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DIFFERENTIAL CONTROL OF SYMPATHETIC FIBRES SUPPLYING HINDLIMB SKIN AND MUSCLE BY SUBRETROFACIAL NEURONES IN THE CAT

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SUMMARY

1. Simultaneous recordings were made from postganglionic sympathetic fibres supplying hindlimb skin and skeletal muscle in chloralose-anaesthetized, artificially ventilated cats. Single-fibre activity was either isolated by dissection or discriminated from few-fibre preparations of fascicles in the left superficial peroneal or sural nerve (innervating hairy skin) and common peroneal nerve (innervating muscle). Vasoconstrictor fibres were identified by their spontaneous activity as well as their responses to stimulation of the lumbar sympathetic chain and to changes in baroreceptor activity. The baroreceptors were then denervated by bilateral section of the vagi, carotid sinus and aortic nerves.

2. In five cats, neurones in the region of the subretrofacial nucleus were activated chemically by microinjections of 2–10 nl 0·5 M-sodium gluatmate from a micropipette inserted into the ventral surface of the medualla. Both skin and muscle vasoconstrictor fibres were activated by glutamate injections into this region on either side of the medulla. Arterial pressure also rose.

3. Glutamate injections at forty-two sites evoked a positive response, defined as an increase in cutaneous and/or muscle vasoconstrictor fibre activity of at least 25%. This response was evoked only in the cutaneous fibre at sixteeen of these sites ('skin points'), only in the muscle fibre at seven sites ('muscle points'), and in both fibres in the remainder ('mixed points'). The largest percentage increases in activity of either type of fibre were obtained from mixed points.

4. The blood pressure rises following glutamate stimulation of muscle points were signficantly greater than those produced by stimulation of skin points. Analysis of all positive responses showed that the evoked rise in blood pressure was significantly correlated with muscle sympathetic activity but not with cutaneous sympathetic activity.

5. Glutamate stimulation at different sites could evoke differential responses in skin and muscle vasoconstrictor fibres without any detectable change in the pattern of phrenic nerve discharge.

6. Skin points were grouped in the medial part of the subretrofacial region, and

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muscle points in the lateral part. In addition, for all positive responses there was a highly significant correlation between the ratio of muscle to cutaneous sympathetic activity evoked, and the distance from the mid-line of the corresponding injection site.

7. These results demonstrate a functional differentiation among subretrofacial neurones in their relative control of the sympathetic vasoconstrictor supply to skin and skeletal muscle. Further, they strongly suggest that such neurones are organized topographically.

INTRODUCTION

Much evidence now supports the view that neurones close to the surface of the rostral ventrolateral medulla are a major source of sympathetic vasomotor drive (Feldberg, 1976; Reis, Ross, Ruggiero, Granata & Joh, 1984; Dampney, Goodchild & Tan, 1985). Studies in the rabbit (Dampney, Goodchild, Robertson & Montgomery, 1982; Dampney *et al.* 1985), cat (McAllen, 1986*b*) and rat (Reis *et al.* 1984) have shown that the sites within the ventrolateral medulla which evoke large pressor responses to microinjections of excitant amino acids are located just ventral to the retrofacial nucleus. This region, which has been termed the 'subretrofacial nucleus' (McAllen, 1986*a*; Dampney, Goodchild & McAllen, 1987) contains a group of bulbospinal neurones which project directly to the intermediolateral cell column in the thoracolumbar spinal cord (Amendt, Czachurski, Dembowsky & Seller, 1978; Blessing, Goodchild, Dampney & Chalmers, 1981; Ross, Ruggiero, Joh, Park & Reis, 1984; Dampney, Czachurski, Dembowsky, Goodchild & Seller, 1987).

Chemical stimulation of the subretrofacial nucleus activates sympathetic nerves supplying the heart, blood vessels and adrenal medulla (Dampney *et al.* 1985; McAllen, 1986*c*) but apparently not sympathetic nerves innervating non-cardiovascular organs such as sweat glands, smooth muscle of the gut or pupillodilator muscles (McAllen, 1986*c*). It is still uncertain, however, whether neurones within the subretrofacial nucleus have a generalized action on the sympathetic outflow to the cardiovascular system, or whether individual neurones may preferentially or exclusively control the activity of sympathetic nerves innervating particular end-organs.

Suggestions that there may be functional specificity among subretrofacial neurones have come from the finding that chemical stimulation of some points within this region may have divergent actions on heart rate, blood pressure and individual vascular beds or sympathetic nerves (Lovick & Hilton, 1985; McAllen, 1986*c*). Other interpretations are possible, however. For example, both the magnitude and direction of the heart rate changes are variable, but this is largely due to a vagal bradycardia that overlays any primary sympathetic responses (McAllen, 1986*c*). Furthermore, the changes in blood pressure and haemodynamic state induced directly by subretrofacial neurones would undoubtedly trigger secondary reflexes (mediated by baroreceptors and cardiopulmonary afferents) that are known to affect vasomotor responses unequally (Folkow & Neil, 1971). Finally, in the case of vascular resistance measurements, there is the additional likelihood of interference by non-uniform actions on vessles by humoral agents such as adrenaline, which may be released in substantial amounts (McAllen, 1986*c*).

The present experiments were designed to overcome these objections and test the hypothesis that subgroups of subretrofacial neurones preferentially control the activity of the sympathetic vasomotor nerves which innervate a specific type of vascular bed. We therefore recorded from functionally indentified fibres (those supplying skin and skeletal muscle in the hindlimb) under conditions where baroreceptors and vagal reflexes were removed. Activity was recorded from both types of fibre simultaneously, so that their responses to identical stimuli could be compared. Groups of subretrofacial neurones were then activated by very small ($\leqslant 10$ nl) injections of sodium glutamate into different sites at the ventrolateral surface of the medulla.

Some of these results have been published in abstract form (Dampney & McAllen, 1986).

METHODS

Experiments were performed on five cats anaesthetized with α-chloralose (70 mg/kg) given intravenously after an intramuscular injection of ketamine hydrochloride (12 mg/kg). One animal was paralysed with gallamine triethiodide (10 mg/kg initial dose), though all were ventilated artificially during the experiment at a level that kept end-tidal CO_2 (monitored by a Beckman LB1 analyser) close to 4%. Rectal temperature was monitored with a thermistor probe and kept around 38·5 °C by an electric blanket.

After tracheostomy and cannulation of the right femoral artery and vein, the animal was mounted supine in a stereotaxic frame. The ventral medulla was then exposed as described previously (McAllen, Neil & Loewy, 1982). Briefly, the oesophagus and larynx were vascularly isolated from the neck before being divided between stout ligatures, and the cranial segment was retracted to expose the underlying muscles. These were then scraped clear from the atlanto-occipital membrane and the periostium up to the level of the tympanic bullae. The parts of occipital and sphenoid bones overlying the ventral medulla were removed with rongeurs and a dental drill to approximately 5 mm from the mid-line on each side. At this stage the dura was left intact.

The left lumbar sympathetic trunk was exposed retroperitoneally through a flank incision by dissection of the psoas muscle. A sleeve electrode pair (McAllen & Spyer, 1978) was placed around the trunk just rostral to the L6 ganglion, surrounded with Parafilm and covered with molten low melting-point (42 °C) wax. The wound edges were approximated and the electrodes left *in situ*.

An incision was made in the lateral aspect of the left leg to expose the peroneal nerves. A fascicle of the common peroneal nerve supplying the peroneal muscles, and (in two cats) a fascicle of the superficial peroneal nerve supplying the skin or (in three cats) the sural nerve, were indentified and dissected. A paraffin pool was made in the leg (Fig. 1*A*), and the arrangement prepared for recording with pairs of platinum wire electrodes over dissecting platforms made from laryngeal mirrors. The two electrodes were connected to differential amplifiers and the signals displayed on an oscilloscope. The territory innervated by each fascicle was determined anatomically by tracing its branches by dissection, then tested physiologically by recording its afferent response. By both criteria fascicles selected from the common peroneal nerve supplied only muscle, while those selected from the superficial peroneal nerve or the sural nerve supplied only skin. The territory innervated by superficial peroneal nerve fascicles, however, while consisting mostly of hairy skin of the ankle and dorsum of the foot, included a small, variable part of the pad. The sural nerve, however, was confirmed to supply only hairy skin on the back of the leg.

Once the territory of its innervation had been determined, each fascicle was crushed distally, the perineurium opened, and dissected filaments placed over the recording electrodes. Sympathetic fibres were identified in single or few-units preparations by their response to stimulation of the lumbar sympathetic trunk (Fig. 1*B*). Action potentials from single fibres in each nerve were then discriminated using Neurolog NL 200 spike triggers, while their spike shape was monitored on a variable persistence storage oscilloscope (Fig. 1*C*). Cutaneous and muscle sympathetic activity, together with blood pressure and a voice/event channel were recorded on magnetic tape for later analysis. Blood pressure was recorded from the femoral artery with a transducer, and heart rate

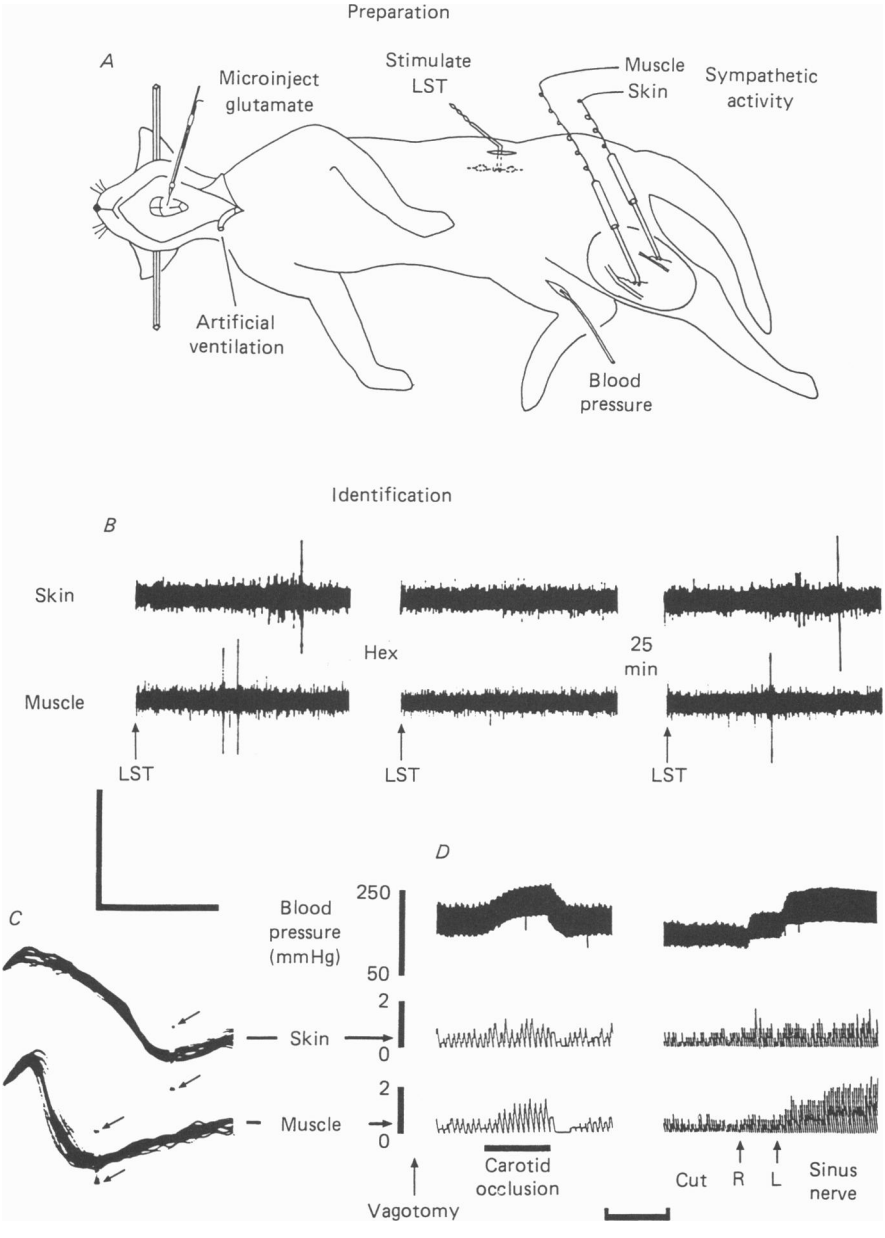

Fig. 1. For legend see opposite.

derived from it by a purpose-built unit. These as well as standard pulses derived from discriminated cutaneous and muscle sympathetic fibre action potentials were displayed on a Grass polygraph.

Once a discriminable, spontaneously active single unit had been isolated from each nerve, the dura was removed from the ventral medulla and artificial ventilation was begun. The units were then tested for their response to occlusion, release of occlusion and traction of the carotid arteries. Following these tests, the baroreceptors were denervated by cutting the vagi and both carotid sinus and aortic nerves. In one cat the superficial peroneal (cutaneous) fibre was lost soon after baroreceptor denervation. A new unit was then isolated which, while it could not be tested for

baroreceptor sensitivity, obeyed the other criteria for identification as a cutaneous vasoconstrictor fibre. Since these are almost certainly adequate (see Discussion), data from this experiment (comprising one muscle, three skin and six mixed points: see Results) were included. Eliminating the entire experiment would not have altered any conclusion or the statistical significance of any result.

Microinjections of 2–10 nl sodium glutamate solution (0·5 M, pH 7·2–7·4), were made in the ventral medulla via a glass micropipette, which was positioned by a micromanipulator, such that its tip was 0·5 mm below the surface. Injections were made by applying air pressure and the volume measured by determining the displacement of the meniscus (McAllen, 1986*b*). The co-ordinates of insertion points were measured by micromanipulator readings and plotted for each animal on a map of brain surface structures. The reference points (also measured by micromanipulator readings and confirmed with greater precision post-mortem after stripping off the pia mater) were the mid-line, caudal border of the trapezoid body, the most rostral hypoglossal rootlets and the lateral longitudinal sulcus (see Fig. 4). This method allowed points on the surface to be localized with an error of not more than 0·1 mm. A composite map of points taken from all five experiments was constructed from the five individual maps on a 'best fit' basis for the surface landmarks.

In addition, one or more selected points in each experiment were marked by injection of approximately 30 nl 2% Pontamine Blue dye. The medulla was fixed post-mortem by immersion in 10% formalin, and dye spots were localized histologically from 50 μm frozen sections counterstained with neutral red. This allowed the centres of these selected injection sites to be mapped in relation to nuclei, particularly the retrofacial nucleus and inferior olive.

Both types of sympathetic fibre could be activated by stimulation of either the ipsilateral or contralateral sides of the ventral medulla. There was no apparent difference in the response patterns obtained. The descending pathways from the medulla to hindlimb skin and muscle sympathetic fibres decussate caudal to the cervical spinal cord, since both can be activated by electrical stimulation on either side of the cervical cord in spinal cats (R. A. L. Damphey & R. M. McAllen, unpublished observations). Data from ipsilateral and contralateral injections were therefore pooled.

Neural recordings were analysed from tape, each channel separately, using a time-window discriminator to detect single unit activity, which was counted in 3 or 5 epochs with a Neurolog NL 601 pulse integrator. During this, discrimination of the single unit was monitored continuously (Fig. 1*C*). Responses were analysed only if (i) single units could be descriminated and (ii) the volume of sodium glutamate solution injected was 10 nl or less. When these criteria were met, the number of spikes occurring during the 15 s period at the peak of the response was counted, and compared with the mean firing rate preceding the injection. In one cat the slow, irregular discharge of a cutaneous vasoconstrictor unit necessitated counting responses over a 30 s period.

Comparisons of unpaired measurements were made using the Wilcoxon rank sum test, and correlations of paired measurements were made by calculating Spearman's rank correlation coefficient (Colton, 1974). All values are presented as the mean $\pm$ S.D.

Fig. 1. *A*, diagram of experimental preparation. *B*, oscilloscope traces of simultaneous single-unit recordings from fascicles of the sural (upper trace: 'skin') and common peroneal (lower trace: 'muscle') nerves firing in response to electrical stimulation of the lumbar sympathetic trunk (LST). The 'muscle' sympathetic fibre initially responds with two action potentials. Between the first and second panels hexamethonium bromide (Hex; 1 mg/kg) was given intravenously. Traces shown in the second panel were taken approximately 2 min and those in the third panel 25 min later. Calibration bars: 50 μV, 250 ms. *C*, the same pair of units as in *B* shown superimposed on a faster time-scale (here the bar = 0·5 ms). Arrows mark the time–voltage window through which spikes had to pass to be discriminated and counted. *D*, chart record of counted action potentials of these two fibres (as indicated) and systemic blood pressure (upper trace) showing the effects of bilateral carotid occlusion and section of the sinus nerves. Time bar: 1 min in left panel, 2 min in right panel. The spike counters were reset every 5 s and the calibrations are given in spikes/s.

RESULTS

Identification and characterization of sympathetic postganglionic units

Simultaneous recordings were made from filaments split from cutaneous (superficial peroneal or sural) and muscle (common peroneal) nerve fascicles in the left hindlimb, and single units identified by their response to stimulation of the ipsilateral lumbar sympathetic trunk (Fig. 1*B*). This response was abolished by hexamethonium bromide (1–2 mg/kg I.V.) in the three cats tested (e.g. Fig. 1*B*). The response latencies to stimulation of the sympathetic trunk and the conduction distance between the stimulating and recording electrodes were determined. From these measurements the overall conduction velocities were calculated to be $0{\cdot}73 \pm 0{\cdot}06$ m/s ($n = 5$) for cutaneous sympathetic fibres, and $0{\cdot}77 \pm 0{\cdot}29$ m/s ($n = 5$) for muscle sympathetic fibres. Both fibre types increased their activity on carotid occlusion and decreased it when the occlusion was removed (Fig. 1*D*). Denervating baroreceptors by section of vagi, aortic and sinus nerves also increased the activity of both fibre types (Fig. 1*D*). All these manoeuvres affected muscle more than cutaneous sympathetic activity. After baroreceptor denervation the mean resting blood pressure was in the range 120–190 mmHg.

Choice of stimulus

As in previous studies (Dampney *et al.* 1985; McAllen, 1986*b*, *c*), we used microinjections of 0·5 M-sodium glutamate to stimulate cell bodies but not axons (Goodchild, Dampney & Bandler, 1982), although in the present study we injected smaller volumes (2–10 nl) to achieve better spatial resolution. This was demonstrated by the fact that in each of three control experiments the largest pressor response (*ca.* 70 mmHg) was obtained from a site as little as 0·5 mm away from sites yielding no detectable response. We also confirmed that glutamate microinjections repeated at the same pressor site produced blood pressure increases of similar magnitude, provided the interval between injections exceeded 10–15 min. Microinjections of 0·5 M-sodium chloride or 50 mM-potassium chloride at such sites had no detectable effect on blood pressure. In confirmation of previous studies in rabbit (Goodchild *et al.* 1982) and rat (Willette, Barcas, Krieger & Sapru, 1983), lower concentrations of glutamate produced responses of smaller magnitude, but always in the same direction.

Effects of glutamate stimulation of subretrofacial neurones on sympathetic activity

The ventral medulla was explored with a micropipette containing sodium glutamate: the left side only in one cat, but both sides in the other four. Microinjections were made with the pipette tip 0·5 mm below the surface. At forty-two sites these evoked a positive response, which we define as an increase in muscle and/or cutaneous sympathetic fibre activity greater than 25% of the pre-stimulus control level. At nineteen of these sites (referred to here as 'mixed points'), they elicited positive responses in both fibre types (e.g. Fig. 2*A*). At sixteen sites ('skin points'), they produced a positive response in only the cutaneous fibre (e.g. Fig. 2*B*), while at seven others ('muscle points'), positive responses were produced in only the muscle fibre (e.g. Fig. 2*C*). At least one point of each type was found in every cat.

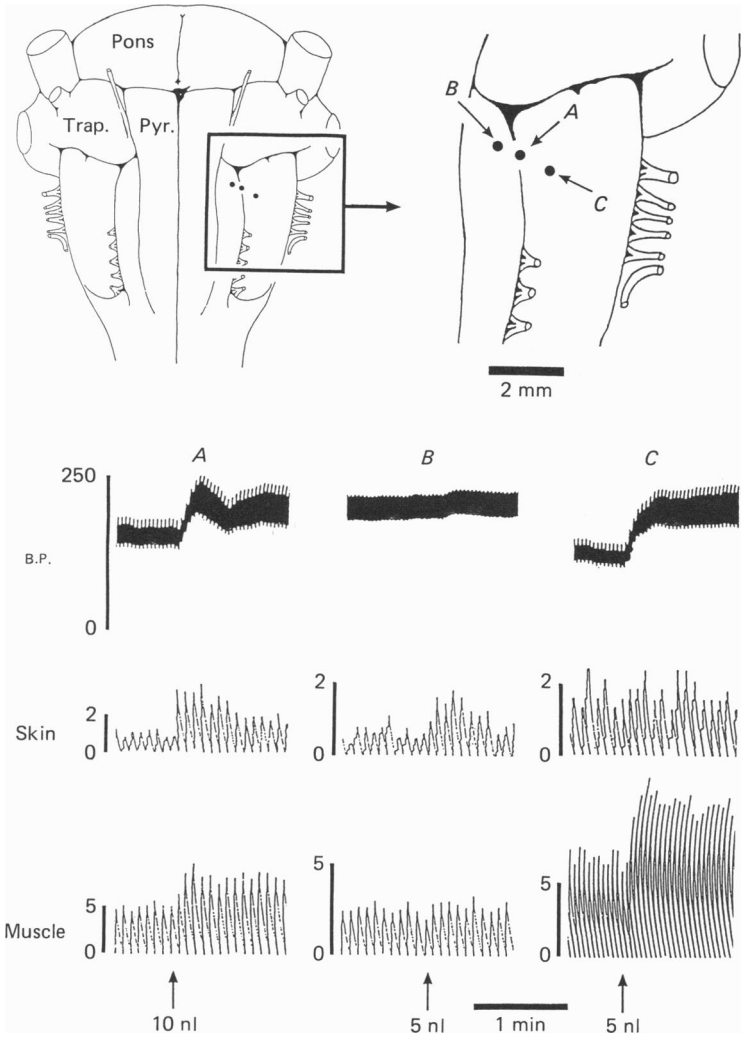

Fig. 2. Above: ventral view of cat brain stem showing three glutamate injection sites (not from the same animal), corresponding to the traces shown below (the inset, at higher magnification, indicates which). Labelled are the Pons, trapezoid body (Trap.) and pyramid (Pyr.) Below: traces show (from above) blood pressure (B.P.) in mmHg. and simultaneous activity records (calibrated in spikes/s) of single sympathetic fibres supplying skin and muscle. Times and volumes of glutamate injections are indicated below by arrows. *A*, injection into a mixed point; *B*, a skin point; *C*, a muscle point.

Injections at forty-one sites within the same general region failed to evoke a positive reponse in either nerve.

The mean increase in cutaneous sympathetic activity for the skin points was $51 \pm 29\%$, but for the mixed points it was $91 \pm 61\%$. This difference was statistically significant ($P < 0{\cdot}01$). In the case of muscle sympathetic activity, the mean increase was $56 \pm 21\%$ for the muscle points, and $72 \pm 53\%$ for the mixed points, but this difference was not statistically significant ($P > 0{\cdot}4$).

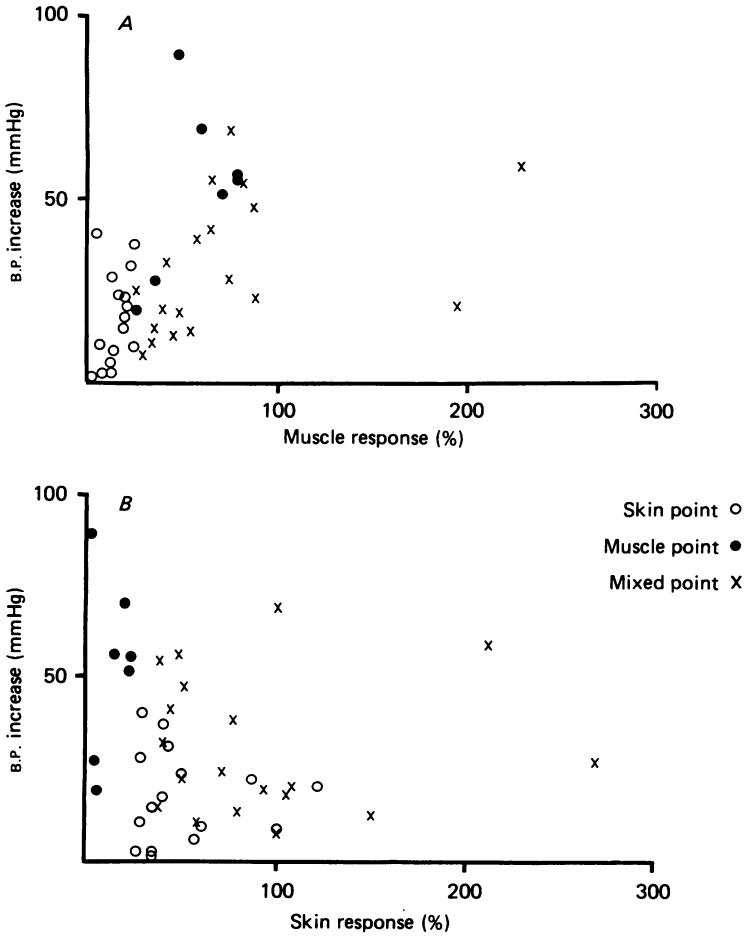

Fig. 3. Graphs, for all positive responses, of the rise in blood pressure following glutamate injections against the corresponding increase in muscle sympathetic activity (*A*) and skin sympathetic activity (*B*) expressed as a percentage of control levels. Data from skin, muscle and mixed points are shown by the symbols indicated.

Correlation between pressor and sympathoexcitatory responses

The pressor response produced by stimulation of the muscle points was 52 ± 24 mmHg, whereas for the skin points it was 17 ± 13 mmHg. This difference was highly significant ($P < 0{\cdot}005$). In addition, for all positive responses the evoked change in mean blood pressure was plotted against the percentage increase in muscle (Fig. 3*A*) and cutaneous (Fig. 3*B*) sympathetic activity. The evoked changes in blood pressure showed a strong positive correlation to the associated changes in muscle sympathetic activity (Spearman's rank correlation coefficient (r_s) of 0·622), which was highly significant ($P < 0{\cdot}001$), but a weak negative correlation to changes in cutaneous sympathetic activity (r_s of $-0{\cdot}207$), which was not statistically significant ($P > 0{\cdot}1$).

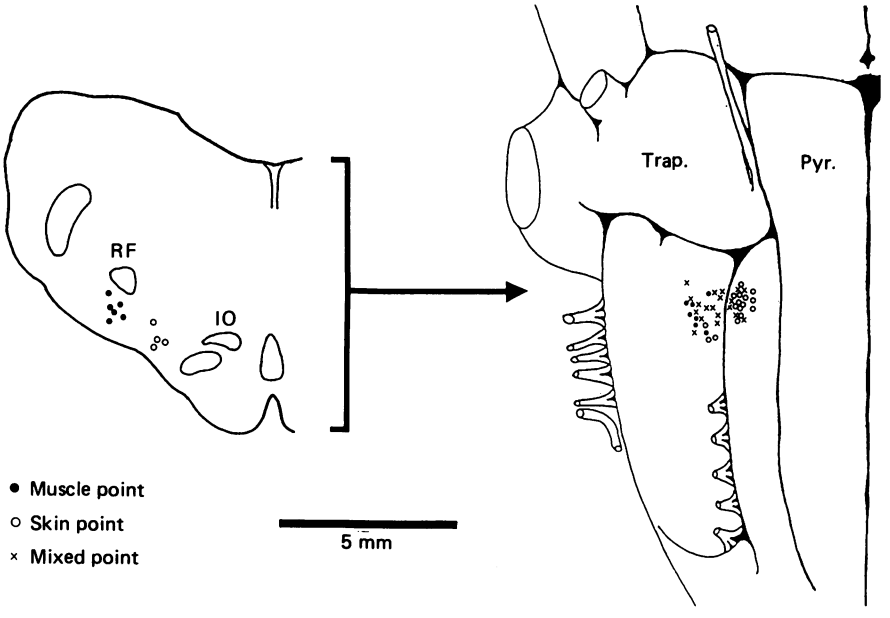

Fig. 4. Right: ventral view of half brain stem (abbreviations as in Fig. 2) on which are plotted all injection sites giving positive responses: skin, muscle and mixed points as indicated. Left: cross-section map, taken at the level indicated, showing the marked locations of six muscle points and four skin points. Abbreviations: IO, inferior olive; RF, retrofacial nucleus.

Location of sympathoexcitatory sites

The locations of all the sites producing positive responses were plotted in relation to surface landmarks (see Methods) onto the same side of a standard view of the ventral medullary surface (Fig. 4). A striking finding was that the muscle points were grouped laterally, while the skin points were more medial. The position of five muscle points and four skin points marked with Pontamine Blue are illustrated on a coronal section of the medulla, taken at the level of the rostral pole of the inferior olive (Fig. 4). The muscle points were located just ventral to the retrofacial nucleus, while the skin points were found medial to this region.

The relationship between the pattern of sympathetic responses and site of stimulation was also analysed quantitatively by plotting, for each positive response, the ratio of evoked increase in muscle sympathetic activity to increase in cutaneous sympathetic activity against the distance of the injection site from the mid-line (Fig. 5). The correlation between this ratio and the lateral distance was highly significant ($r_s = 0{\cdot}687$, $P < 0{\cdot}001$).

Dissociation between sympathetic and respiratory responses

In one experiment, phrenic nerve activity was also recorded to test whether evoked changes in the activity of central respiratory neurones might account for differential responses in muscle and cutaneous sympathetic fibres. Figure 6 shows responses evoked by three injections in this cat at the sites indicated. Figure 6*A* and

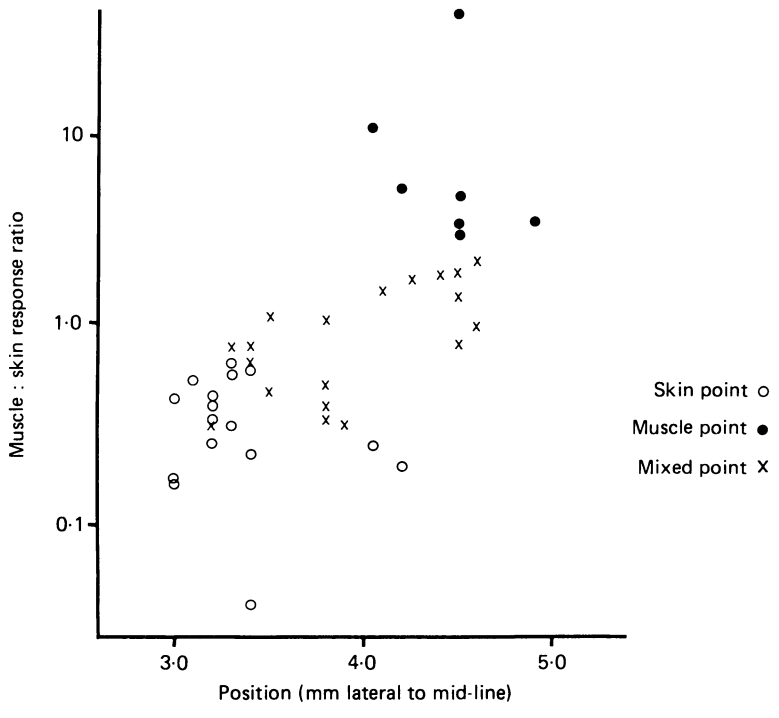

Fig. 5. Graph of the ratio of percentage increases produced in muscle and skin sympathetic fibre activity (log scale) against the lateral position of the injection site, for all positive responses. Skin, muscle and mixed points as indicated.

B show a mixed response and a muscle response, respectively. There was no detectable change in phrenic nerve activity in either case. In confirmation of previous studies (McAllen, 1986*b*), however, injections into sites rostrolateral to the subretrofacial region produced an increase in phrenic nerve discharge (Fig. 6 *C*), due to increases in both respiratory frequency and peak phrenic neural discharge. This respiratory response was accompanied by a small increase in blood pressure (Fig. 6 *C*) but no obvious change in cutaneous or muscle sympathetic activity.

DISCUSSION

Sympathetic nerve recordings

The first point to discuss is what types of fibre were recorded from in these experiments. There are strong reasons for believing that they were sympathetic postganglionic vasoconstrictor fibres to the tissues supplied by their respective nerves (skin and muscle). First, the units studied all responded to stimulation of the ipsilateral sympathetic chain, and in the three cats tested this evoked activity was blocked by hexamethonium. Their calculated overall conduction velocities were comparable to values determined by Jänig (1985) for cutaneous and muscle postganglionic vasoconstrictor fibres.

Secondly, all the units studied were spontaneously active. The only known classes

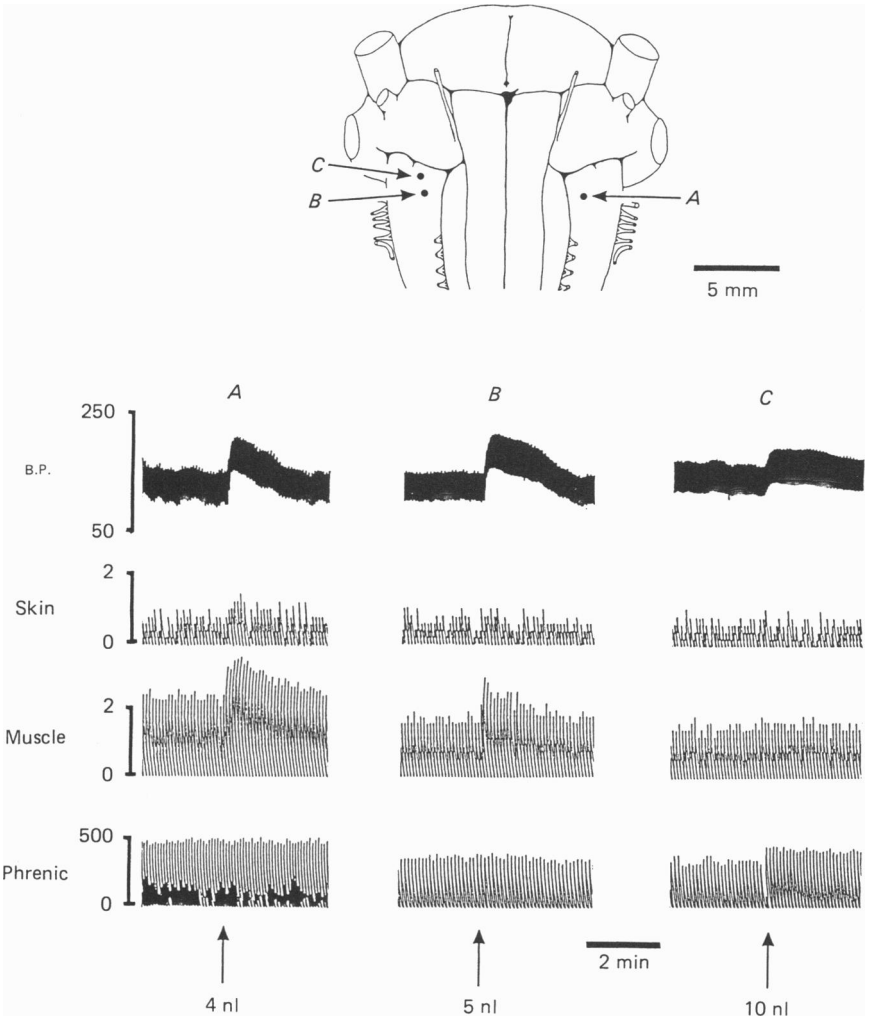

Fig. 6. Results from a single experiment in which phrenic nerve activity was recorded. Above: ventral view of brain stem, as in Fig. 2, with injection sites indicated. Below: corresponding chart records (from above); blood pressure in mmHg, single cutaneous sympathetic, single muscle sympathetic and whole phrenic nerve activity recordings calibrated in spikes/s. Times and volumes of glutamate injections are indicated below by arrows.

of sympathetic fibre supplying hindlimb skeletal muscle are vasoconstrictor and (cholinergic) vasodilator fibres. Of these two fibres types, only the vasoconstrictors show spontaneous activity in anaesthetized animals (Jänig, 1985). The sympathetic supply to the hairy skin in the hindlimb consists entirely of vasoconstrictor fibres, which also show spontaneous activity (Jänig, 1985). At least in anaesthetized animals, no tonic acitivity is shown by any other known type of sympathetic fibre innervating the hindlimb except sudomotor fibres, whose territory is restricted to the pad (Jänig, 1985). While it is true that the (cutaneous) superficial peroneal fascicles

sometimes included a minor part of the pad in their territory, and so theoretically may have carried a few sudomotor fibres, Jänig (1985) did not find this fibre type here. This objection does not apply to the sural nerve, which innervates only hairy skin. Fibres split from this nerve (in three cats) responded in a manner indistinguishable from those in the superficial peroneal nerve (two cats).

Finally, the responses of fibres split from muscle and cutaneous nerves to changes of arterial baroreceptor input were entirely consistent with the properties of muscle and cutaneous sympathetic fibres as determined by others (Jänig, Kümmel & Wilprich, 1980). We therefore conclude that our recordings from cutaneous nerves were of cutaneous vasoconstrictor activity, and those from muscle nerves were of muscle vasoconstrictor activity.

Chemical stimulation of subretrofacial neurones

The advantages and limitations of the method of glutamate microinjection as a means of exciting central neurones have been discussed elsewhere (Goodchild *et al.* 1982). While there is good evidence that glutamate excites cell bodies and dendrites, but not axons of passage (Curtis & Ryall, 1966; Fries & Zieglgansberger, 1974; Goodchild *et al.* 1982), it can also evoke a period of depression following this excitation (Crawford & Curtis, 1964; Engberg, Flatman & Lambert, 1979). Such biphasic responses, though, do not appear to have affected our results. There is now abundant evidence that activating subretrofacial neurones increases sympathetic activity and blood pressure (see Introduction). This we have confirmed in the present experiments, where the excitatory sympathetic responses occurred immediately after glutamate was injected, and generally lasted a minute or more.

The excitation was not due to the effects of injection volume or sodium ions since injections of equal volumes of equimolar sodium chloride were without effect. Further, it appears unlikely to be due to secondary release of potassium ions, since 50 mM-potassium chloride was similarly ineffective.

Differential activation of cutaneous and muscle vasoconstrictor nerves

Our results show clearly that microinjections of sodium glutamate at different sites within the ventrolateral medulla could produce differentiated patterns of cutaneous and muscle sympathetic excitation, depending on the site of injection. The simplest explanation for this finding is that there are subgroups of vasomotor neurones within the subretrofacial region which exert a preferential, or even exclusive, control of sympathetic outputs to specific vascular beds. Before this is accepted, however, alternative explanations should be considered.

First, it could be argued that all subretrofacial neurones drive muscle and cutaneous sympathetic nerve activity in a uniform fashion, but that changes in blood pressure resulting from sympathetic excitation activate peripheral baroreceptors, which may then reflexly affect sympathetic activity in a non-uniform way. This possibility is ruled out, however, by the fact that the carotid sinus, aortic and vagal nerves were cut.

A second possibility is that medial and lateral glutamate microinjections might have different actions on respiration which, in turn, could exert unequal effects on cutaneous and muscle vasoconstrictor activity. Since all animals were vagotomized

and artificially ventilated, we are concerned here only with the direct actions of central respiratory drive on vasomotor neurones. These actions most commonly take the form of an excitatory input, which is closely related to phrenic nerve (i.e. inspiratory) activity (Preiss & Polosa, 1977), and indeed this type of influence has been found on hindlimb muscle vasoconstrictor neurones, though only weakly on skin vasoconstrictor neurones (Jänig *et al.* 1980). When phrenic discharge was measured in the present study, however, it became evident that the responses seen in muscle or cutaneous sympathetic nerves could not be attributed to changes in central respiratory drive. In confirmation of previous findings (McAllen, 1986*b*), glutamate injections into neighbouring areas of the ventral medulla could increase phrenic activity, yet leave sympathetic nerves unaffected. Furthermore, other injections activated muscle and cutaneous sympathetic nerves differentially without causing any detectable change in phrenic activity.

Finally, it could be argued that non-uniform sympathetic responses could be generated by a common population of medullary neurones, if these were connected to output pathways of different threshold. Thus, a lower degree of stimulation (e.g. by a non-focal microinjection) might activate only one outflow, but stronger stimulation activate both. This mechanism, however, could not explain how in the same animal a much greater relative excitation in the cutaneous fibre could be evoked by one injection, and in the muscle fibre by another. Responses of both types were observed in every cat. The same argument applies to the possibility that changes in the excitability of medullary neurones, due to toxic effects of glutamate, could produce differential patterns of sympathetic response. We conclude that there must be more than one population of subretrofacial neurones driving sympathetic vasoconstrictor nerves.

Specificity of subretrofacial neurone actions

It is possible that there are neurones which exclusively regulate the activity of muscle sympathetic fibres without any influence on cutaneous sympathetic activity, and vice versa. Alternatively, such neurones may exert a more powerful control over one type of sympathetic output than another. Our results do not distinguish between these possibilities, nor do they exclude the possible existence of additional neurones with unselective actions. It is interesting, however, that while evoked increases in muscle sympathetic activity were strongly correlated with rises in blood pressure, evoked increases in cutaneous sympathetic activity were not, and often occurred in isolation. Therefore, the control by subretrofacial neurones of cutaneous sympathetic discharge, at least, may be highly specific.

In this context it is also noteworthy that Barman, Gebber & Calaresu (1984), in an electrophysiological study of this region of the medulla, found single units whose spontaneous activity was correlated with sympathetic nerve discharge. Some showed activity more strongly correlated with that of the external carotid than renal nerve, some the reverse, and others equally with both. Their results therefore also support the idea of selectivity among ventral medullary neurones controlling sympathetic nerves but, because of limitations of the correlation method and whole-nerve recordings, similarly fail to distinguish whether such selectivity is relative or absolute.

Since the hindlimb sympathetic fibres receive inputs from preganglionic neurones which arise from similar spinal segments, it seems probable that the specificity of control of sympathetic activity by subretrofacial neurones is related to the tissue innervated and not to the segmental level of the outflow. Functional specificity of subretrofacial neurones, such that a particular neural pool drives vasoconstrictor fibres to only one type of vascular bed throughout the body, could account for the observation in man that muscle vasoconstrictor activity in the arm is highly correlated with muscle vasonstrictor activity in the leg, but not with cutaneous sympathetic activity in the arm (Jänig, Sündlof & Wallin, 1983). It also makes it easier to account for the differential control of vascular beds in different end-organs by reflexes (e.g. the baroreceptor reflex: Ninomiya, Nisimaru & Irisawa, 1971) or during more complex behaviours (e.g. thermoregulatory responses: Simon & Riedel, 1975).

Spatial separation of skin and muscle points.

It was a consistent finding that muscle sympathetic fibres were activated preferentially by lateral, and cutaneous fibres by medial glutamate injections. It should be emphasized, however, that the position of an injection site does not tell us exactly the location of the neurones excited. All that can be said is that their glutamate-sensitive parts (i.e. soma or dendrites) are within a limited radius of that site. It is possible, for example, that all subretrofacial neurones are intermingled, but those driving skin vasoconstrictor fibres extend their dendrites more medially, while those driving muscle have processes directed laterally. It seems more probable, however, that neurones preferentially driving muscle vasoconstrictor fibres are located in the lateral part of the nucleus and those serving skin in the medial part. Thus, glutamate injected medial or lateral to the nucleus would spread in higher concentration to the subretrofacial neurones located in the nearer side of the nucleus. The histologically localized injection sites for muscle and skin points are compatible with this view, as is the observation that the largest increases in either fibre type were evoked from sites in between (mixed points).

In his recent review, Jänig (1985) posed the following question to be answered by future research: 'Can one discriminate between different types of descending spinal pathways with respect to functionally identified preganglionic neurones?' At least for subretrofacial neurones controlling hindlimb postganglionic vasoconstrictor fibres, the answer appears to be 'yes'.

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