Effects of preoptic warming on subretrofacial and cutaneous vasoconstrictor neurons in anaesthetized cats

R. M. McAllen and C. N. May

Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria 3052, Australia

1. Sympathetic and subretrofacial neuron responses to preoptic warming were studied in chloralose- or Saffan- anaesthetized, paralysed cats.

2. Warming a thermode in the preoptic region inhibited the activity of cutaneous vasoconstrictor fibres supplying hairy skin. Muscle vasoconstrictor fibre activity recorded at the same time was either unaffected or raised.

3. Small injections of sodium glutamate (5 nl, 0·1 M) were made into the region of the subretrofacial nucleus in the ventrolateral medulla. The part of that region where glutamate injections evoked brisk increases in cutaneous vasoconstrictor fibre activity was chosen for further study.

4. Extracellular single unit recordings were made in that area from seventy-seven subretrofacial neurons, which were identified by their barosensitivity (inhibition by carotid blind sac inflation). Forty-seven of them were antidromically activated by stimulation in the spinal cord.

5. The activity of twenty subretrofacial neurons (twelve proven bulbospinal) was significantly reduced by periods of preoptic warming. Cutaneous vasoconstrictor activity recorded at the same time also fell. Forty-nine subretrofacial neurons (thirty-five proven bulbospinal) were unaffected or excited by periods of preoptic warming that inhibited cutaneous vasoconstrictor fibres. The response of eight neurons was unclear.

6. No difference in either mean firing rate or axonal conduction velocity was found between neurons inhibited by preoptic warming and other subretrofacial neurons.

7. The subretrofacial neurons inhibited by warming were found intermingled with those unaffected or excited. Marked recording sites of warm-inhibited neurons were clustered around the ventromedial border of the subretrofacial nucleus.

8. In two cats, bilateral inhibition of subretrofacial neurons by surface application of 1 M glycine reduced cutaneous vasoconstrictor fibre activity to 32 and 44% of control levels.

9. The results suggest that specific cutaneous vasoconstrictor premotor neurons exist in the subretrofacial nucleus. These apparently provide most of the background excitatory drive to cutaneous vasomotor neurons. Central warming stimuli may act, at least in part, by withdrawing that drive.

The sympathetic nervous system is able to co-ordinate and convey highly differentiated patterns of activity to its various target organs. Even amongst the subgroup of postganglionic neurons that supply and constrict blood vessels there are clear functional differences. For example, sympathetic fibres which supply vessels in skeletal muscle are highly barosensitive and are excited by stimulation of either nociceptors or arterial chemoreceptors; those supplying hairy skin are much less barosensitive, and are *inhibited* by nociceptor or chemoreceptor stimuli (Jänig, 1988). It seems that individual postganglionic vasomotor neurons are differentiated into separate functional channels or classes, each of which is dedicated to supplying the vessels of a particular type of tissue (Jänig, 1985, 1988).

Preganglionic neurons also appear to be functionally specific, although on this point the evidence is less conclusive. Nevertheless, activity patterns characteristic of (e.g.) muscle or cutaneous vasoconstrictor neurons may be recognized among them (Jänig & Szulczyk, 1981; Gilbey & Stein, 1991; Boczek-Funcke, Dembowsky, Häbler, Jänig, McAllen & Michaelis, 1992). Additional evidence of a histochemical nature supports the contention that the postganglionic

neurons of each class receive inputs from specific types of preganglionic neuron (e.g. Gibbins, 1992; Shafton, Oldfield & McAllen, 1992).

The major source of excitatory drive to sympathetic vasomotor neurons appears to be the premotor cells of the rostral ventrolateral medulla (RVLM) (Calaresu & Yardley, 1988; Guyenet, Haselton & Sun, 1989; Guyenet, 1990). In cats, these cells form a compact group – the subretrofacial (SRF) nucleus (Dampney, 1990; Polson, Halliday, McAllen, Coleman & Dampney, 1992). They supply excitatory drive to cardiac, vasomotor and adrenal outflows, but apparently not other types of sympathetic neuron (McAllen, 1986*b*). Catecholamine-synthesizing cells (C1 adrenaline group) as well as other neuron types are included in this population (Polson *et al.* 1992).

It is still a matter of active debate whether, and to what extent, different functional channels may be discerned among the SRF premotor neuron population. Can an individual SRF neuron be labelled, for example, as 'muscle vasoconstrictor' in the same way that this can be done with pre- or postganglionic neurons? Or is the vasomotor drive which individual SRF neurons provide distributed to a number of different vasomotor outflows? In keeping with the second view, when microinjections of glutamate were used to activate groups of neurons at different sites in the rat RVLM, these were found to drive different sympathetic outflows in constant, fixed proportion (Beluli & Weaver, 1991). But when similar experiments were done on cats, responses favouring one or other vasomotor outflow could be found when different subregions of the SRF were activated (Lovick, 1987; Dampney & McAllen, 1988; McAllen & Dampney, 1990; Dean, Seagard, Hopp & Kampine, 1992; McAllen & May, 1994). Such findings demonstrate that, at least in the cat, there is some degree of target selectivity among the RVLM premotor neuron population. But they do not show whether that target selectivity is relative or absolute for individual SRF neurons, nor do they eliminate the possible existence of other SRF neurons with more generalized vasomotor actions (Guyenet *et al.* 1989).

The present experiments were done to throw light on the organizational principle of sympathetic premotor neurons in the cat, by attempting to identify target-dedicated neurons in among the SRF cell population. The approach taken is analogous to that used by Jänig and colleagues on peripheral sympathetic fibres, whereby neurons of each class are recognized by their responses to reflex tests. But because most of the reflex tests that would be expected to work on medullary neurons lacked the necessary discriminative power for our purpose, we chose to use thermal stimuli. Reduction of activity by warming should discriminate between the vasoconstrictor pathway to skin and those to all other tissues.

Preliminary reports of this work have been published in abstract form (May & McAllen, 1990; McAllen & May, 1993).

METHODS

General

Successful experiments were performed on twenty-two cats of either sex (2·8–4·5 kg), anaesthetized with either α-chloralose ($n = 18$) or the steroid anaesthetic mixture Saffan (9 mg ml^{-1} alphaxalone + 3 mg ml^{-1} alphadolone acetate; Pitman-Moore Inc., North Ryde, NSW 2113, Australia) ($n = 4$). A preliminary series (5 cats anaesthetized with chloralose, 1 with Saffan) studied muscle and cutaneous vasoconstrictor fibre responses to preoptic warming. The main experimental series used fourteen cats (11 anaesthetized with chloralose, 3 with Saffan). Two further cats were used to measure cutaneous vasoconstrictor fibre responses to inhibition of subretrofacial neurons.

Cats were premedicated with ketamine hydrochloride (11 mg kg^{-1} I.M.). They were then anaesthetized intravenously either with α-chloralose (70 mg kg^{-1}), in which case small doses of pentobarbitone (12 mg) or chloralose (50–100 mg) were given I.V. if necessary to supplement their anaesthesia, or with a continuous infusion of Saffan (0·3–2 mg kg^{-1} h^{-1} I.V.), in which case depth of anaesthesia was titrated by the infusion rate. A deep level of Saffan anaesthesia was maintained during the preparation, and this was adjusted to a lighter (though still 'surgical') level for the experiment.

During recording periods, animals were paralysed with bolus doses of pancuronium bromide (2 mg I.V.). At intervals during the experiment, this was allowed to wear off, so the anaesthetic level could be tested conventionally by withdrawal reflexes. At other times, the anaesthetic level was monitored by reference to the miotic state of the pupil (Boczek-Funcke *et al.* 1992), and by the pressor response to noxious stimulation. For cats anaesthetized with Saffan, care was always taken to check that anaesthesia had stabilized to an adequate level with the current rate of infusion before the neuromuscular blocking agent was administered.

All animals were given a tracheostomy and ventilated artificially with oxygen-enriched air, maintaining end-tidal CO_2 between 3·5 and 4·6%. Rectal temperature was kept close to 38 °C by a servo-controlled blanket. The right femoral artery and vein were cannulated, for the measurement of blood pressure and the administration of anaesthetic. The bladder was catheterized and drained.

Preparation

The animal was mounted supine in a stereotaxic frame. Two adjacent thoracic spines were exposed and secured to the frame with a clamp. The medulla was then exposed from a ventral approach, as described previously (McAllen, Neil & Loewy, 1982). Briefly, the oesophagus and larynx were vascularly isolated from the neck, divided and retracted. The base of the skull was cleared of muscle, and opened rostrally from the atlanto-occipital membrane to the tympanic bullae with rongeurs and a dental drill, exposing approximately 5 mm either side of the mid-line. At this stage the dura over the medulla was left intact.

The mandible was divided at the mid-line, and both halves retracted laterally. The tongue, pharynx and larynx were removed. The optic chiasm was exposed ventrally by removing the soft palate and drilling the intervening bone. The overlying dura was incised, and an ophthalmic cautery used to stop bleeding from dural sinuses. A stainless steel thermode of 2 mm diameter, containing a heating coil and

mounted in a micromanipulator, was lowered into the preoptic area either just rostral to the optic chiasm, pressing caudally between the cerebral hemispheres, or through an incision in the chiasm, pressing rostrally in the third ventricle. (Warming in either position effectively lowered cutaneous vasoconstrictor activity: see below.) A thermocouple was attached to the outside of the thermode close to the tip. This was used either to monitor (early experiments) or to servo-control (later experiments) the surface temperature of the thermode. On two occasions, a breakdown of the heating coil's insulation, such that it made contact with the surface tubing and caused electrical interference, forced us to insulate the outside of the probe with nail varnish. In these cases, we needed to raise the temperature of the thermocouple (which was inside the varnish) above 55 °C to be effective; we could not measure the surface temperature outside the varnish. On other occasions, the probe's surface temperature was raised to between 45 and 53 °C.

For experiments of the main series, pairs of stainless-steel stimulating electrodes were inserted into the dorsolateral funiculus of the spinal cord, either at the T4 segment via a dorsal laminectomy (3 cats), or from a ventral approach at the C3–C4 level, after drilling through the vertebra (11 cats). Electrodes were glued in place with cyanoacrylate cement and covered with low-melting-point wax.

In the main series, one carotid sinus was prepared as a blind sac, by tying all branches except the external and common carotid arteries. A pneumatic occluder was placed around the common carotid; it was occluded for baroreceptor tests. The external carotid was cannulated towards the sinus, and connected to a pressurized reservoir of heparinized saline (10 units ml^{-1}) for baroreceptor tests. Sinus pressure was measured from a side tube.

Before recording, animals were given a bilateral pneumothorax to reduce brain movement associated with respiration, and the dura was opened over the medulla.

Nerve recordings

The left peroneal nerves, and in 5/6 preliminary experiments also the sural nerve, were exposed via a lateral leg incision. Fascicles supplying peroneal muscle and/or hairy skin were dissected clear for about 15 mm and laid intact over a dissecting platform. A pool was constructed from the skin edges and filled with mineral oil. Activity from each whole fascicle was recorded in continuity by a pair of platinum hooks in order to check its receptive field. Fascicles were verified as supplying either muscle or hairy skin, but not both. They were then crushed distally, desheathed and prepared for dissection.

Few-fibre activity was recorded differentially from dissected filaments of these nerves via platinum electrodes, amplified and filtered (bandpass 10–1000 Hz with a 50 Hz notch). Muscle (MVC) or cutaneous (CVC) vasoconstrictor activity was identified in recordings from these filaments by its location (i.e. the territory supplied by that fascicle) and its barosensitivity (carotid blind sac inflation in the main experimental series, release of carotid occlusion or carotid sinus stretch in other experiments (Fig. 1*A*).

In one experiment, whole nerve activity was recorded from the central cut end of the right phrenic nerve, exposed low in the neck. It was recorded via a pair of platinum wire hooks under a paraffin pool. The signal was amplified and filtered (bandpass 200–3000 Hz).

Medullary recordings

In order to locate the appropriate medullary neurons before recording, 5 nl volumes of sodium glutamate (0·1 M) were pressure injected through a glass micropipette 0·7 mm beneath the brain surface at different sites over the ventrolateral medulla (Goodchild, Dampney & Bandler, 1982). The region from which brisk CVC responses were evoked (as in Fig. 2*A d*) was prepared for extracellular single unit recording. Small patches were opened in the pia mater, through which glass-insulated tungsten microelectrodes were inserted with a stepping drive. Signals were recorded differentially, amplified and filtered (bandpass 300–5000 Hz). Single SRF neurons were identified by their barosensitivity, as described in the Results section. Antidromic activation by spinal stimulation was only accepted as proven if it satisfied the critically timed collision test (Lipski, 1981).

Central neuron and peripheral fibre spikes were monitored on a variable persistence storage oscilloscope and discriminated with window discriminators. Discriminator pulses were counted on pulse integrators. Raw activity and integrated spike counts, along with blood pressure, sinus pressure and a voice/event channel, were recorded on magnetic tape for later analysis. Data were also recorded on an electrostatic chart recorder.

At the end of some experiments, direct anodal current (approx. 2 mA for 1 min) was passed through the recording electrode. The electrode was removed and approximately 50 nl Pontamine Blue dye was pressure injected into the same spot. The cat was killed with an overdose of pentobarbitone sodium, and the medulla was removed and immersed overnight in 4% paraformaldehyde. Forty micrometre transverse frozen sections were taken of the region containing the mark. Marked sites were located microscopically and drawn with the help of a computer-based mapping program (MAGELLAN; Halasz & Martin, 1984).

Analysis

Discriminated spike signals were analysed off-line from tape, using the CED 'SPIKE2' program (Cambridge Electronic Design, Cambridge, UK) to generate sequential histograms of SRF neuron or sympathetic fibre firing over 5 or 10 s time bins. A custom-written program measured the firing rate over the 1 min control period and calculated the cumulative sum (cusum) relative to this base value (Imamura & Onoda, 1983). Significant changes in firing rate were detected by the cusum test (Imamura & Onoda, 1983), taking $P < 0·01$ to indicate statistical significance. (Previous experience showed this test was prone to give false positive results, such as during control periods, at the $P < 0·05$ level. This was not so at the $P < 0·01$ level, which reliably detected all responses visible to the eye.)

RESULTS

Effects of preoptic warming on cutaneous vasoconstrictor (CVC) and muscle vasoconstrictor (MVC) fibre activity

In one Saffan- and five chloralose-anaesthetized cats, simultaneous few-fibre recordings were made from postganglionic vasoconstrictor fibres supplying hindlimb

muscle (MVC, deep peroneal nerve) and hairy skin (CVC, sural or superficial peroneal nerve). Their barosensitivity was checked, as illustrated in Fig. 1*A*: both fibre types were excited by bilateral carotid occlusion, and were silenced by its release while blood pressure was still high. As expected, MVC responses to baroreceptor stimuli were stronger than CVC responses (Jänig, 1985, 1988).

The surface temperature of a preoptic thermode was increased in different tests to between approximately 45 and 53 °C. The position and temperature of the probe were adjusted until warming caused a reversible decrease in CVC activity. The location of the probe to obtain inhibition of CVC activity was quite critical, even small shifts being accompanied by changes in threshold and effect. At suboptimal sites, CVC activity was sometimes excited (along with MVC activity) before it (alone) was inhibited.

Warming effective sites in six cats (49 tests) decreased CVC activity by 76·2 ± 2·1% (mean ± S.E.M.). MVC activity did not fall significantly during any test: on average it increased by 7·7 ± 1·9%. Occasionally it fell slightly after the warming was discontinued. Preoptic warming sometimes caused a small initial increase in blood pressure, followed by a gradual fall, which closely mirrored the fall in CVC activity. In 4/6 cats those changes were small, amounting to less than 10 mmHg. In the remaining two cats, blood pressure gradually fell during preoptic warming periods by 10 ± 7 and 26 ± 6 mmHg (means ± S.D. for 16 and 10 tests, respectively). Blood pressure and CVC activity fell in parallel; MVC activity increased.

An example of CVC, MVC and blood pressure responses to an episode of preoptic warming is shown in Fig. 1*B*.

Medullary neuron recordings

Recordings of subretrofacial (SRF) neuron activity were made from fourteen cats (11 anaesthetized with chloralose, 3 with Saffan: main series). In all cases, a thermode had been placed in a preoptic site which, when warmed,

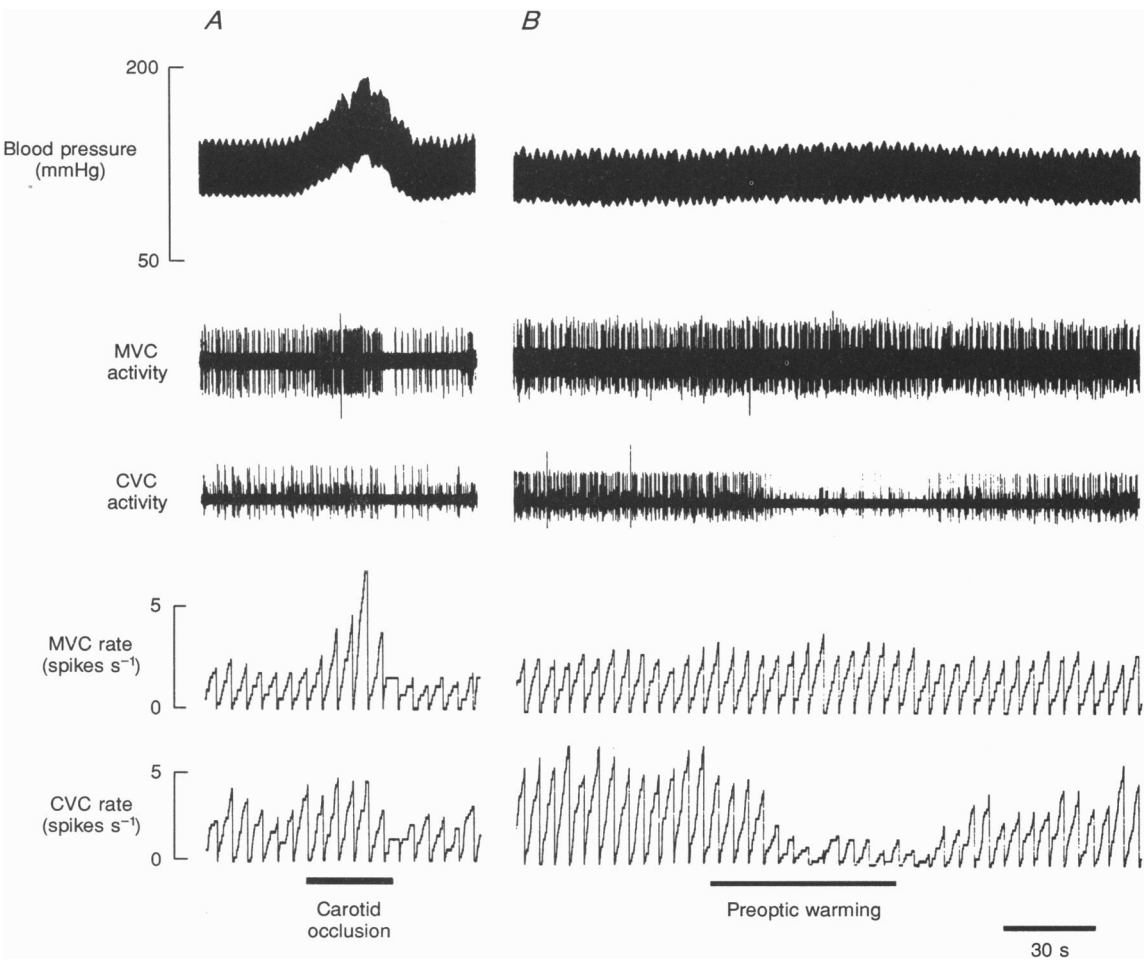

Figure 1. MVC and CVC responses to carotid occlusion and preoptic warming
Record from one experiment; traces from above: blood pressure; MVC few-fibre recording; CVC few-fibre recording; MVC spike count; CVC spike count. Note that both MVC and CVC activity increased (MVC more) during bilateral carotid occlusion, and both were inhibited on its release (*A*). Note also the effect of preoptic warming on CVC activity and the lack of effect on MVC activity (*B*).

effectively lowered peripheral CVC activity (superficial peroneal nerve) but caused little change in blood pressure (sometimes a small rise, never a significant fall). MVC activity was not recorded.

Before central recording, the medullary region containing neurons that drove CVC activity was located with microinjections of glutamate. Figure 2 shows an example of this procedure. Sodium glutamate (500 pmol in 5 nl) was injected 0·7 mm beneath the ventral medullary surface at the four sites shown. The injection at site *d* (immediately adjacent to the SRF nucleus) evoked a brisk increase in CVC activity along with a substantial blood pressure rise. The CVC response to the injection at site *c* (*ca* 0·3 mm ventrolateral to the SRF nucleus) was more sluggish, though more selective, as indicated by the smaller pressor response. Irrespective of the magnitude of the accompanying pressor response, recordings were aimed at regions that evoked brisk, strong CVC responses (in this case, site *d*).

In extracellular recordings from this region, seventy-seven single SRF neurons were identified by their location, their spontaneous activity (4·2 ± 2·3 spikes s^{-1}; mean ± S.D.) and their barosensitivity (inhibition by carotid sinus inflation to > 200 mmHg). The latter stimulus also always caused at least transient inhibition of the CVC activity recorded at the same time. Of the seventy-seven SRF neurons, forty-nine were shown to posess a spinal axon, since they could be activated antidromically by electrical stimulation in the spinal cord (proven by the collision test). Their characteristics were as described elsewhere for this neuron population (e.g. McAllen, 1986*a*).

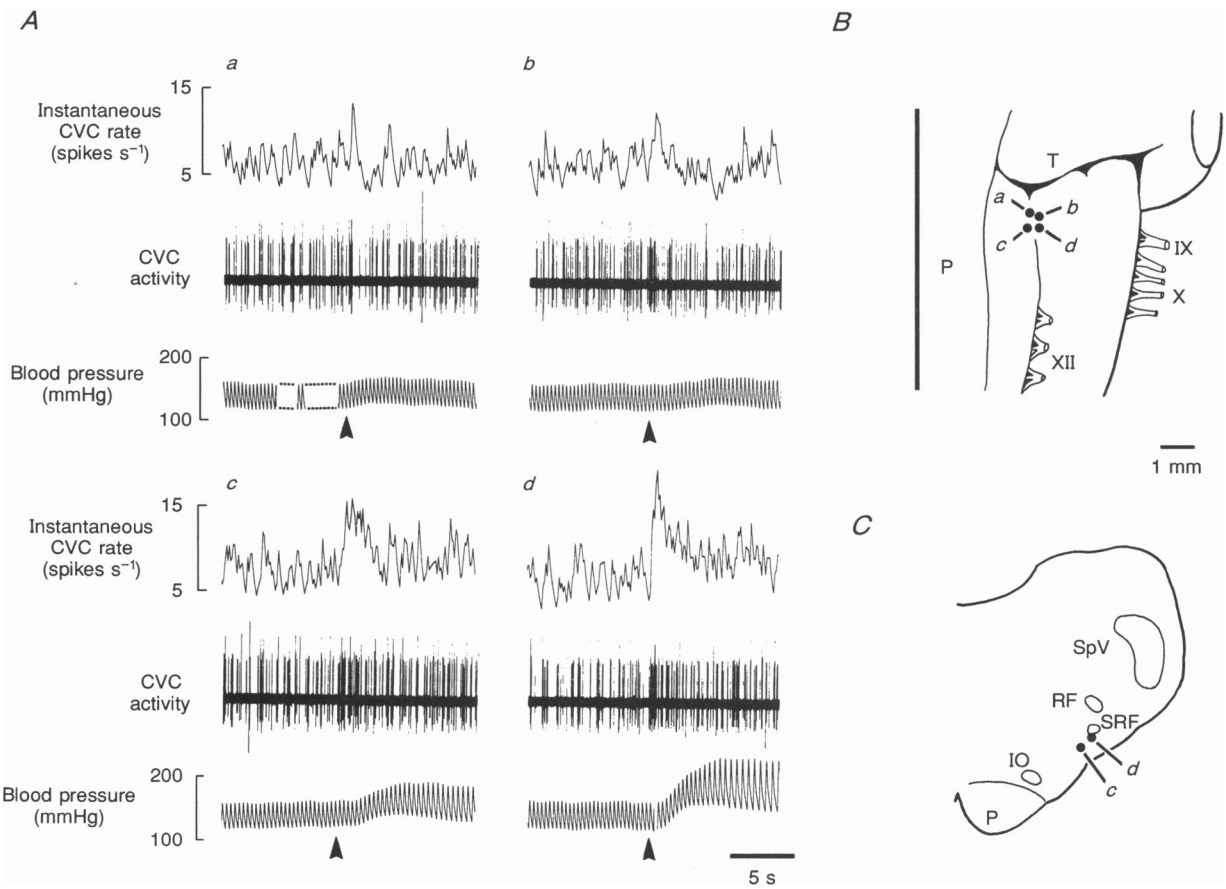

Figure 2. Location of CVC-driving medullary neurons

A shows records of CVC and blood pressure responses to injections of glutamate (500 pmol in 5 nl) into 4 sites 0·7 mm beneath the ventrolateral medullary surface. Traces (from above) show instantaneous CVC spike rate, CVC few-fibre activity and blood pressure. Note the brisk CVC response in *A d*, compared with the slower response in *A c* (where the accompanying pressor response is less). (Missing segments of the blood pressure trace in *A a* have been replaced by dotted lines.) Injection sites were reconstructed histologically, and their locations are shown on a ventral surface view (*B*) and transverse section (*C*) of hemimedulla; *a*–*d* denote sites of the corresponding injections. Abbreviations: IO, inferior olive; P, pyramidal tract; RF, retrofacial nucleus; SpV, spinal trigeminal tract; SRF, subretrofacial nucleus; T, trapezoid body; IX, X, XII, respective cranial nerve roots.

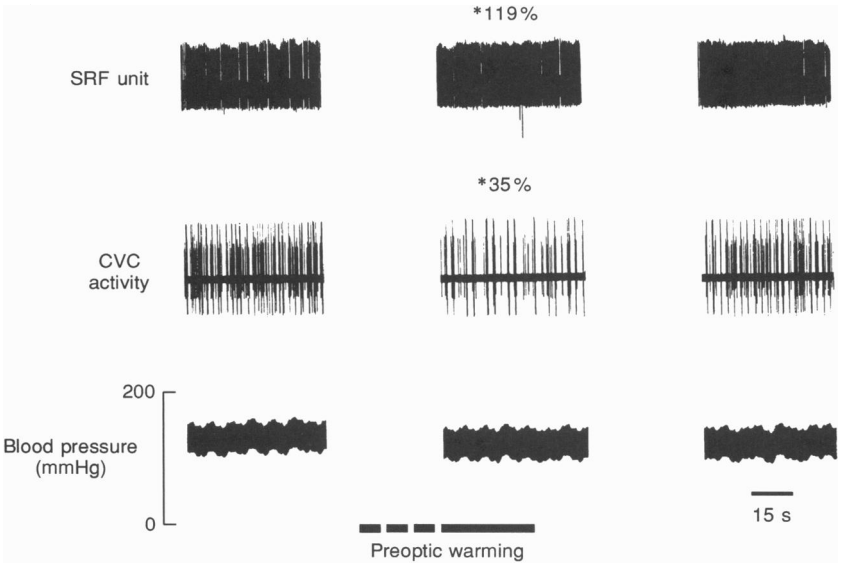

Figure 3. Example of an SRF neuron excited by preoptic warming
Record of SRF neuron activity (upper trace), CVC fibre activity (middle trace) and blood pressure (lower trace) responses to preoptic warming. Significant changes in firing rate are denoted by asterisks and the firing rate reached given as a percentage of control activity. This SRF neuron was excited while the CVC fibres were inhibited. Gaps between panels were 13 min (for 6 of which the preoptic area was warmed) and 1 min, respectively.

Responses of SRF neurons to preoptic warming

The seventy-seven SRF neurons were each tested with at least one episode of preoptic warming sufficient to cause a significant reduction in CVC few-fibre activity. Twenty SRF neurons (12 with proven spinal axons) responded with a significant decrease in firing rate, which appeared to be a primary response to the stimulus ('warm-inhibited neurons'). Their activity decreased by $40 \pm 29\%$ (mean $\pm$ S.D.; 45 tests), while the corresponding decrease in CVC activity monitored at the same time was $46 \pm 15\%$. Forty-nine SRF neurons (35 proven bulbospinal) were unaffected or activated by the stimulus ('unaffected/excited neurons'). Their activity increased on average by $21 \pm 45\%$ (52 tests), while CVC activity decreased by $54 \pm 21\%$ in response to the same tests. For the remaining eight SRF neurons, the

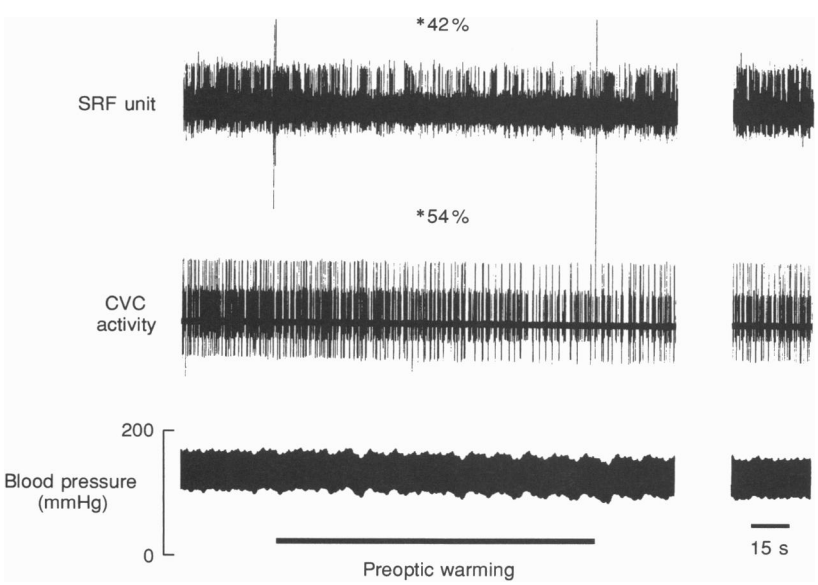

Figure 4. Example of an SRF neuron inhibited by preoptic warming
Record of another SRF neuron (from the same experiment as in Fig. 3) responding to preoptic warming. Traces as in Fig. 3. In this case the SRF neuron was inhibited. The gap between panels was 2 min.

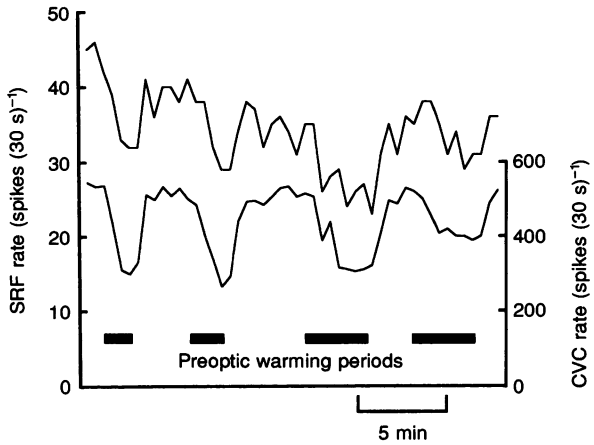

Figure 5. Example of an SRF neuron responding to several episodes of preoptic warming
Plot of the responses of a warm-inhibited SRF neuron (upper line) and CVC few-fibre activity (lower line) to successive periods of preoptic warming (indicated by bars). Note different scales.

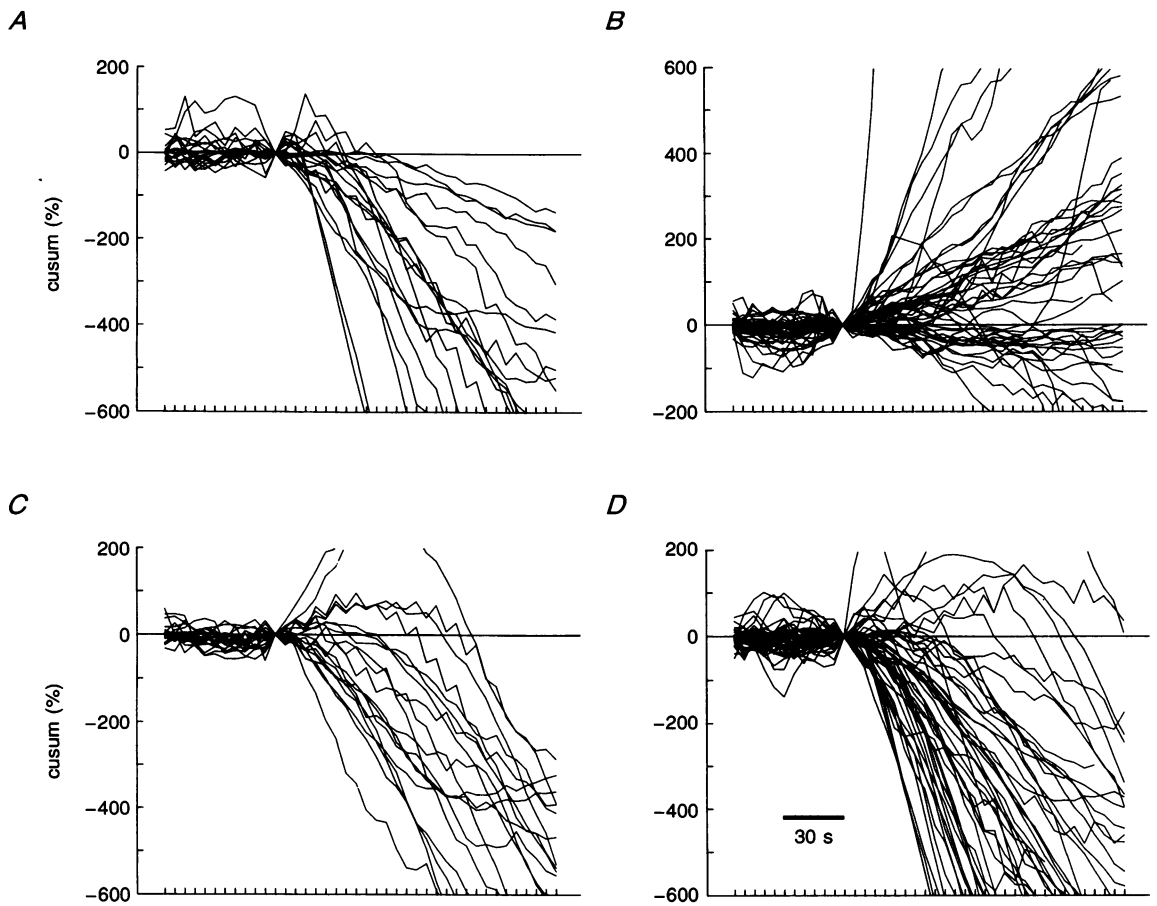

Figure 6. Cumulative sum plots of SRF neuron and CVC fibre responses to preoptic warming
Cumulative sum (cusum) plots of the responses to preoptic warming of 20 warm-inhibited SRF neurons and 49 unaffected/excited SRF neurons are shown in *A* and *B*, respectively. *C* and *D* show the corresponding plots of CVC fibre activity in response to the same tests (14 few-fibre preparations). The activity was counted in 5 s bins, of which the first 12 were the control period. The cusum measures the cumulative excess or deficit in spike activity compared with the mean firing rate in the control period. It is expressed as a percentage of the control 5 s spike count.

confounding factor of blood pressure changes made it impossible to be sure whether or not they were inhibited primarily by preoptic warming or reflexly by the blood pressure response. Examples of SRF neuron responses to preoptic warming are shown in Figs 3–5, along with the corresponding CVC responses.

Individual warm-inhibited neurons and peripheral CVC fibres showed a range of sensitivities and time courses in their response to preoptic warming. The grouped data are displayed as normalized cusum plots in Fig. 6; warm-inhibited neuron responses are shown in Fig. 6*A*, those of unaffected/excited neurons in Fig. 6*B*, and the CVC fibre responses to the same tests in Fig. 6*C* and *D*.

Differences between warm-inhibited SRF neurons and others

We found no significant difference between warm-inhibited and unaffected/excited neurons with respect to their axonal conduction velocities or their on-going firing rates (Student's *t* test; Fig. 7). Both were within the ranges previously reported for SRF neurons in the cat (Barman & Gebber, 1985; McAllen, 1986*a*).

Because the degree of cardiac rhythmicity in the on-going discharge of most CVC neurons has been reported to be weak or absent, compared with the strong rhythmicity shown by other types of vasoconstrictor neuron (Jänig, 1985, 1988), we also looked for differences in cardiac rhythmicity between the two SRF neuron populations. Under our experimental conditions, however, the activity of CVC fibres supplying hairy skin was itself often quite strongly modulated with the cardiac cycle (Fig. 8*A* and *C*). Cardiac rhythmicity was weak or absent in the discharge of 45% of warm-inhibited neurons (e.g. Fig. 8*B*), compared with 29% of the unaffected/excited neurons (*P*, n.s., χ^2 test). No warm-inhibited neuron showed strong cardiac rhythmicity unless this was also present at the same time in the peripheral CVC activity.

Location of warm-inhibited neurons

Warm-inhibited neurons were found intermingled with unaffected/excited neurons. But not uncommonly, a second warm-inhibited neuron was found in the same or an adjacent electrode track to the first, suggesting that these neurons may be grouped. Nine recording sites of warm-inhibited neurons were reconstructed from marked tracks, and these are shown in Fig. 9. They were found most commonly at rostral levels of the SRF nucleus, around the ventromedial edge of the main cell group (Fig. 9).

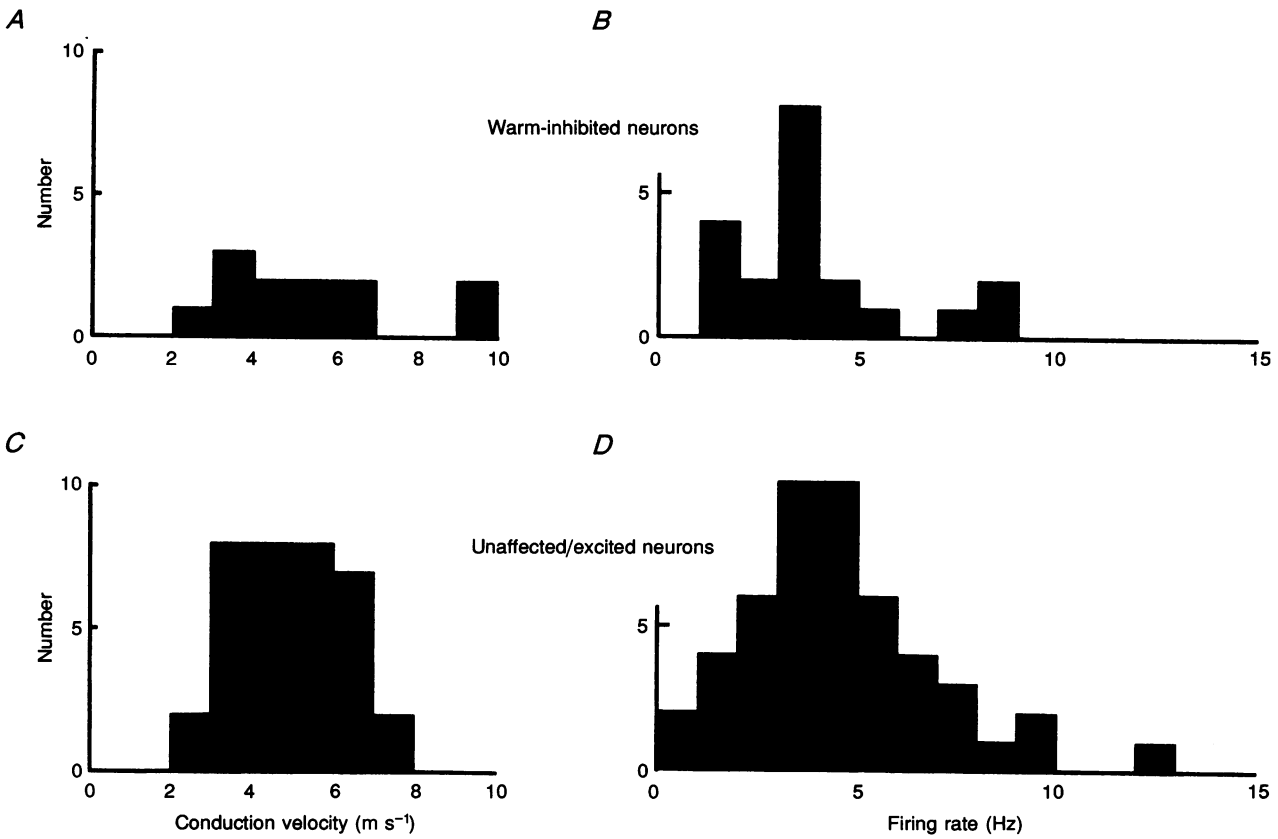

Figure 7. Mean firing rates and conduction velocities of SRF neurons
Histograms of axonal conduction velocity (left) and mean firing rate (right) of warm-inhibited SRF neurons (above) and unaffected/excited SRF neurons (below). *A*, $n = 12$; *B*, $n = 20$; *C*, $n = 35$; *D*, $n = 49$.

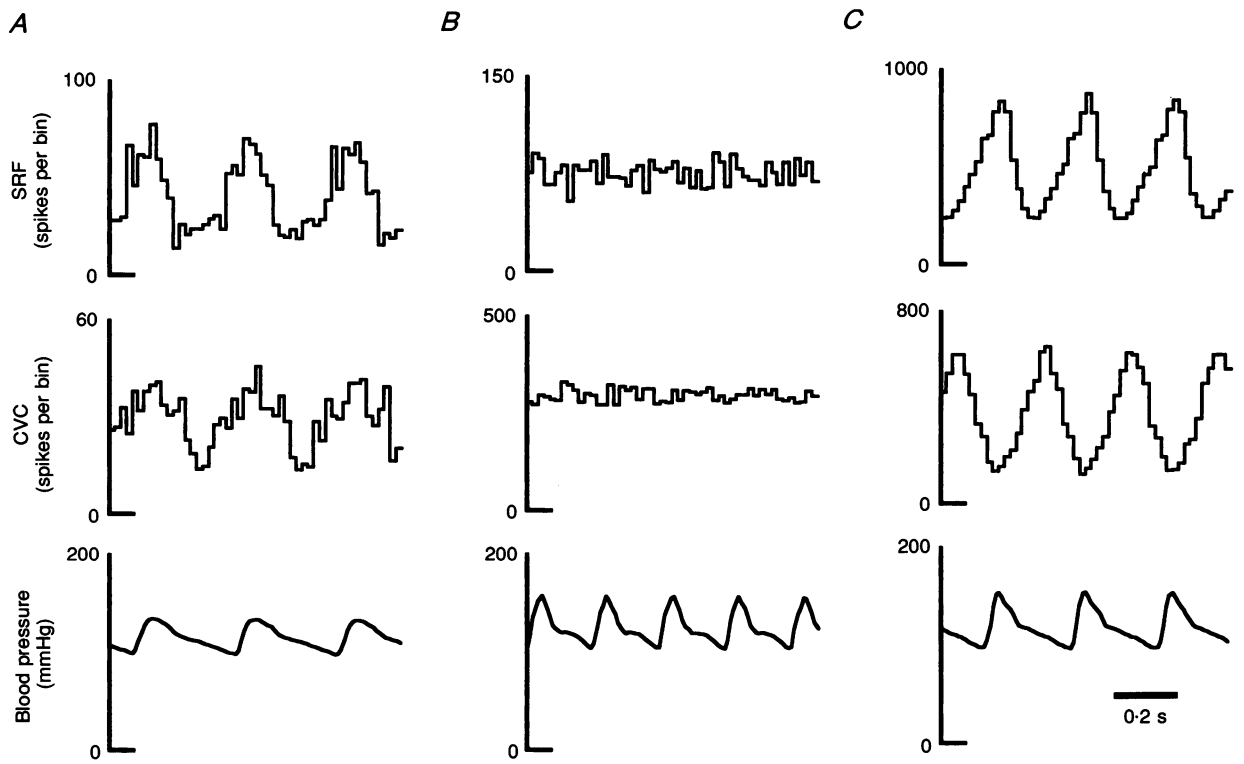

Figure 8. Cardiac rhythmicity of SRF and CVC neurons
Cardiac cycle-triggered histograms (cross correlograms) for SRF neurons (upper traces) and CVC activity recorded at the same time (middle traces). The cycle-triggered averages of the blood pressure are shown in the lower traces. *A*, an SRF neuron which was excited by preoptic warming (630 cycles); *B* and *C*, two warm-inhibited SRF neurons (4000 and 2800 cycles, respectively).

Tonic CVC drive from the ventrolateral medulla

CVC activity was recorded from two cats while neuronal activity in the ventrolateral medulla was bilaterally inhibited by surface application of 1 M glycine (McAllen, 1985). This reduced their mean blood pressures from 95 to 70 mmHg and from 118 to 83 mmHg, while it reversibly reduced their on-going CVC activity to 32 and 44% of control levels, respectively. In both cats this was done while

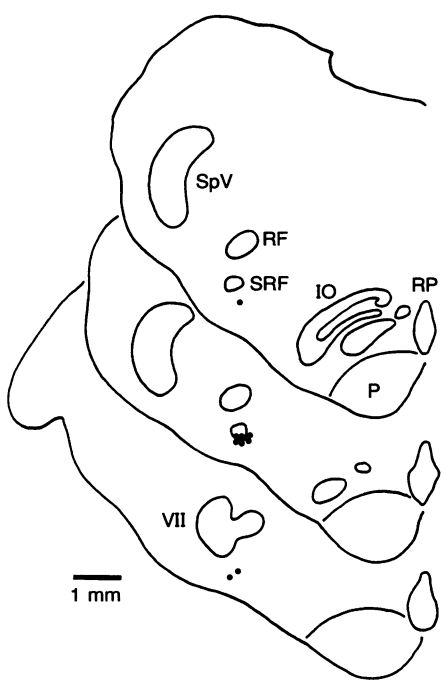

Figure 9. Location of warm-inhibited SRF neurons
Recording sites of 9 warm-inhibited SRF neurons, plotted on 3 transverse sections of medulla 750 μm apart. Abbreviations: IO, inferior olive; P, pyramidal tract; RF, retrofacial nucleus; RP, nucleus raphe pallidus; SpV, spinal trigeminal tract; SRF, subretrofacial nucleus; VII, facial nucleus.

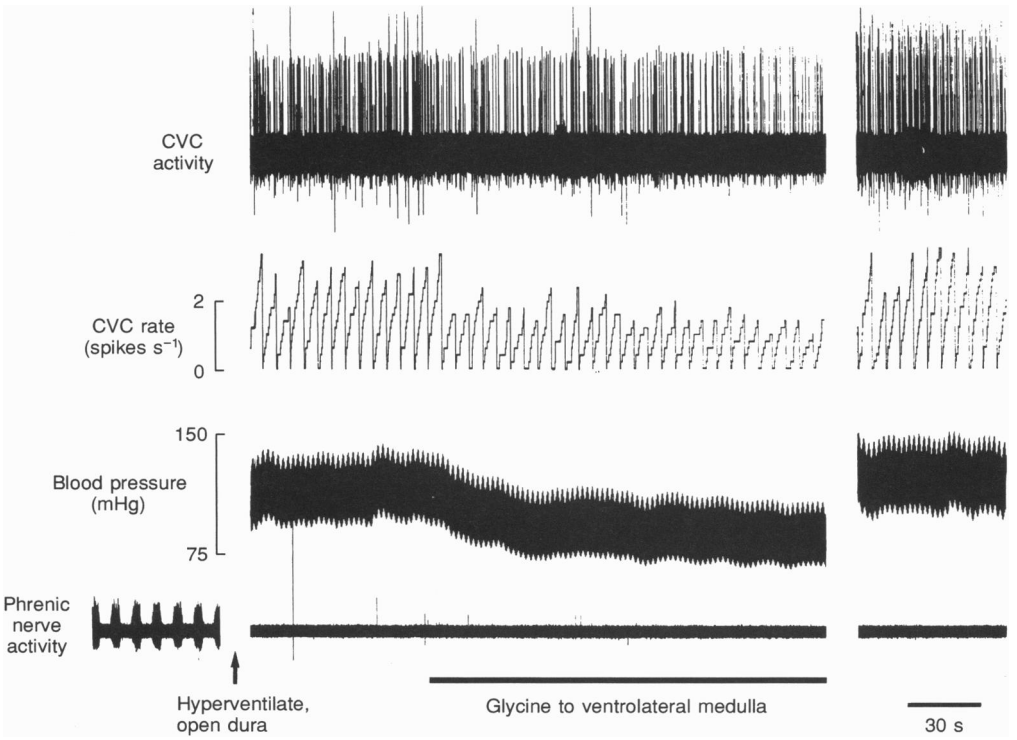

Figure 10. Tonic CVC drive from ventrolateral medullary neurons
Chart record of an experiment in which 1 M glycine was applied bilaterally to the ventrolateral medullary surface. Traces (from above) show CVC fibre activity, CVC spike count, blood pressure and phrenic nerve activity. The phrenic trace is shown initially while central respiratory activity was present (end-tidal CO_2, 4·6%). The dura over the ventral medulla was then removed and the animal hyperventilated to a CO_2 level of 3·5%, abolishing central respiratory activity (gap in trace *ca* 30 min). The final panel, showing recovery, was taken 20 min after the glycine was removed (21 min after end of middle panel).

expired CO_2 was reduced to 3·5%. This was demonstrated to be below the apnoeic threshold in the case illustrated (Fig. 10), where phrenic nerve activity was recorded.

DISCUSSION

These experiments were undertaken with the aim of identifying target-specific vasomotor pathways in recordings from the premotor neurons of the ventrolateral medulla. To the best of our knowledge this is the first systematic study of its kind. Our results suggest that there is a population of SRF neurons which specifically drives *cutaneous* sympathetic vasoconstrictor neurons. This conclusion is based on the observations that (1) preoptic warming inhibited CVC but not MVC activity, and (2) preoptic warming also inhibited the activity of about a quarter of the barosensitive neurons recorded in a region of the SRF nucleus that drove CVC activity. Additionally, we showed that CVC activity depends heavily on drive from the ventrolateral medulla – presumably SRF neurons – because most CVC activity disappeared when this area was inhibited bilaterally with glycine.

Preliminary experiments demonstrated that the preoptic warming stimulus selectively inhibited CVC activity. In those experiments, MVC activity was used as an index of non-cutaneous vasomotor drive. In the main experimental series, the probe was positioned with care in sites which inhibited CVC activity while causing either no change or a small rise in blood pressure. It seems reasonable to assume that non-cutaneous vasomotor outflows were not inhibited, and the stimulus had the required specificity for our purpose. Other workers using similar stimuli have reached the same conclusion (Grewe, Jänig, Kümmel & Varma, 1982; Jänig, 1985).

Anaesthesia depresses thermoregulatory reflexes (Sessler, 1991). The challenge was therefore to obtain an anaesthetized preparation in which CVC activity responded physiologically to preoptic warming. In some animals, possibly for technical reasons, these could not be found, and the experiment was abandoned. Use of Saffan anaesthesia in four animals made no apparent difference, so the simpler method of chloralose anaesthesia was resumed, and the results from both anaesthetics are considered together.

If the specificity of the preoptic warming stimulus is accepted, it follows that, if SRF neurons are sympathetic premotor neurons, the warm-inhibited neurons probably belong to a specific cutaneous vasoconstrictor pathway.

But the functions of the unaffected/excited group are less clear. Anaesthetic depression, as well as our caution in raising thermode temperature, is likely to have caused us to classify some SRF neurons as insensitive to preoptic warming, when in reality they would have responded to stronger stimuli, or responded to the same stimulus in the unanaesthetized state. An admixture of false negatives to that category may have obscured any systematic differences between the two populations. On the other hand, it is probable that SRF neurons with properties strongly divergent from those of CVC fibres – such as those which were significantly excited by preoptic warming – belonged to non-cutaneous vasomotor pathways.

Another reason that might have caused us to underestimate the population of medullary neurons in the CVC pathway is that we only studied the ventrolateral medulla, and only barosensitive neurons. However, the finding that most CVC drive was removed by glycine simply applied to the ventrolateral medullary surface – an approach which would probably not have accessed all SRF neurons – suggests that, under these conditions, neurons elsewhere played no more than a minor role. (The respiratory neurons of the ventrolateral medulla are unlikely to have contributed to the result, because central respiratory drive had been removed by hyperventilation.)

It remains a possibility that non-barosensitive neurons could contribute drive to the CVC pathway. Indeed most CVC neurons are less barosensitive than other vasomotor neurons, and many may show little or no cardiac rhythmicity (Jänig, 1985, 1988; see also Fig. 8*B*). But a stronger baroreceptor test, inflation of a carotid blind sac, at least transiently inhibited all the CVC fibre activity recorded in the present experiments. The SRF neurons studied here were defined as barosensitive by the same criterion.

The present findings complement those from an earlier study (Dampney & McAllen, 1988), in which it was found that glutamate injections into different sites over the SRF region in barodenervated cats could preferentially or exclusively activate either CVC or MVC outflows. In that study, exclusive CVC responses were obtained from injections made ventromedial to the SRF nucleus, although the strongest CVC responses were obtained from more lateral injections (i.e. close to the SRF nucleus), and were accompanied by MVC responses (Dampney & McAllen, 1988). (An analogous result, taking the rise in blood pressure to indicate non-CVC vasomotor responses, may be seen here in Fig. 2*C* and *D*.) This was interpreted as showing that CVC-driving neurons (or perhaps their dendrites) were positioned more medially than MVC-driving neurons; but, because of the method's limited spatial resolution, it was unclear whether the CVC-driving neurons belonged to the SRF nucleus proper, or whether they formed a separate group beyond its confines. The present data from unit recordings – a method with intrinsically better spatial resolution – indicate that the SRF neurons in the CVC pathway are situated within, or very close to, the main SRF cell group, around its ventromedial border. SRF neurons at this site (perhaps via radially spreading dendrites) would be activated in preference to other SRF cells by glutamate diffusing in from the ventromedial direction. This arrangement therefore accounts for the finding that more selective CVC responses were obtained from glutamate injections made somewhat ventromedial to the SRF nucleus (Dampney & McAllen, 1988; cf. Fig. 2*C*, this study).

Taken together, the present results suggest that a specific CVC pathway may be discerned among the sympathetic premotor neurons of the SRF nucleus. They thus provide independent support for the view (Dampney & McAllen, 1988) that vasomotor pathways to different tissues are independently represented not only among post- and preganglionic neurons (Jänig & Szulczyk, 1981; Jänig, 1985, 1988; Gilbey & Stein, 1991), but also among the premotor neurons in the ventrolateral medulla.

The putative CVC premotor neurons identified here were barosensitive, and were inhibited by preoptic warming. They sent axons to the spinal cord (anatomical studies would suggest directly to the intermediolateral cell column; Dampney, Czachurski, Dembowsky, Goodchild & Seller, 1987). They (and conceiveably other neurons in the immediate vicinity) sent excitatory drive to CVC neurons, which was responsible for most of the resting tone in that outflow. We may therefore infer that at least part of the way central warming stimuli inhibit cutaneous vasoconstriction is by reducing the descending drive from SRF neurons. It remains to be tested whether there is a contribution from other pathways, and whether the vasoconstrictor response to cooling is due to reversing that process.

REFERENCES

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

Beluli, D. J. & Weaver, L. C. (1991). Differential control of renal and splenic nerves without medullary topography. *American Journal of Physiology* **260**, H1072–1079.

Boczek-Funcke, A., Dembowsky, K., Häbler, H.-J., Jänig, W., McAllen, R. M. & Michaelis, M. (1992). Classification of preganglionic neurones projecting into the cat cervical sympathetic trunk. *Journal of Physiology* **453**, 319–339.

Calaresu, F. R. & Yardley, C. P. (1988). Medullary basal sympathetic tone. *Annual Review of Physiology* **50**, 511–524.

Dampney, R. A. L. (1990). The subretrofacial nucleus: its pivotal role in cardiovascular regulation. *News in Physiological Sciences* **5**, 63–67.

Dampney, R. A. L., Czachurski, J., Dembowsky, K., Goodchild, A. K. & Seller, H. (1987). Afferent connections and spinal projections of the vasopressor region of the rostral ventrolateral medulla in the cat. *Journal of the Autonomic Nervous System* **20**, 73–86.

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., SEAGARD, J. L., HOPP, F. A. & KAMPINE, J. P. (1992). Differential control of sympathetic activity to kidney and skeletal muscle by ventral medullary neurons. *Journal of the Autonomic Nervous System* **37**, 1–10.

GIBBINS, I. L. (1992). Vasoconstrictor, vasodilator and pilomotor pathways in sympathetic ganglia of guinea pigs. *Neuroscience* **47**, 657–672.

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.

GOODCHILD, A. K., DAMPNEY, R. A. L. & BANDLER, R. A. (1982). A method for evoking physiological responses by stimulation of cell bodies, but not axons of passage, within localized regions of the central nervous system. *Journal of Neuroscience Methods* **6**, 351–363

GREWE, W., JÄNIG, W., KÜMMEL, H. & VARMA, S. (1982). Effects of central thermal stimuli on activity in cutaneous and muscle vasoconstrictor neurones supplying cat hindlimb. *Pflügers Archiv* **394**, R53..

GUYENET, P. G. (1990). Role of the ventral medulla oblongata in blood pressure regulation. In *Central Regulation of Autonomic Functions*, ed. LOEWY, A. D. & SPYER, K. M., pp. 145–167. Oxford University Press, New York, Oxford.

GUYENET, P. G., HASELTON, J. R. & SUN, M.-K. (1989). Sympathoexcitatory neurons of the rostroventrolateral medulla and the origin of sympathetic vasomotor tone. *Progress in Brain Research* **81**, 105–116.

HALASZ, P. & MARTIN, P. R. (1984). A microcomputer-based system for semi-automatic analysis of histological sections. *Proceedings of the Royal Microscopical Society* **19**, 312P.

IMAMURA, K. & ONODA, N. (1983). The cumulative sum analysis of spike discharges in a small sample. *Japanese Journal of Physiology* **33**, 135–138.

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.

JÄNIG, W. (1988). Pre- and postganglionic vasoconstrictor neurons: differentiation, types and discharge properties. *Annual Review of Physiology* **50**, 525–539.

JÄNIG, W. & SZULCZYK, P. (1981). The organization of lumbar preganglionic neurons. *Journal of the Autonomic Nervous System* **3**, 177–191.

LIPSKI, J. (1981). Antidromic activation of neurones as an analytical tool in the study of the central nervous system. *Journal of Neuroscience Methods* **4**, 1–32.

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

MCALLEN, R. M. (1985). Mediation of the fastigial pressor response and a somatosympathetic reflex by ventral medullary neurones in the cat. *Journal of Physiology* **368**, 423–433.

MCALLEN, R. M. (1986*a*). Identification and properties of subretrofacial bulbospinal neurones – a descending cardiovascular pathway in the cat. *Journal of the Autonomic Nervous System* **17**, 151–164.

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

MCALLEN, R. M. & DAMPNEY, R. A. L. (1990). Vasomotor neurons of the rostral ventrolateral medulla are organized topographically with respect to type of vascular bed but not body region. *Neuroscience Letters* **110**, 91–96.

MCALLEN, R. M. & MAY, C. N. (1993). Thermoresponsiveness among subretrofacial neurons. *Proceedings of the Australian Neuroscience Society* **4**, 66p.

MCALLEN, R. M. & MAY, C. N. (1994). Differential drives from rostral ventrolateral medullary neurons to three identified sympathetic outflows. *American Journal of Physiology* (in the Press).

MCALLEN, R. M., NEIL, J. J. & LOEWY, A. D. (1982). Effects of kainic acid applied to the ventral surface of the medulla oblongata on vasomotor tone, the baroreceptor reflex and hypothalamic autonomic responses. *Brain Research* **238**, 65–76.

MAY, C. N. & MCALLEN, R. M. (1990). Selective inhibition of cutaneous vasoconstrictor neurons by preoptic heating in anaesthetised cats. *Proceedings of the Australian Neuroscience Society* **1**, 107p.

POLSON, J. W., HALLIDAY, G. M., MCALLEN, R. M., COLEMAN, M. J. & DAMPNEY, R. A. L. (1992). Rostrocaudal differences in morphology and neurotransmitter content of cells in the subretrofacial vasomotor nucleus. *Journal of the Autonomic Nervous System* **38**, 117–138.

SESSLER, D. I. (1991) Central thermoregulatory inhibition by general anesthesia. *Anesthesiology* **75**, 557–559.

SHAFTON, A. D., OLDFIELD, B. J. & MCALLEN, R. M. (1992). CRF-like immunoreactivity selectively labels preganglionic sudomotor neurons in cat. *Brain Research* **599**, 253–260.

Acknowledgements

This work was supported by a grant-in-aid from the National Heart Foundation of Australia and by a block grant to the Howard Florey Institute from the National Health and Medical Research Council of Australia. We are grateful to Daphne Hards, Michelle Giles, Andrew McCubbin and David Trevaks for technical help.

Received 17 March 1994; accepted 17 July 1994.