Sympathetic activity recorded from the rat caudal ventral artery *in vivo*

Christopher D. Johnson and Michael P. Gilbey

Department of Physiology, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF

1. In twenty-five sodium pentobarbitone (α-chloralose supplemented)-anaesthetized, artificially ventilated and paralysed rats, postganglionic sympathetic single unit activity was recorded at the level of the adventitia of the caudal ventral artery of the tail using a focal recording technique.

2. Ten units were identified as being sympathetic in nature, as they were activated following electrical stimulation of the lumbar sympathetic chain. The on-going activity of seven of these was blocked by hexamethonium (6–12 mg kg^{-1}).

3. The units were not under tonic baroreceptor modulation, as indicated by the lack of pulse modulation of discharge. Respiratory modulation was apparent, with neurones firing mainly during expiration (phrenic silence), and activity was influenced also by the lung inflation cycle. Whole-body warming decreased unit activity.

4. Interspike interval and autocorrelation analysis showed that unit discharge was dominated by the respiratory rhythm and that units tended to discharge in bursts (often duplets). It is suggested that the intraburst interval may be determined by a hypothetical sympathetic oscillator.

5. This study presents the first analysis of single unit activity recorded *in vivo* from sympathetic fibres innervating an identified blood vessel.

Complex patterns of sympathetic nerve activity are generated by the central nervous system in response to reflex inputs or as components of complex behavioural activity, e.g. those produced by stimulation of the upper airways with smoke (Peterson, Coote, Gilbey & Futuro-Neto, 1983), desynchronized sleep-like periods in the decerebrate cat (Futuro-Neto & Coote, 1982), somatic stimuli (Jänig, 1985) and central respiratory drive (Zhou & Gilbey, 1992). The techniques used in these studies have failed to identify the precise target(s) to which the various activities are directed. For example, even if activity is correctly identified as being skin vasoconstrictor (see Jänig, 1985), the vessel innervated has not been identified. This has been a substantial hinderance to understanding further the physiological organization of the sympathetic nervous system.

In this report, data are presented that represent the first analysis of single unit sympathetic activity supplying an identified blood vessel. This has been achieved by the *in vivo* application of an adaptation of the technique of focal extracellular recording used by Cunnane and co-workers *in vitro* (see Brock & Cunnane, 1987; Åstrand, Brock & Cunnane, 1988; Evans, 1990; Cunnane & Moss, 1993). In this way, the nature of unit activity directed specifically at the caudal ventral artery of the rat tail has been examined. The importance of tonic baroreceptor activity and respiratory-related activities in determining the patterning and frequency of activity in the sympathetic supply to this vessel has been examined. In addition, the effect of raising whole-body temperature on unit activity has been investigated because of the involvement of the rat tail circulation in thermoregulation (for references see O'Leary, Johnson & Taylor, 1985). Some results obtained using more conventional recording techniques are also reported, as they provide information relevant to the interpretation of data obtained with the focal recording technique.

Preliminary accounts of this work have been published as abstracts (Johnson & Gilbey, 1993*a*, *b*, *c*).

METHODS

Experiments were carried out on twenty-five male Sprague–Dawley rats (200–350 g) anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) and supplemented with α-chloralose (5–10 mg) when required, as judged from recordings of heart rate, blood pressure, phrenic nerve activity, size of pupils, and palpebral and paw-pinch reflexes. The muscle relaxant gallamine triethiodide (16 mg kg^{-1}) was administered during data collection. Within this period animals were allowed to recover from neuromuscular block and the depth of anaesthesia checked.

A carotid artery and a jugular vein were cannulated to monitor arterial pressure and administer drugs, respectively. Artificial ventilation (rate, 90–120 min^{-1}) was performed using

O_2-enriched room air. Tracheal pressure (5–10 mmHg) and end-tidal CO_2 were monitored continuously. Arterial blood samples were taken periodically and arterial pH and gas tensions kept within the following ranges: pH, 7·3–7·45; arterial P_{CO_2}, 35–48 mmHg; arterial $P_{O_2} > 100$ mmHg. Animals were given a pneumothorax and an end-expiratory pressure of 2–3 cmH_2O was applied to the expiratory line to prevent atelectasis. Following a ventral laparotomy, a thermocouple was placed over the abdominal aorta to monitor and regulate core temperature (37·5 ± 0·5 °C in the control state and up to 40 °C during whole-body warming) by a heating blanket wrapped around the animal.

Preparation of nerves

Following the laparotomy, the lumbar sympathetic chains were exposed and a silver wire bipolar electrode wrapped around them between the third and fifth lumbar ganglia. Both sympathetic chains and electrodes were embedded in insulating material (Provil, Bayer Dental, Germany) and the laparotomy repaired. These electrodes were used to stimulate the chain and thereby evoke activity in sympathetic fibres projecting into the tail.

Phrenic nerve activity was recorded so that phrenic-related sympathetic activity could be assessed. A ventral incision was made above the left clavicle, which was removed, and the left phrenic nerve dissected free. The distal end of the nerve was crushed and a silver wire bipolar electrode wrapped around it and the whole embedded in insulating material as described above.

The tail was positioned in a Perspex bath for dissection. The fluid in the bath was at room temperature (20–23 °C). In one set of experiments, a ventral collector nerve was exposed, cut distally, desheathed and covered in paraffin. Activity was either recorded from the whole nerve (6 rats) or from dissected fibres in order to record single or multi-unit activity (9 rats). Conventional bipolar platinum wire electrodes were used to record from the nerve; one pole of the electrode was placed on the cut central end and the other on the crushed distal end; a ground electrode was placed in close proximity. In another set of experiments (10 rats), where sympathetic activity was recorded from the caudal ventral artery using a focal extracellular recording technique, the artery was exposed and the superficial connective tissue removed, but the adventitia left intact. The Perspex bath was filled with a standard Krebs solution (Åstrand *et al.* 1988). Krebs solution-filled glass electrodes (tip diameter < 80 μM) pulled from capillary tubing were placed on the vessel. To produce a 'seal' between the tip of the electrode and the blood vessel, gentle suction was applied to the electrode via the side-arm of the electrode holder. A ground electrode was placed in close proximity.

Drugs

Hexamethonium bromide (Sigma, UK; 6–12 mg kg^{-1} in saline) was administered I.V. and α,β-methylene adenosine 5′-triphosphate (Sigma; 10^{-5} M) was added to the stock Krebs solution.

Data collection and analysis

All neuronal discharges were recorded through high impedance headstages (NL 100, Neurolog, Digitimer Ltd, UK), amplified and filtered. Nerve activities were monitored on an oscilloscope and VDU linked to an IBM computer. Single unit sympathetic activity was discriminated using a spike processor (D130, Digitimer). Nerve discharges, ECG, arterial blood

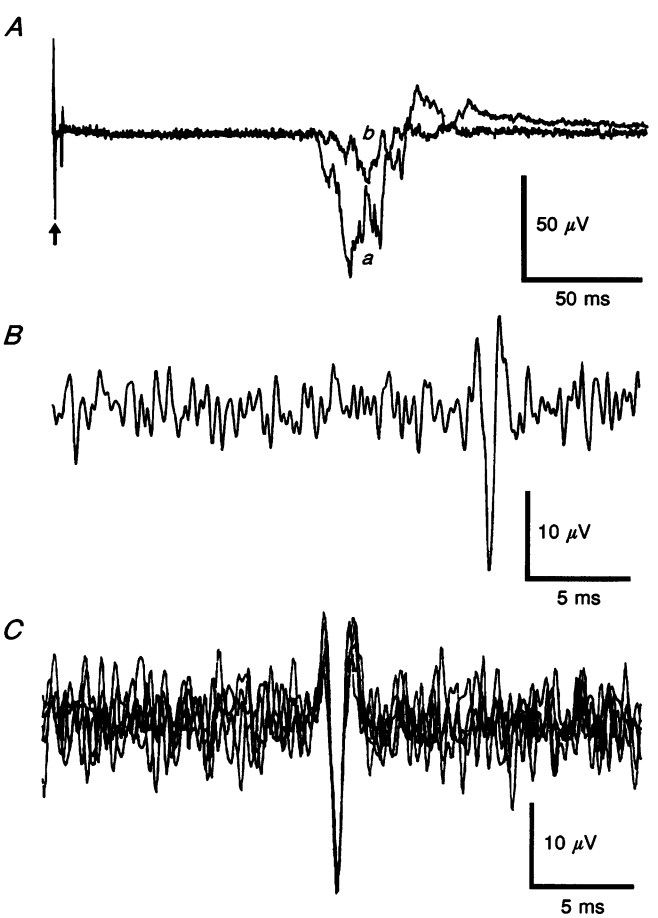

Figure 1.
A a, activity evoked in response to sympathetic chain stimulation (indicated by arrow; 100 trials, 1 Hz, 1 ms pulse, supramaximal stimulus) recorded from the central end of a cut ventral collector nerve. *A b*, response was reduced by hexamethonium (6 mg kg^{-1}, I.V.). *B*, activity evoked (1 trial; sweep delay, 300 ms from stimulus) in response to sympathetic chain stimulation (stimulus parameters as above) recorded from caudal ventral artery using focal recording technique. *C*, the unit shown in *B* was discriminated so that its on-going activity could be analysed. Five superimposed sweeps are shown. Each sweep was triggered by a transistor-to-transistor logic (TTL)-pulse generated from the discriminated unit.

pressure, tracheal pressure and a record of abdominal temperature were stored on tape. On- and off-line analyses were carried out using an interface (1401) and software (Spike 2) supplied by Cambridge Electronic Design, Cambridge, UK. Phrenic-, ECG-, arterial pulse pressure- and tracheal pressure-triggered histograms were generated and degree of modulation graded as previously described (Gilbey & Stein, 1991). Data are presented as means ± S.E.M. Statistical significance was assessed using one-way ANOVA. All n values refer to either the number of ventral collector nerves from which evoked potentials were recorded, the number of units recorded from teased fibres or the number of focally recorded units.

RESULTS

Stimulation of the lumbar sympathetic chains (1 Hz, 1 ms pulse, supramaximal stimulus voltage) elicited responses (bandwidth, 5–1000 Hz) in a whole ventral collector nerve, which when averaged was seen as an evoked potential. That this potential represented activity in sympathetic efferents was confirmed by the observation that it was abolished or greatly reduced (range, 68–100 %; median, 80 %; $n = 6$) by I.V. injection of the nicotinic ganglion blocker hexamethonium (6–12 mg kg^{-1} I.V.; Fig. 1*A*). The mid-range latency of each averaged response (100–230 ms) was used to calculate an estimated 'conduction velocity'; for six such responses (each from a different animal), these were 0·46 ± 0·03 m s^{-1}.

As on-going sympathetic activity cannot be recorded from a whole ventral collector nerve, fibres were teased from the nerve. The latencies of activity evoked, following stimulation of the sympathetic chain, in these fibres produced estimated 'conduction velocities' of 0·45 ± 0·04 m s^{-1} ($n = 9$). Evoked activity in all cases was blocked by hexamethonium. Hexamethonium-sensitive on-going activity was recorded from six single fibres in different preparations.

With the focal recording technique, evoked activity was recorded from seven hexamethonium-sensitive units (Fig. 1*B*). The latencies of these evoked responses gave estimated 'conduction velocities' of 0·40 ± 0·04 m s^{-1}. On-going activity was recorded from five of these (Fig. 1*C*). On-going activity was also recorded from three units that were hexamethonium resistant, which had estimated 'conduction velocities' of 0·52, 0·43 and 0·48 m s^{-1}.

The estimated 'conduction velocities' using the three techniques were not significantly different ($P = 0·621$).

Characteristics of focally recorded unitary events

As excitatory junction currents (post-junctional events associated with neuroeffector transmission) can be recorded using this technique, activity was observed when filters were set to a bandwidth of 0·1–1500 Hz and in the presence of α,β-methylene adenosine 5′-triphosphate (a purinergic P_{2x} agonist which desensitizes the receptors and thereby

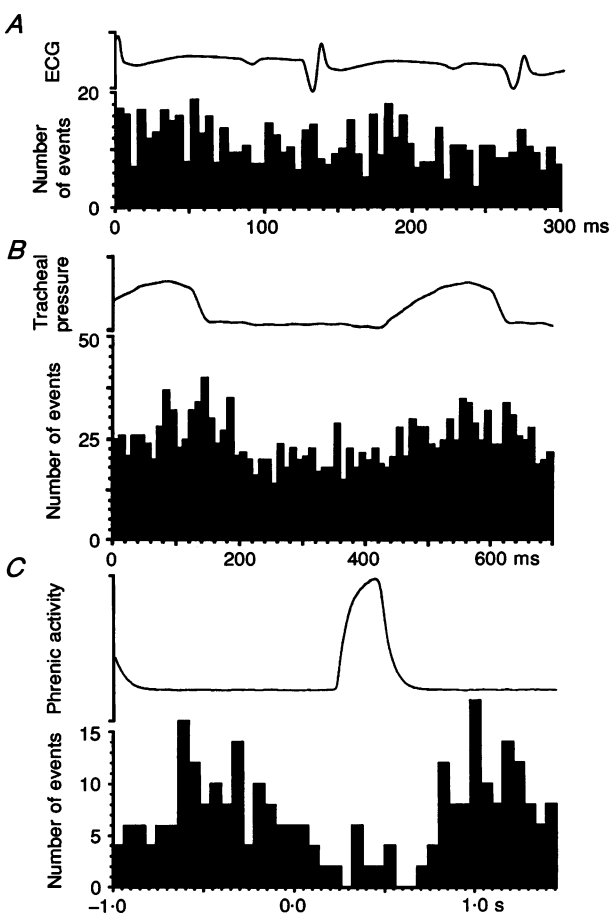

Figure 2. Histograms showing the relationship of the on-going discharge of a focally recorded unit to cardiac cycle (*A*), lung inflation (*B*) and phrenic bursts (*C*)
A, ECG-triggered histogram (5 ms bins, 1000 trials). Top trace, averaged ECG over same period. *B*, tracheal pressure-triggered histogram (10 ms bins, 1000 trials). Top trace, averaged tracheal pressure over same period. *C*, phrenic-triggered histogram (60 ms bins, 100 trials). Top trace, rectified and smoothed phrenic nerve activity averaged over same period. Phrenic average has been shifted to the right by 300 ms to compensate for phase shift caused by conduction delay in sympathetic pathway (see text).

blocks excitatory junction currents; see Åstrand *et al.* 1988). Under these conditions, unitary events were recorded which had durations in the range 2–4 ms, were typically triphasic (Fig. 1*C*), and followed 1 Hz sympathetic chain stimulation (not seen with excitatory junction currents). The unitary events were thus confirmed as being action potentials.

Cardiac-related modulation of discharges

Systolic blood pressures in all experiments were in the range 100–140 mmHg. Sympathetic discharge was examined for cardiac-related activity by constructing ECG- or arterial pulse-triggered histograms. Neither the on-going activity recorded from fibre preparations ($n = 3$) nor that recorded using the focal recording technique ($n = 6$) showed any clear cardiac-related activity. Figure 2*A* shows a typical example.

Phrenic-related activity

The activity of the five focally recorded units analysed (3 hexamethonium sensitive, 2 hexamethonium resistant) had phrenic-related discharges. As there is a long delay in the sympathetic pathway (peripheral (see above) plus central, approximately 100 ms; see Guyenet & Brown, 1986), an allowance was made for the phase shift between phrenic and sympathetic nerve activities. Allowing for this phase shift, peak firing of units was during phrenic silence, with the period of depression of activity during the phrenic discharge (Fig. 2*C*).

Lung inflation-related activity

The tracheal pressure recording (peak pressure, 5–10 mmHg; rate, 90–120 cycles min^{-1}) was used as the trigger to generate histograms to examine modulation of sympathetic activity related to the lung inflation cycle. Modulation was seen in three focally recorded units (1 of 4 hexamethonium-sensitive and 2 of 3 hexamethonium-resistant units; Fig. 2*B*).

Interspike interval and autocorrelation analysis of unit activity

This analysis was carried out to examine the firing 'frequency' distribution and the possible presence of rhythmic discharges. The focally recorded on-going activity of all eight units analysed in this manner showed early peaks (median of modal intervals, 0·1–0·15 s; range, 0·05–0·20 s) in their interspike interval histograms. However, in five out of eight cases, although these intervals were similar to the pulse interval they were not coincident with it (see Fig. 3*A*). Recordings from five of these units were made simultaneously with recordings of phrenic nerve activity. These had a peak in their interspike interval histograms coincident with the interphrenic burst interval (see Fig. 3*A*). Autocorrelation analysis showed the discharge of the units to have a rhythm dominated by that of the respiratory cycle (as indicated by phrenic nerve discharge; Fig. 3*B*). Units tended to discharge in 'bursts' of duplets at the respiratory frequency, which is indicated by the similar early peaks in both interspike interval and autocorrelation histograms.

Effect of whole-body warming on activity

Activity (2 of 3 fibres and 4 of 4 hexamethonium-sensitive units, and 1 of 1 hexamethonium-insensitive units) was

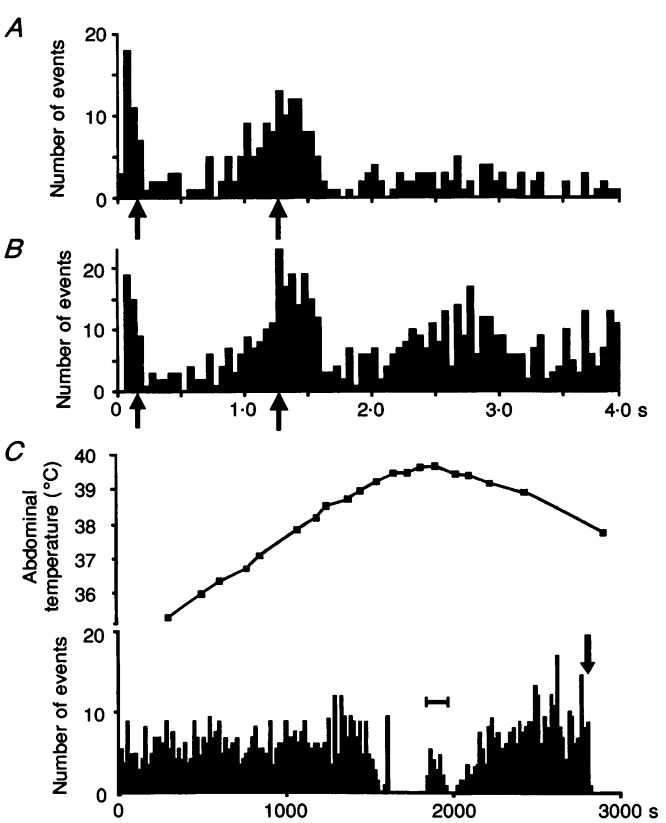

Figure 3.
A and *B*, interspike interval and autocorrelation histograms of a focally recorded unit. *A*, interspike interval histogram (50 ms bins, 400 intervals). The arrow on the left indicates modal pulse interval and that on the right modal phrenic burst interval. *B*, autocorrelation histogram (50 ms bins, 400 sweeps). Arrows as above. The rhythm of unit discharge is dominated by that of the phrenic bursts. *C*, rate histogram (20 s bins) illustrating the influence of an increase in whole-body temperature on the discharge of a focally recorded unit. When abdominal temperature (top trace) reached 39 °C, activity declined to zero. Activity could still be evoked from the chain (indicated by bar) and returned to control levels as temperature fell. Activity was blocked by hexamethonium (6 mg kg^{-1}, I.V., given at arrow).

unaffected until a 'critical' core temperature was reached, which varied between 38 and 39 °C. At this temperature there was an abrupt 'switch-off' in activity, which was maintained (Fig. 3*C*). As temperature began to fall, after the blanket was switched off, the activity returned to control levels. Blood pressure remained constant throughout the heating procedure.

DISCUSSION

Stimulation of the lumbar sympathetic chain evoked responses in whole ventral collector nerves, teased fibres and units recorded from the ventral caudal artery with latencies which furnished 'conduction velocities' which were not significantly different from one another. Hexamethonium could block these evoked responses, which is consistent with lumbar chain stimulation activating the preganglionic supply to postganglionic sympathetic neurones innervating the tail whose cell bodies are located primarily in sacral (S) and coccygeal (Co) ganglia (Sittiracha, McLachlan & Bell, 1987). On the basis of the above and a report showing that all afferents from the tail pass into spinal segments S2–Co3 (Sittiracha *et al.* 1987), all units focally recorded from the ventral caudal artery can be considered sympathetic. Not all activity, evoked or on-going, was susceptible to 12 mg kg^{-1} hexamethonium given I.V. This is not surprising, as it has been observed that doses of up to 36 mg kg^{-1} hexamethonium can be required to abolish the potential evoked in the ventral collector nerve following sympathetic chain stimulation (authors' unpublished observations). Furthermore, some of the hexamethonium resistance may be explained by non-nicotinic transmission.

The unit activities recorded using the focal recording system represented neuronal action potentials rather than excitatory junction currents, as they had durations of 2–4 ms and were present when α,β-methylene adenosine 5′-triphosphate was added to the Krebs solution; in the presence of this drug, excitatory junction currents are blocked (Åstrand *et al.* 1988). They also followed faithfully 1 Hz stimulation of the chain which is not seen with excitatory junction currents (Åstrand & Stjärne, 1989).

As sympathetic fibres run along the caudal ventral artery for a few millimetres before innervating it (Sittiracha *et al.* 1987), the recorded activity was destined for this target. Thus this study presents the first analysis of single unit activity recorded *in vivo* from sympathetic fibres innervating an identified blood vessel, in this case the caudal ventral artery. The principal rhythm in the discharge of units was that of the frequency of phrenic bursts. The additional peak in the interspike interval histograms in the range 0·05–0·20 s relates to the intraburst interval which, as it was not necessarily coincident with the pulse interval, may be determined by a hypothetical sympathetic oscillator (Gebber, Barman & Zviman, 1989). These 'bursts' of action potentials probably lead to more effective neuroeffector transmission than that which would occur with single action potentials (Brock & Cunnane, 1992).

In the rat, the thermoregulatory control of tail blood flow is an important homoeothermic mechanism. O'Leary *et al.* (1985) concluded that the increase in tail vascular conductance during body heating was purely via withdrawal of vasoconstrictor drive. The data from these experiments show unequivocally that there is withdrawal of sympathetic drive to the caudal ventral artery in response to hyperthermia. The activity recorded was not under tonic baroreceptor modulation, as indicated by its lack of clear pulse-related modulation. In all animals, artificial ventilation was effected at rates higher than the frequency of phrenic bursts, so phrenic discharge primarily reflects central respiratory (inspiratory) drive (see Gilbey, Numao & Spyer, 1986; Häbler, Jänig, Krummel & Peters, 1993). Therefore, the focally recorded units received an excitatory drive which was greatest during expiration. These 'caudal ventral artery' units therefore have similar discharge characteristics in these two respects (i.e. respiratory modulation but no tonic baroreceptor input) to activity recorded from the saphenous nerve supplying hairy skin (Häbler *et al.* 1993) and some sympathetic preganglionic neurones recorded from the lower thoracic and upper segments of the spinal cord projecting into the lower lumbar chain (Zhou & Gilbey, 1992). Häbler *et al.* (1993) postulated that the non-respiratory-modulated units recorded by Zhou & Gilbey (1992) might project to the tail. This study shows that non-modulated units are unlikely to innervate the caudal ventral artery, but non-respiratory-modulated activity may be directed at other parts of the tail circulation. Another possibility which cannot be excluded is that non-respiratory-modulated and respiratory-modulated sympathetic preganglionic neurones converge onto the same postganglionic neurone.

In conclusion, this study has defined some of the characteristics of activity in sympathetic fibres innervating the caudal ventral artery of the rat tail. It remains to be determined how these activities compare to those in the sympathetic supply to other blood vessels of the tail and other circulations.

REFERENCES

Åstrand, P., Brock, J. A. & Cunnane, T. C. (1988). Time course of transmitter action at the sympathetic neuroeffector junction in rodent vascular and non-vascular smooth muscle. *Journal of Physiology* **401**, 657–670.

Åstrand, P. & Stjärne, L. (1989). On the secretory activity of single varicosities in sympathetic nerves innervating the tail artery. *Journal of Physiology* **409**, 207–220.

Brock, J. A. & Cunnane, T. C. (1987). Relationship between the nerve action potential and transmitter release from sympathetic postganglionic nerve terminals. *Nature* **326**, 605–607.

BROCK, J. A. & CUNNANE, T. C. (1992). Electrophysiology of neuroeffector transmission in smooth muscle. In *Autonomic Neuroeffector Mechanisms*, ed. BURNSTOCK, G. & HOYLE, C. H. V., pp. 121–211. Harwood Academic Publishers, Reading, UK.

CUNNANE, T. C. & MOSS, V. A. (1993). Measurement of nerve impulse traffic and blood vessel diameter in rat mesenteric blood vessels *in vivo*. *Journal of Physiology* **259**, 188*P*.

EVANS, R. J. (1990). Neural control of mesenteric arteries. D.Phil. Thesis, University of Oxford.

FUTURO-NETO, H. A. & COOTE, J. H. (1982). Desynchronized sleep-like pattern of sympathetic activity elicited by electrical stimulation of sites in the brainstem. *Brain Research* **252**, 269–276.

GEBBER, G. L., BARMAN, S. M. & ZVIMAN, M. (1989). Sympathetic activity remains synchronized in presence of a glutamate antagonist. *American Journal of Physiology* **256**, R722–732.

GILBEY, M. P., NUMAO, Y. & SPYER, K. M. (1986). Discharge patterns of cervical sympathetic preganglionic neurones related to central respiratory drive in the rat. *Journal of Physiology* **378**, 253–265.

GILBEY, M. P. & STEIN, R. D. (1991). Characteristics of sympathetic preganglionic neurones in the lumbar spinal cord of the cat. *Journal of Physiology* **432**, 427–443.

GUYENET, P. G. & BROWN, D. L. (1986). Nucleus paragigantocellularis lateralis and lumbar sympathetic discharge in the rat. *American Journal of Physiology* **250**, R1081–1094.

HÄBLER, H. J., JÄNIG, W., KRUMMEL, M. & PETERS, O. A. (1993). Respiratory modulation of the activity in postganglionic neurons supplying skeletal muscle and skin of the rat hindlimb. *Journal of Neurophysiology* **70**, 920–930.

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.

JOHNSON, C. D. & GILBEY, M. P. (1993*a*). Modulation of sympathetic activity in identified fibres innervating blood vessels in the rat tail. *International Union of Physiological Sciences* **XXXII**, 173.43.

JOHNSON, C. D. & GILBEY, M. P. (1993*b*). Recordings of activity from sympathetic fibres innervating blood vessels in the rat tail. *Journal of Physiology* **467**, 14*P*.

JOHNSON, C. D. & GILBEY, M. P. (1993*c*). Recordings from sympathetic fibres innervating blood vessels. *Clinical Autonomic Research* **3**, 69.

O'LEARY, D. S., JOHNSON, J. M. & TAYLOR, W. F. (1985). Mode of neural control mediating rat tail vasodilation during heating. *Journal of Applied Physiology* **59**, 1533–1538.

PETERSON, D. F., COOTE, J. H., GILBEY, M. P. & FUTURO-NETO, H. A. (1983). Differential pattern of sympathetic outflow during upper airway stimulation with smoke. *American Journal of Physiology* **245**, R433–437.

SITTIRACHA, T., MCLACHLAN, E. M. & BELL, C. (1987). The innervation of the caudal artery of the rat. *Neuroscience* **21**, 647–659.

ZHOU, S. Y. & GILBEY, M. P. (1992). Respiratory-related activity of lower thoracic and upper lumbar sympathetic preganglionic neurones in the rat. *Journal of Physiology* **451**, 631–642.

Acknowledgements

The study was supported by a Wellcome Trust Project Grant. The authors wish to thank Dr Zhou for assistance, Dr T. C. Cunnane for his encouragement and Professor K. M. Spyer for his general support.

Received 10 February 1994; accepted 10 March 1994.