenteric, but not of renal nerves, in spinal cats imply that projections from the RVLM provide tonic drive to renal, but not to mesenteric nerves (Stein & Weaver, 1988). This hypothesis is supported by results of a recent investigation in which bilateral microinjections of the inhibitory amino acid glycine into the RVLM elicited greater reduction in renal, than in mesenteric nerve activity (Yardley *et al.* 1988). However, discharge of renal nerves was only reduced by 30% in that study, suggesting that a substantial portion of descending excitatory drive remained effective after microinjection of glycine. To ascertain the extent to which on-going sympathetic nerve discharge depends upon tonic drive from the RVLM it is necessary to remove as much of this drive as possible. This was accomplished in the present study by inhibiting the discharge of neurones within the RVLM by bilateral application of glycine to the ventral surface of the rostal medulla.

Although excitatory inputs from the RVLM may not be essential for the maintenance of discharge of certain sympathetic nerves, such inputs may determine the periodicity of their discharge. Postganglionic sympathetic nerves are known to discharge with periodicities of 2–6 Hz (Barman & Gebber, 1980; Gebber & Barman, 1980). This slow rhythm is generated within brain stem circuits and is transmitted to sympathetic nerves through descending bulbospinal pathways which originate in the RVLM (Barman & Gebber, 1983). In addition, discharges of pairs of sympathetic nerves which continue to fire after spinal cord transection have been shown to be strongly correlated when the neuraxis is intact, but not after spinal cord transection, indicating that synchrony in the discharge of these nerves depends on descending supraspinal inputs (Ardell, Barman & Gebber, 1982; Taylor & Schramm, 1987).

The purpose of the present investigation was to assess the influence of the excitatory projection from the RVLM on the firing of mesenteric and renal nerves. The specific goals were to determine if inhibition of tonic activity (blockade) of structures within the RVLM caused greater decreases in the overall discharge of renal nerves than that of mesenteric nerves, while reducing the 2–6 Hz rhythm in firing of both nerves similarly. In addition, it was hypothesized that blockade of the RVLM would unmask tonically active sympatho-inhibitory systems which may contribute to the decreases in nerve discharge. This hypothesis was tested by comparing magnitudes of nerve discharge observed after spinal cord transection to those seen following RVLM blockade. A preliminary report of this work has appeared as a published abstract (Stein, Yardley, Fitzsimons & Weaver, 1987).

METHODS

Preparation of animals. Eighteen cats (1·8–4·4 kg) were anaesthetized with intravenously administered α-chloralose (80 mg kg^{-1}, Sigma Chemical Company, USA). Supplementary doses (8–20 mg kg^{-1}) were given as needed. Body temperature was maintained at 37 °C with a heating pad. Both femoral arteries and a femoral vein were cannulated, and a tracheostomy tube was inserted, low in the neck. A catheter was passed through the urethra to allow continuous emptying of the urinary bladder. Gallamine triethiodide (4 mg kg^{-1}, Rhone-Poulenc, Canada) was given to induce muscle relaxation during surgery. Additional gallamine (4 mg kg^{-1}) was administered as needed during the experiment after assessment of the animal's plane of anaesthesia. This assessment was based on the cat's palpebral and paw-pinch reflexes, the stability of the blood pressure, and the size of the pupils. Cats were artificially respired with room air. End-tidal CO_2 was continuously monitored (CO_2 analyser, model LB-2, Sensormedics, USA) and maintained at 3·0–4·5%. Arterial blood samples were periodically withdrawn to measure pH and blood gas pressures (blood gas analyser, model 165, Corning Medical, USA). Acceptable values were: pH, 7·35–7·45; P_{a,CO_2}, 25–40 mmHg; and P_{a,O_2} > 80 mmHg. Acid–base disorders were corrected by infusing sodium bicarbonate, by adjusting the respiratory volume and/or rate, or by respiring the animals with oxygen-enriched room air. A lactated Ringer solution was infused slowly throughout the experiment to compensate for fluid loss.

The ventral surface of the brain was exposed as described by Guertzenstein (1973). Cats were placed in a stereotaxic frame (David Kopf Instruments, USA), in a supine position. The oesophagus and larynx were severed and reflected rostrally through the mouth. Following lateral retraction of overlying muscles, the basioccipital bone was carefully removed with rongeurs as close to the tympanic bulla as possible without damaging the nerves coursing through the jugular foramen. The dura and arachnoid were left intact until immediately before the application of glycine. At this time they were cut longitudinally and retracted laterally to expose the ventral surface of the medulla. The first cervical segment of the spinal cord was exposed ventrally by removing the vertebral arch of the atlas and the odontoid process of the axis with rongeurs.

The renal and mesentric arteries were exposed via a retroperitoneal approach. Renal and mesenteric nerves were identified and small bundles were dissected free from surrounding tissue and severed. The nerve bundles were desheathed and the central ends were placed on bipolar platinum–iridium electrodes for recording multifibre electrical activity. Exposed nerves were immersed in warm mineral oil and the surrounding tissue was coated with petroleum jelly to prevent dehydration. A pneumothorax was made to eliminate artifacts in the neural recordings caused by movement associated with ventilation. In four cats, sino-aortic denervation and vagotomy were performed by severing the vagus and glossopharyngeal nerves as they passed into the jugular foramen.

Data acquisition. Systemic arterial pressure was monitored using Gould-Statham P-23 ID transducers. Heart rate was computed from the arterial pressure pulse using a Grass Instruments tachograph (7P4H). In nine experiments, neural activity was amplified at a bandwidth of 30 Hz–3 kHz (Grass Instruments P511 preamplifers). In nine other experiments, activity was amplified at a wider bandwidth of 1 Hz–3 kHz to reveal the low-frequency components of the signal. At this bandwidth bursts of nerve discharge appear as slow oscillations in voltage (see Figs 4 and 5). All neural discharge was monitored on oscilloscopes, and the rectified, integrated neural signals (Grass Instruments 7P10 integrators) were displayed along with arterial pressure and heart rate on a Grass Instruments polygraph. All physiological signals were recorded on electromagnetic tape (Racal, model 7DS, UK).

Experimental protocol. The inhibitory amino acid glycine (BDH Chemicals, Canada) was dissolved in normal saline at a concentration of 200 mg ml^{-1}; the pH was adjusted to 7·35–7·45 and the solution was warmed to 37 °C. Small cotton pledgets (1–2 mm diameter) soaked with the glycine solution were topically applied to both sides of the ventral medulla, 2–4 mm lateral to the mid-line and 2 mm caudal to the trapezoid bodies. This corresponds to the 'glycine-sensitive area' described by Guertzenstein & Silver (1974). Activity of renal and mesenteric nerves was simultaneously recorded to determine if excitatory drive from the RVLM selectively influences the magnitude or frequency characteristics of on-going discharge of these nerves. In four cats, responses to the application of glycine were evaluated while supporting arterial pressure with intravenous infusions of phenylephrine (2·0–10·0 μg kg^{-1} min^{-1}; Neo-Synephrine, Sterling, Canada), and, after

denervation of systemic pressoreceptors, to ensure that the observed changes in the periodicity of nerve firing were not secondary to decreases in arterial blood pressure. Cotton pledgets soaked with warm physiological saline were applied as controls. Pledgets were removed after 5 min and the ventral surface of the medulla was rinsed with approximately 30 ml warm physiological saline. After recovery of neural discharge and blood pressure to control levels the application of glycine was repeated. During the nadir of the decrease in nerve discharge following this second application of glycine, the spinal cord was transected at the first cervical segment to determine if sympatho-inhibition contributed to the decrease in nerve activity. In thirteen of eighteen cats, conduction in the cervical spinal cord was blocked prior to the transection by the injection of 0·3 ml of 2% lidocaine (Rugby Laboratories, USA) directly into the spinal cord. The completeness of the transection was verified post-mortem. After spinal cord transection, mean arterial blood pressure was maintained at, or above, 85 mmHg by intravenous infusions of phenylephrine (5·0–10·0 μg kg^{-1} min^{-1}). Recovery of nerve activity after blocking spinal cord conduction would have suggested that the application of glycine to the ventral surface of the medulla unmasked tonically active bulbospinal sympatho-inhibitory pathways. A similar protocol was used by Alexander (1946), who demonstrated that on-going cardiac nerve discharge, which had been completely abolished by transection of the neuraxis at the level of the obex, resumed partially, following a low bulbar transection.

Data analysis. Nerve activity was integrated over 10 s intervals using a PDP 11/23 computer as described previously (Stein & Weaver, 1988), and is expressed as μV. s $(10\ s)^{-1}$. Neural signals that had been recorded at the wide bandwidth of 1 Hz–3 kHz were filtered at a bandwidth of 100 Hz–2 kHz (differential amplifier, Frederick Haer, USA) before integration. Background electrical noise in the neural recordings was determined after ganglionic blockade with hexamethonium (5 mg kg^{-1}, i.v.; Sigma Chemical Company), or after crushing the nerves at the end of the experiment. This background noise was subtracted from the neural signal when the data were quantified. Changes from control in nerve activity, arterial pressure and heart rate were tested with blocked analysis of variance. Mean values were compared with a test of least significant differences. Differences were considered significant when the probability value was less than 5%, and variability was expressed as pooled standard error derived from the analysis of variance. The non-parametric Friedman test was used to compare magnitudes of mesenteric nerve responses to magnitudes of simultaneously recorded renal nerve responses (Sokol & Rohlf, 1969).

Power density spectra of the neural signals recorded at the wide bandwidth were constructed by an RC Electronics Computer-scope system. The signals were filtered with a low-pass frequency of 50 Hz and sampled at a rate of 1 kHz. This procedure prevented aliasing of the high-frequency components of the signal (Malmstadt, Enke & Crouch, 1981). The spectra of each nerve were normalized by expressing relative power density in arbitrary units proportional to watts, and total power in the 1–6 Hz frequency range was computed. Changes in total power in this frequency range were used as indices of changes in the slow rhythm in nerve firing. Comparisons between percentage changes in total power of paired renal and mesenteric nerve activities were made using the Friedman test. This test was also used to compare percentage changes in total power of the density spectra to percentage changes in integrated nerve activity (Sokal & Rohlf, 1969).

RESULTS

In seventeen of eighteen cats, nerve activity was tested for pressoreceptor sensitivity by evaluating responses to increases in arterial pressure, elicited by intravenous infusions of phenylephrine (5–20 μg kg^{-1}). All renal nerves and sixteen of seventeen mesenteric nerves tested had discharge inhibited by stimulation of pressoreceptors, and thus probably contained vasomotor fibres. An increase in mean arterial pressure of 43 ± 13 mmHg significantly reduced the discharge of renal and mesenteric nerves from 41 to 4 μV.s $(10\ s)^{-1}$ (pooled S.E.M. = 6·5) and from 26 to 13 μV.s $(10\ s)^{-1}$ (pooled S.E.M. = 1·9), respectively.

Bilateral application of glycine-soaked cotton pledgets to the ventral surface of the rostral medulla in fourteen of eighteen cats elicited decreases in nerve discharge,

blood pressure and heart rate which began within seconds, and reached minimum levels within 2–6 min. In the example shown in Fig. 1, firing of the renal nerve decreased more than that of the mesenteric nerve; this relationship was consistently observed in every cat tested. Mean discharge of renal and of mesenteric nerves decreased from 32 to 7 μV.s (10 s)$^{-1}$ (pooled S.E.M. = 3·5) and from 32 to 21 μV.s (10 s)$^{-1}$ (pooled S.E.M. = 1·7), respectively (Fig. 2). The decrease in firing of renal

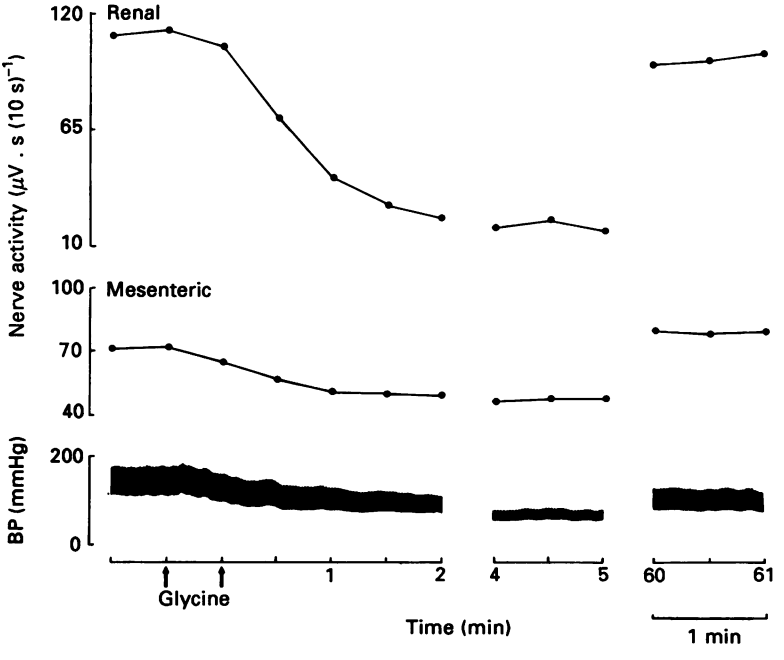

Fig. 1. Response of one cat to bilateral application of cotton pledgets soaked in glycine (200 mg ml^{-1}, arrows) to the ventral surface of the rostral medulla. Integrated activity (expressed as μV.s (10 s)$^{-1}$) of renal and mesenteric nerves is illustrated in the top two panels. Systemic arterial pressure (BP) is illustrated in the bottom panel. Time in minutes is indicated beneath these panels. The pledgets were removed after 5 min. Recovery values 60 min after application of glycine are shown on the far right.

nerves was significantly greater than that of mesenteric nerves. These neural responses were accompanied by significant reductions in mean arterial pressure, from 126 to 68 mmHg (pooled S.E.M. = 3·4, Fig. 2) and heart rate, from 250 to 222 beats min^{-1} (pooled S.E.M. = 3·2). The durations of these responses were variable. Discharge of mesenteric nerves returned to control within 19$\pm$5 min (mean$\pm$S.E.M.) after removal of the pledgets. However, renal nerve activity, arterial blood pressure and heart rate remained decreased for 33$\pm$5 min, 36$\pm$6 min and 32$\pm$6 min, respectively, after removal of the pledgets. As a control, cotton pledgets soaked with warm physiological saline were applied to the medulla in seven cats. This procedure did not affect nerve activity, blood pressure or heart rate.

To determine if decreases in nerve activity observed during RVLM blockade could be attributed to descending sympatho-inhibitory systems the application of glycine was repeated, and the spinal cord was severed at the first cervical segment at the

nadir of the decrease in nerve activity (3·5 ± 0·3 min after application of glycine). An increase in nerve discharge immediately after transection of the spinal cord would demonstrate the presence of tonically active sympatho-inhibitory systems. The magnitude of renal nerve discharge after transection of the spinal cord was not significantly different from that during blockade of the RVLM (Fig. 3), indicating that the reduction in renal nerve firing after application of glycine to the RVLM was not due to tonic sympatho-inhibition. In contrast, firing of mesenteric nerves

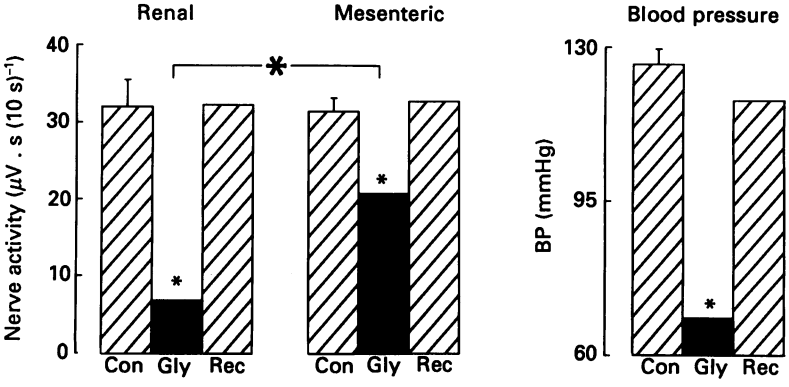

Fig. 2. Responses of sympathetic activity and mean arterial pressure to bilateral application of glycine to the ventral surface of the rostral medulla in fourteen cats. Bars represent averages of integrated discharge of renal and mesenteric nerves (expressed as μV.s (10 s)$^{-1}$) and mean arterial pressure (mmHg) during 1 min control periods (Con), during 1 min of maximum change from control after application of glycine (Gly) and during 1 min recovery periods (Rec). Variability is indicated by pooled standard error of means. Maximum changes from control occurred 4–6 min after application of glycine. Recovery values were recorded 1 h after application of glycine. Small asterisks indicate significant difference from control. The reduction in renal nerve activity was significantly greater than that of mesenteric nerve activity as indicated by the large asterisk.

increased after transection of the spinal cord in ten of fourteen cats. In two cats discharge of mesenteric nerves decreased, and in two others, discharge of mesenteric nerves did not change after spinal cord transection. The mean magnitudes of mesenteric nerve discharge ($n = 14$) recorded at 5, 30 and 60 min after transection were not significantly different from that recorded during the control period (Fig. 3), suggesting that sympatho-inhibitory systems may have contributed to the diminished mesenteric nerve firing during RVLM blockade.

After transection of the spinal cord, mean arterial pressure decreased and stabilized at 49 ± 5 mmHg, after which (3–5 min after transection) phenylephrine was infused to support blood pressure. This level of blood pressure after spinal cord transection was significantly less than that observed during RVLM blockade (68 ± 5 mmHg), suggesting that the application of glycine to the ventral medulla did not eliminate all descending sympatho-excitatory drive. However, in five cats, the blood pressures after severing the spinal cord (mean ± S.E.M. = 55 ± 11 mmHg) were within 5 mmHg of those seen during RVLM blockade (mean ± S.E.M. = 57 ± 10 mmHg). The neural responses among this subgroup of cats were similar to those of the entire population; discharge of renal and mesenteric nerves decreased by

77 ± 13 and 25 ± 8%, respectively, after application of glycine to the ventral surface of the medulla.

Responses to glycine application and subsequent spinal cord transection were tested in four of the eighteen cats after sino-aortic denervation and vagotomy. Before denervation, RVLM blockade caused decreases in the discharge of renal ($n = 3$) and mesenteric ($n = 3$) nerves, from 24 ± 8 to 10 ± 6 μV.s $(10\ s)^{-1}$ and from 19 ± 10 to 16 ± 9 μV.s $(10\ s)^{-1}$, respectively. After severing the buffer nerves this procedure

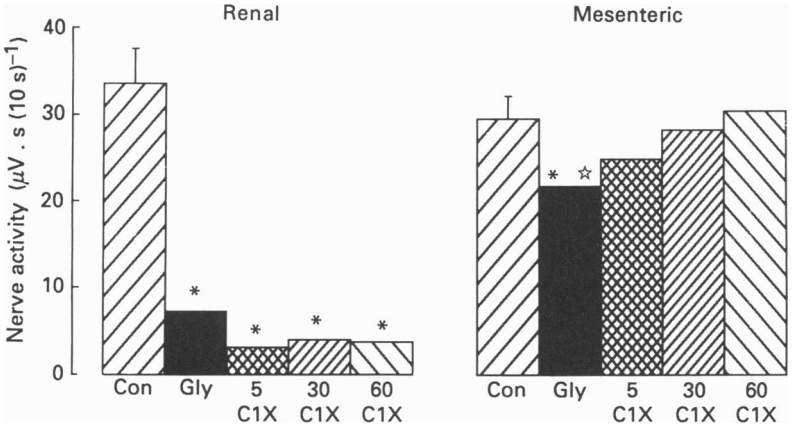

Fig. 3. On-going discharge of sympathetic nerves after bilateral application of glycine to the ventral surface of the rostral medulla and after high cervical spinal cord transection in fourteen cats. Bars represent mean integrated discharge of renal and mesenteric nerves during 1 min control periods (Con), during 1 min of maximum change from control after application of glycine (Gly), and 5, 30 and 60 min following spinal cord transection at the first cervical segment (C1X). The spinal cord was transected $3{\cdot}5 \pm 0{\cdot}3$ min after application of glycine. Variability is indicated by pooled standard error of means. Asterisks indicate significant difference from control. Star indicates significant difference from activity 60 min following C1X. Following C1X renal nerve discharge remained depressed but mesenteric nerve activity increased, returning to control levels.

produced similar results; renal nerve discharge decreased from 29 ± 11 to 10 ± 5 μV.s $(10\ s)^{-1}$ and firing of mesenteric nerves changed from 12 ± 6 to 8 ± 4 μV.s $(10\ s)^{-1}$. One hour following high cervical spinal transection, mean discharges of these three renal and three mesenteric nerves were 5 ± 3 and 11 ± 8 μV.s $(10\ s)^{-1}$, respectively. Thus, responses in sino-aortic denervated and vagomized cats were similar to those cats with intact buffer nerves.

In nine cats, discharge of renal and mesenteric nerves was recorded at a bandwidth of 1 Hz–3 kHz to reveal the slow frequency periodicities in nerve discharge. Neurograms of activity recorded at such a bandwidth are illustrated in traces *A* of Figs 4 and 5. The same intervals of nerve discharge, filtered at a bandwidth of 100 Hz–2 kHz, are illustrated in traces *B*, showing only the high-frequency components of the signal. Both renal and mesenteric nerves discharged in bursts (Figs 4 and 5, Control, traces *B*) which appeared as slow oscillations in discharge when activity was recorded at the wide bandwidth (traces *A*). Power spectral analyses revealed that the major frequencies of the oscillations in nerve discharge ranged from 1 to 6 Hz (Figs 4 and 5, Control, traces *C*). As reported by others (Barman & Gebber, 1980), the

power density spectra usually contained a peak at, or near, the heart rate. This peak was not a pulse artifact as it was no longer present after transection of the spinal cord. Moreover, this peak disappeared following denervation of the buffer nerves in four cats. In the density spectra illustrated in Figs 4 and 5, the cardiac-related peak is not prominent, probably due to the lack of strong baroreceptor influences in this

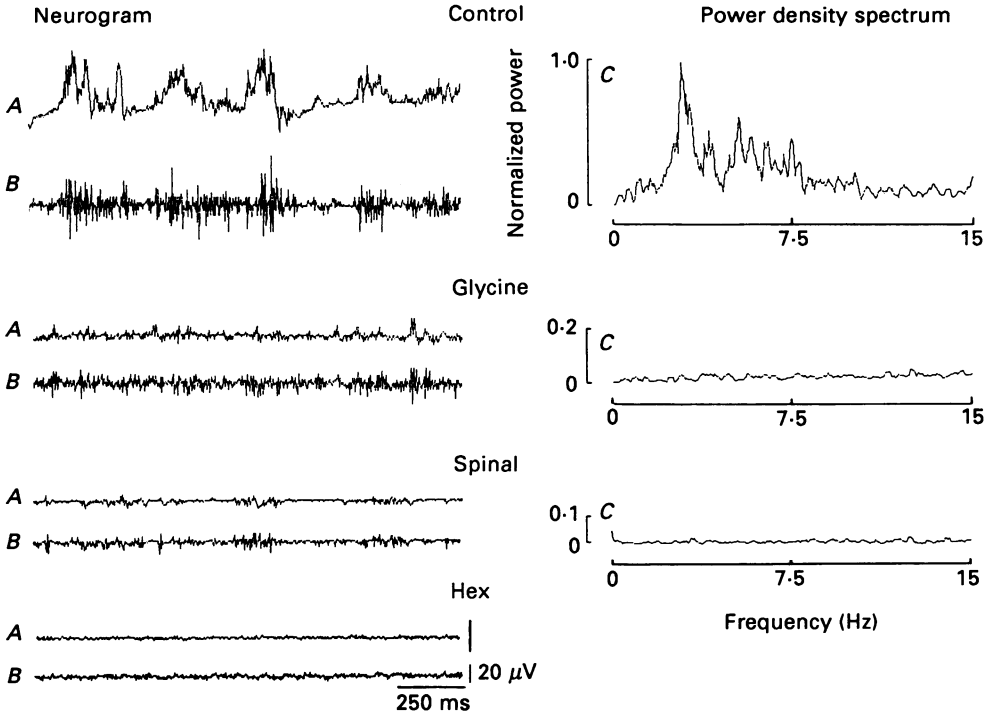

Fig. 4. Neurograms of on-going discharge and corresponding power density spectra of one renal nerve during a control period (Control), during the maximum change from control following application of glycine to the ventral surface of the rostral medulla (Glycine) and 1 h following high cervical spinal cord transection (Spinal). Trace *A* of each panel illustrates a 1·5 s neurogram of nerve activity amplified at a bandwidth of 1 Hz–3 kHz. The same interval of nerve discharge, filtered at a bandwidth of 100 Hz–2 kHz, is shown in trace *B* of each panel. Background noise level in the nerve recording after ganglionic blockade with hexamethonium (Hex) is shown at the bottom of the figure. Vertical and horizontal calibrations are indicated. Trace *C* of each panel illustrates power density spectra of the 1 Hz–3 kHz discharge. Density spectra are normalized so that relative power (proportional to watts) is plotted against frequency. Note differences in scale of the density spectra. Application of glycine and transection of the spinal cord reduced filtered nerve discharge to 18 and 15% of control, respectively (traces *B*), and reduced total power of the spectra to 1 and 0% of control, respectively (traces *C*).

animal. In this cat, a phenylephrine-induced increase in mean arterial pressure from 128 to 187 mmHg inhibited the firing of renal and mesenteric nerves by only 43 and 32%, respectively. Occasionally, slower periodicities in the density spectra, at, or near, the respiratory rate were observed, but, as it was not clear if these periodicities were artifacts caused by movement associated with ventilation, they were not analysed further. Faster periodicities of 10 Hz which have been described

by other investigators (Gootman & Cohen, 1981) were never observed in the present study.

Application of glycine to the RVLM consistently elicited decreases in the slow rhythm in nerve firing (Figs 4 and 5, Glycine, traces *A*). Changes in total power of the density spectra in the 1–6 Hz frequency range were used as quantitative indices

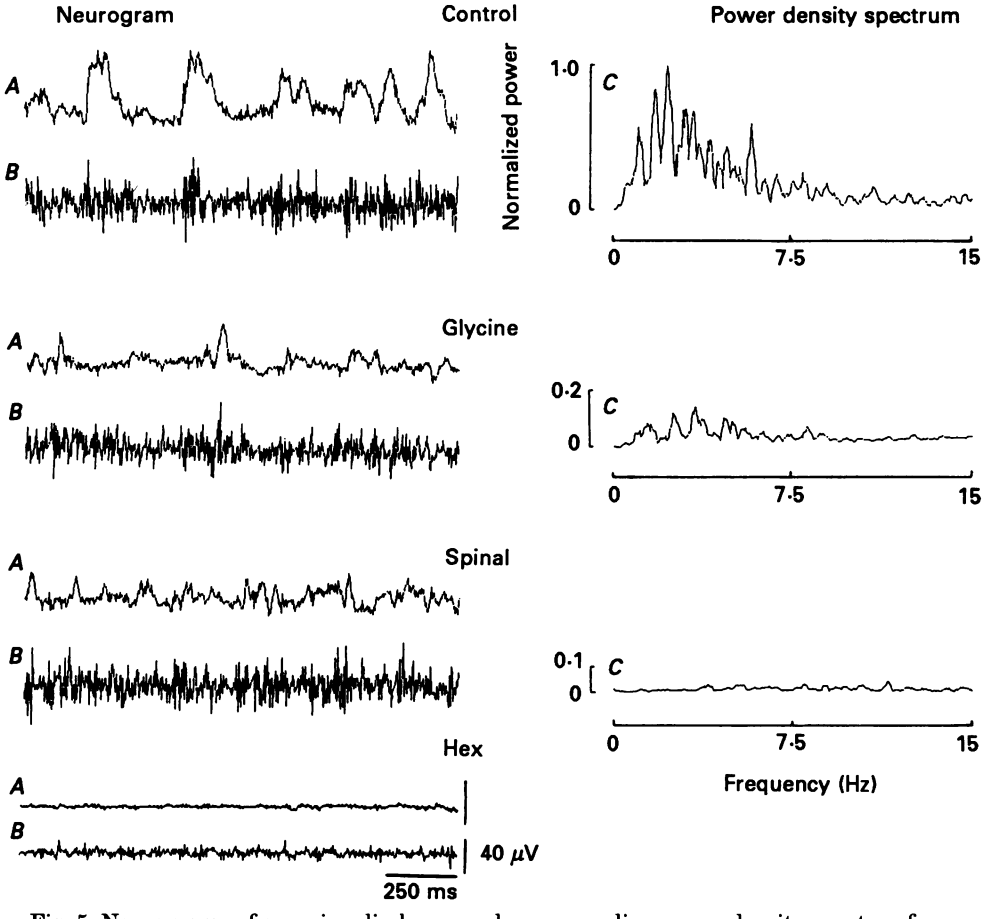

Fig. 5. Neurograms of on-going discharge and corresponding power density spectra of one mesenteric nerve during a control period (Control), during the maximum change from control following application of glycine to the ventral surface of the rostral medulla (Glycine) and 1 h following high cervical spinal cord transection (Spinal). Activity was recorded in the same cat as the renal nerve discharge shown in Fig. 4. Format as in Fig. 4. Application of glycine and transection of the spinal cord reduced filtered nerve discharge to 73 and 95% of control, respectively (traces *B*), and reduced total power of the density spectra to 24 and 2% of control, respectively (traces *C*).

of changes in the slow rhythm in nerve firing. Changes in overall nerve discharge were assessed by quantifying changes in integrated discharge of the filtered (100 Hz–2 kHz) neural signals. In the density spectrum shown in Fig. 4 (Glycine, trace *C*), total power of the renal nerve discharge was reduced to 1% of control. Integration of the filtered neural signal revealed that activity of this renal nerve was reduced to

18% of control during the RVLM blockade (Fig. 4, Glycine, trace *B*). Similarly, total power of the density spectrum of mesenteric nerve discharge shown in Fig. 5 (Glycine, trace *C*) was reduced to 24% of control, whereas integrated filtered nerve discharge was only decreased to 73% of control following application of glycine to the medulla (Glycine, trace *B*). Non-parametric statistics indicated that the

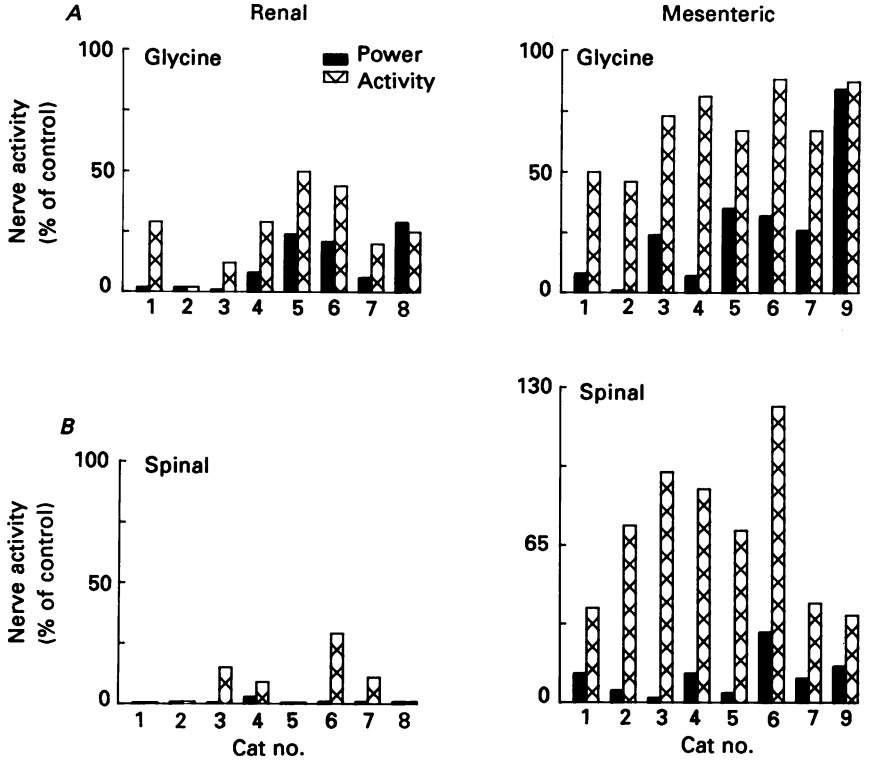

Fig. 6. Effects of application of glycine to the ventral surface of the rostral medulla and of high cervical spinal transection on on-going discharge of renal and mesenteric nerves in nine cats. Measurements were taken 3–4 min after application of glycine and 1 h following spinal cord transection. Activity of renal and mesenteric nerves was recorded simultaneously in cats 1–7. In cat 8 only renal, and in cat 9 only mesenteric, nerve activity was recorded. Bars represent total power of the density spectra (filled bars) and integrated nerve activity (cross-hatched bars) expressed as percentage of control (prior to application of glycine). Non-parametric statistics indicated the following: after glycine application, Δ renal power > Δ renal activity, Δ mesenteric power > Δ mesenteric activity, Δ renal activity > Δ mesenteric activity, Δ renal power = Δ mesenteric power; after spinal transection, Δ renal activity = Δ renal power, Δ mesenteric power > Δ mesenteric activity, Δ renal activity > Δ mesenteric activity, Δ renal power > Δ mesenteric power.

percentage decreases in total power of the renal and mesenteric nerve density spectra (i.e. slow rhythm in nerve firing) were significantly greater than the percentage decreases in integrated nerve discharge (Fig. 6*A*). Although integrated discharge of renal nerves was diminished more than that of mesenteric nerves, the reductions in total power of the renal and mesenteric nerve spectra were not significantly different from each other (non-parametric Friedman test).

As the inhibition of activity of neurones in the RVLM caused pronounced decreases in arterial blood pressure, the reductions in the slow rhythm in nerve firing may have been secondary to the unloading of pressoreceptors. This possibility was assessed in four cats by evaluating neural responses to RVLM blockade while infusing phenylephrine to maintain arterial blood pressure within 10 mmHg of control levels, and also, after sino-aortic denervation and vagotomy (Table 1). Changes in total power seen during responses to glycine, in which arterial pressure

TABLE 1. Changes in total power of the density spectra (slow rhythm in nerve firing) and in mean arterial pressure following topical application of glycine to the RVLM bilaterally

		BP decreased		BP supported		Denervated	
Nerve	Cat no.	% Δ Power	Δ MAP	% Δ Power	Δ MAP	% Δ Power	Δ MAP
Renal	1	−79	−33	−79	−5	−84	−55
	2	−94	−55	−92	−2	−92	−18
	3	−71	−12	−89	−1	−82	−74
Mesenteric	1	−68	−33	−73	−5	−46	−55
	2	−74	−55	−73	−2	−87	−18
	4	−16	−12	−16	−7	−69	−78

% Δ Power, percentage change from control in total power of the density spectra in the 1–6 Hz frequency range; Δ MAP, change in mean arterial pressure (mmHg); BP decreased, arterial pressure was allowed to decrease following application of glycine; BP supported, arterial pressure was supported by intravenous infusion of phenylephrine (2–10 $\mu g\ kg^{-1}\ min^{-1}$); denervated, 60 min after sino-aortic denervation and vagotomy.

was allowed to decrease, were not different from those seen during the support of arterial blood pressure. Moreover, reductions in total power seen after denervation of systemic pressoreceptors were similar to, or greater than, those seen in cats with intact buffer nerves. Therefore, decreases in pressoreceptor inputs during the responses to glycine probably did not significantly contribute to the loss of rhythmicity in sympathetic nerve discharge.

Following transection of the spinal cord at the first cervical segment the slow rhythm in firing of most nerves was virtually abolished. In the density spectrum shown in Fig. 4, total power of renal discharge in the 1–6 Hz frequency range was abolished (Spinal, trace *C*). Integrated discharge of the filtered renal nerve signal was reduced to 15% of control after the spinal cord was severed (Spinal, trace *B*). Similarly, total power of the spectrum of mesenteric nerve activity illustrated in Fig. 5 was reduced to 2% of control after spinal cord transection (Spinal, trace *C*). However, integrated discharge of the mesenteric nerve was maintained at 95% of control (Fig. 5, Spinal, trace *B*). Nearly all mesenteric nerve discharge which persists after severing the spinal cord ceases after subdural injections of lidocaine in the thoracic and lumbar regions of the spinal cord (R. D. Stein & L. C. Weaver; unpublished), indicating that the majority of this activity is not generated peripherally (Szurszewski, 1981), rather, it is transmitted to mesenteric nerves via preganglionic nerves. Non-parametric statistics indicated that the percentage decreases in the slow rhythm (i.e. power) in renal nerve discharge were not significantly different from the percentage decreases in integrated renal nerve

activity (Fig. 6*B*). In contrast, although the slow rhythm in firing of mesenteric nerves was significantly reduced, mean integrated activity of the filtered mesenteric nerve signal was not significantly affected by spinal cord transection (Fig. 6*B*).

DISCUSSION

The sympathetic nervous system is capable of producing distinct patterns of nerve discharge to control selectively the functions of various effector organs. In contrast to Cannon's (1930) concept of a system which is characterized by 'general diffuse action', it is now widely recognized that different elements within the sympathetic nervous system can display different patterns of discharge, and that descending projections may selectively influence certain sympathetic outflows (Barman, Gebber & Calaresu, 1984; Jänig, 1985; Meckler & Weaver, 1985, 1988; Dampney & McAllen, 1988; Stein & Weaver, 1988).

The results of the present investigation support the contention that tonic excitatory drive from the RVLM is not essential for the maintenance of on-going discharge of all components of sympathetic outflow. Whereas firing of renal nerves appears to be contingent upon such drive, activity of mesenteric nerves can be generated within spinal cord systems. The RVLM has been shown to be topographically organized (Lovick, 1987). The neuronal pool which affects sympathetic outflow to the kidney appears to be located superficially, in the anterior end of the RVLM, whereas those neurones affecting mesenteric vasoconstrictor activity appear to be found superficially in the posterior portion of the region (Lovick, 1987). As the goal of this study was to abolish all excitatory drive from this region, glycine was topically applied using cotton pledgets large enough to cover, yet small enough to be restricted to, the region of the RVLM (1–2 mm diameter). In five cats, in which this procedure apparently eliminated all descending excitatory drive to sympathetic vasomotor neurones, the decrease in discharge of renal nerves was significantly greater than that of mesenteric nerves. Therefore, it is unlikely that differences in renal and mesenteric responses observed after the application of glycine resulted from unequal distribution of glycine to renal and mesenteric neuronal pools within the RVLM.

The unequal effects of RVLM blockade on the discharge of renal and mesenteric sympathetic outflows may reflect differences in the passive electrophysiological properties of their preganglionic neurones. Sympathetic preganglionic neurones have been classified based on differences in their electrophysiological properties, but it is not known whether such differences relate to functional specificity (Dembowsky, Czachurski & Seller, 1986). If sympathetic preganglionic neurones presynaptic to mesenteric neurones have lower thresholds for activation, or lower resting membrane potentials than do those impinging on renal neurones, fewer synaptic inputs would be required to activate mesenteric neurones, and thus their discharge would be more likely to withstand the removal of an excitatory input than would that of renal neurones. In addition, it is conceivable that preganglionic neurones presynaptic to mesenteric neurones may not receive as many inputs from the RVLM as those innervating renal neurones. A recent anatomical study has shown that projections from the RVLM are not uniformly distributed throughout the rosto-caudal extent of the thoraco-lumbar spinal cord (Caverson & Ciriello, 1987).

Although inhibition of tonic activity of neurones within the RVLM elicited greater decreases in the discharge of renal than mesenteric nerves, mesenteric nerve discharge was significantly reduced by 30%. Two other studies have also provided evidence that discharge of mesenteric nerves or preganglionic splanchnic nerves is strongly influenced by RVLM inputs (Hilton *et al.* 1983; Dean & Coote, 1986). These results contrast with our previous findings that firing of mesenteric nerves remains unabated following high cervical spinal cord transection (Stein & Weaver, 1988). This discrepancy may be explained if spinal systems are capable of driving mesenteric, but not renal, nerve discharge when all supraspinal input is removed, and if mesenteric and renal spinal neurones receive inhibitory, as well as excitatory bulbospinal inputs when the neuraxis is intact. Such tonic sympatho-inhibition, independent of baroreceptor inputs, has been demonstrated (Barman & Gebber, 1978; Dembowsky, Czachurski, Amendt & Seller, 1980; McCall & Harris, 1987). Selective removal of the excitatory input from the RVLM would unmask the sympatho-inhibitory influence, resulting in a decrease in nerve firing. This hypothesis was tested by transecting the spinal cord at the first cervical level during the RVLM blockade.

Activity of mesenteric nerves did increase following spinal cord transection in ten of fourteen cats, suggesting that the diminished mesenteric nerve activity during application of glycine to the ventral medulla may have been caused, in part, by descending sympatho-inhibitory inputs. Alternatively, following removal of supraspinal influences latent excitatory synaptic inputs may be expressed (Dostrovsky, Millar & Wall, 1976), and the recovery of mesenteric nerve firing after spinal cord transection may reflect the plasticity of spinal structures controlling a portion of the mesenteric nerve outflow (Taylor & Schramm, 1987). However, such latent synaptic influences should also have been expressed following removal of excitatory drive from the RVLM. As this was not the case, the hypothesis is tenable that the recovery of mesenteric nerve discharge following spinal cord transection may have resulted from a decline of sympatho-inhibitory effects.

The slow oscillations in sympathetic nerve discharge which were evident when activity was amplified at a wide bandwidth have been analysed in detail by Barman & Gebber (1980) and Gebber & Barman (1980). Topical application of glycine to the medulla consistently caused greater percentage decreases in the slow rhythm in nerve firing (i.e. total power of the density spectra in the 1–6 Hz frequency range) than in integrated nerve activity (Figs 4, 5 and 6). The reductions in the slow rhythm in firing of renal and mesenteric nerves were similar. Transection of the spinal cord resulted in greater reductions in the slow rhythm in discharge of both nerves. Therefore, although renal and mesenteric nerves differ in their dependence on supraspinal inputs for the maintenance of on-going discharge, such inputs are necessary to organize firing of both groups of nerves into bursts of action potentials. In the absence of this descending input firing of both renal and mesenteric nerves is random.

At least two hypotheses may account for the loss of rhymicity in the discharge of mesenteric nerves following removal of descending inputs. First, two subgroups of mesenteric neurones may exist. One subgroup may depend upon drive from the RVLM for the maintenance of on-going activity and discharge with a 1–6 Hz periodicity. A second subgroup may have random discharge which is generated

within the spinal cord. After removal of descending influences, only the second group of neurones which have random discharge patterns would remain active. Alternatively, the majority of mesenteric nerve activity may be generated within the spinal cord, and descending inputs from the RVLM may co-ordinate this discharge into slow waves. In the absence of the descending inputs, nerve activity would become random. This second possibility is likely, as single mesenteric fibres which fire with cardiac rhymicity when the neuraxis is intact continue to discharge without such rhymicity after transection of the spinal cord (Stein & Weaver, 1988).

The functional significance of the mesenteric nerve activity that persists after inhibition of activity of neurones within the RVLM, or after transection of the spinal cord, remains to be determined. Although McAllen (1986) reports that projections from the RVLM appear to impinge selectively on vasomotor neurones, this does not imply that every vasomotor neurone depends upon excitatory drive from the RVLM for the maintenance of on-going discharge. Indeed, the decreases in mesenteric and renal nerve activity caused by application of glycine to the medulla (34 and 78%, respectively) were rarely as great in magnitude as those elicited by pressoreceptor stimulation (50 and 90%, respectively), suggesting that some vasomotor neurones remained active after RVLM blockade. Moreover, we have shown that renal fibres and some mesenteric and splenic fibres which continue to fire after severing the spinal cord had activity which was sensitive to pressoreceptor influences when the neuraxis was intact (Meckler & Weaver, 1988; Stein & Weaver, 1988); supposedly these neurones subserve vasomotor functions (Jänig, 1985). Still, following blockade of the RVLM mesenteric vascular conductance is significantly increased (Hilton *et al.* 1983; Willette *et al.* 1987) and arterial pressure falls to spinal levels (the present study; Guertzenstein & Silver, 1974; Hilton *et al.* 1983; Ross *et al.* 1984). Failure of sustained mesenteric nerve activity to support vascular tone could occur because nerve impulses which are delivered randomly produce less vascular tone than do phasically delivered impulses (Nilsson, Ljung, Sjöblom & Wallin, 1985), and RVLM blockade and spinal cord transection did cause mesenteric nerve activity to become irregular. However, even if the sustained nerve activity were sufficient to cause mesenteric vasoconstriction, such vasoconstriction may not be capable of supporting arterial pressure, as spinal transection causes profound decreases in sympathetic outflow to other vascular beds (Meckler & Weaver, 1985; Taylor & Schramm, 1987) and significant decreases in cardiac output (Fitzsimons & Weaver, 1988).

In conclusion, excitatory drive from the RVLM is not essential for the maintenance of on-going activity of *all* sympathetic nerves. Whereas discharge of renal nerves is strongly dependant upon such drive, discharge of mesenteric nerves can be generated within the spinal cord. However, the RVLM does equally influence the frequency characteristics of the discharge of both nerves. Normally, mass activity of both renal and mesenteric nerves is organized into bursts of action potentials with periodicities of 1–6 Hz. In the absence of input from the RVLM nerve discharge becomes random. The functional significance of the persistent mesenteric nerve firing seen after blockade of the RVLM or transection of the spinal cord awaits further investigation.

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REFERENCES

ALEXANDER, R. S. (1946). Tonic and reflex functions of medullary sympathetic cardiovascular centers. *Journal of Neurophysiology* **9**, 205–217.

AMENDT, K., CZACHURSKI, J., DEMBOWSKY, K. & SELLER, H. (1979). Bulbospinal projections to the intermediolateral cell column; a neuroanatomical study. *Journal of the Autonomic Nervous System* **1**, 103–117.

ARDELL, J. L., BARMAN, S. M. & GEBBER, G. L. (1982). Sympathetic nerve discharge in chronic spinal cat. *American Journal of Physiology* **243**, H463–470.

BARMAN, S. M. & GEBBER, G. L. (1978). Tonic sympathoinhibition in the baroreceptor denervated cat. *Proceedings of the Society for Experimental Biology and Medicine* **157**, 648–655.

BARMAN, S. M. & GEBBER, G. L. (1980). Sympathetic nerve rhythm of brain stem origin. *American Journal of Physiology* **239**, R42–47.

BARMAN, S. M. & GEBBER, G. L. (1983). Sequence of activation of ventrolateral and dorsal medullary sympathetic neurons. *American Journal of Physiology* **245**, R438–447.

BARMAN, S. M. & GEBBER, G. L. (1985). Axonal projection patterns of ventrolateral medullospinal sympathoexcitatory neurons. *Journal of Neurophysiology* **53**, 1551–1566.

BARMAN, S. M., GEBBER, G. L. & CALARESU, F. R. (1984). Differential control of sympathetic nerve discharge by the brain stem. *American Journal of Physiology* **247**, R513–519.

CANNON, W. B. (1930). The autonomic nervous system: An interpretation. *Lancet* **i**, 1109–1115.

CAVERSON, M. M. & CIRIELLO, J. (1987). Ventrolateral medullospinal neurons involved in the control of the circulation. In *Organization of the Autonomic Nervous System: Central and Peripheral Mechanisms*, ed. CIRIELLO, J., CALARESU, F. R., RENAUD, L. & POLOSA, C., pp. 227–237. New York: Alan Liss.

DAMPNEY, R. A. L. & MCALLEN, R. M. (1988). Differential control of sympathetic fibres supplying hindlimb skin and muscle by subretrofacial neurones in the cat. *Journal of Physiology* **395**, 41–56.

DEAN, C. & COOTE, J. H. (1986). A ventromedullary relay involved in the hypothalamic and chemoreceptor activation of sympathetic postganglionic neurones to skeletal muscle, kidney and splanchnic area. *Brain Research* **377**, 279–285.

DEMBOWSKY, K., CZACHURSKI, J., AMENDT, K. & SELLER, H. (1980). Tonic descending inhibition of the spinal somato-sympathetic reflex from the lower brain stem. *Journal of the Autonomic Nervous System* **2**, 157–182.

DEMBOWSKY, K., CZACHURSKI, J. & SELLER, H. (1986). Three types of sympathetic preganglionic neurones with different electrophysiological properties are identified by intracellular recordings in the cat. *Pflügers Archiv* **406**, 112–120.

DOSTROVSKY, J. O., MILLAR, J. & WALL, P. D. (1976). The immediate shift of afferent drive of dorsal column nucleus cells following deafferentation: A comparison of acute and chronic deafferentation in gracile nucleus and spinal cord. *Experimental Neurology* **52**, 480–495.

FITZSIMONS, C. L. & WEAVER, L. C. (1988). The relative contributions of decreased cardiac output and decreased peripheral resistance to hypotension in spinal cats. *Canadian Journal of Physiology and Pharmacology* abstract 66, (5) axiii.

GEBBER, G. L. & BARMAN, S. M. (1980). Basis for 2–6 cycle/s rhythm in sympathetic nerve discharge. *American Journal of Physiology* **239**, R48–56.

GOOTMAN, P. M. & COHEN, M. I. (1981). Sympathetic rhythms in spinal cats. *Journal of the Autonomic Nervous System* **3**, 379–387.

GUERTZENSTEIN, P. G. (1973). Blood pressure effects obtained by drugs applied to the ventral surface of the brain stem. *Journal of Physiology* **229**, 395–408.

GUERTZENSTEIN, P. G. & SILVER, A. (1974). Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. *Journal of Physiology* **242**, 489–503.

HILTON, S. M., MARSHALL, J. M. & TIMMS, R. J. (1983). Ventral medullary relay neurones in the pathway from the defence areas of the cat and their effect on blood pressure. *Journal of Physiology* **345**, 149–166.

JÄNIG, W. (1985). Organization of the lumbar sympathetic outflow to skeletal muscle and skin of the cat hindlimb and tail. *Reviews of Physiology, Biochemistry and Pharmacology* **102**, 119–213.

LOVICK, T. A. (1987). Differential control of cardiac and vasomotor activity by neurones in nucleus paragigantocellularis in the cat. *Journal of Physiology* **389**, 23–35.

MCALLEN, R. M. (1986). Action and specificity of ventral medullary vasopressor neurones in the cat. *Neuroscience* **18**, 51–59.

MCCALL, R. B. & HARRIS, L. T. (1987). Sympathetic alterations after midline medullary raphe lesions. *American Journal of Physiology* **253**, R91–100.

MALMSTADT, H. V., ENKE, C. G. & CROUCH, S. R. (1981). *Electronics and Instrumentation for Scientists*. Reading, MA, USA: Benjamin/Cummings.

MECKLER, R. L. & WEAVER, L. C. (1985). Splenic, renal, and cardiac nerves have unequal dependence upon tonic supraspinal inputs. *Brain Research* **338**, 123–135.

MECKLER, R. L. & WEAVER, L. C. (1988). Characteristics of ongoing and reflex discharge of single splenic and renal sympathetic postganglionic fibres in cats. *Journal of Physiology* **396**, 139–153.

NILSSON, H., LJUNG, B., SJÖBLOM, N. & WALLIN, B. G. (1985). The influence of the sympathetic impulse pattern on contractile responses of rat mesenteric arteries and veins. *Acta physiologica scandinavica* **123**, 303–309.

PILOWSKY, P., WEST, M. & CHALMERS, J. (1985). Renal sympathetic nerve responses to stimulation, inhibition and destruction of the ventrolateral medulla in the rabbit. *Neuroscience Letters* **60**, 51–55.

ROSS, C. A., RUGGIERO, D. A, PARK, D. H., JOH, T. H., SVED, A. F., FERNANDEZ-PARDAL, J., SAAVEDRA, J. M. & REIS, D. J. (1984). Tonic vasomotor control by the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of the area containing C1 adrenaline neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. *Journal of Neuroscience* **4**, 474–494.

SOKAL, R. & ROHLF, R. J. (1969). *Biometry. The Principles and Practice of Statistics in Biological Research*. San Francisco: Freeman.

STEIN, R. D. & WEAVER, L. C. (1988). Multi- and single-fibre mesenteric and renal sympathetic responses to chemical stimulation of intestinal receptors in cats. *Journal of Physiology* **396**, 155–172.

STEIN, R. D., YARDLEY, C. P., FITZSIMONS, C. L. & WEAVER, L. C. (1987). Topical application of glycine to the rostral ventrolateral medulla causes unequal decreases in activity of renal and mesenteric sympathetic nerves. *Society for Neuroscience Abstracts* **13**, 810.

SZURSZEWSKI, J. H. (1981). Physiology of mammalian prevertebral ganglia. *Annual Review of Physiology* **43**, 53–68.

TAYLOR, R. F. & SCHRAMM, L. P. (1987). Differential effects of spinal transection on sympathetic nerve activities in rats. *American Journal of Physiology* **253**, R611–618.

WILLETTE, R. N., PUNNEN-GRANDY, S., KRIEGER, A. J. & SAPRU, H. N. (1987). Differential regulation of regional vascular resistance by the rostral and caudal ventrolateral medulla in the rat. *Journal of the Autonomic Nervous System* **18**, 143–151.

YARDLEY, C. P., STEIN, R. D. & WEAVER, L. C. (1988). Inhibition of tonic activity in the rostral ventrolateral medulla causes non-uniform decrease in renal and mesenteric nerve activity. *American Journal of Physiology* (in the Press).