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CHARACTERISTICS OF SYMPATHETIC PREGANGLIONIC NEURONES IN THE LUMBAR SPINAL CORD OF THE CAT

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SUMMARY

1. In anaesthetized cats extracellular recordings have been made from antidromically identified sympathetic preganglionic neurones, located in the 2nd and 3rd lumbar segments, with axons projecting into the left lumbar sympathetic chain beyond the L4 ganglion. Sympathetic preganglionic neurones have been characterized with respect to: axonal conduction velocities, firing patterns in relation to ECG and phrenic nerve activity, responses to noxious stimuli applied to the ipsilateral hindlimb and ionophoretically applied 5-HT.

2. Two hundred and ninety-seven sympathetic preganglionic neurones were studied. Their axonal conduction velocities (0·5–13·9 m/s) were in the B and C fibre range. Sixty-eight had on-going activity and the remainder were quiescent. Of the 229 quiescent sympathetic preganglionic neurones, 111 were activated by the ionophoretic application of glutamate.

3. Of the 100 sympathetic preganglionic neurones analysed for an ECG-related pattern of discharge, forty-nine had no, and fifty-one had an ECG-related pattern of discharge. Both sympathetic preganglionic neurones with on-going activity and glutamate activated cells exhibited ECG-related patterns of discharge.

4. Only six of fifty sympathetic preganglionic neurones had a respiratory-related activity pattern. Three had maximal discharge during expiration and three during inspiration.

5. Forty-one sympathetic preganglionic neurones were examined for their response to noxious stimulation of the ipsilateral hindlimb. Ten had their activity decreased (seven glutamate-activated, three with on-going activity), seven had their activity increased (four glutamate-activated and three with on-going activity) and twenty-four were unaffected. These results demonstrate that both sympathetic preganglionic neurones with on-going activity and glutamate-activated neurones can be influenced by noxious input. Ten sympathetic preganglionic neurones had properties consistent with them having a skin vasoconstrictor function and three with muscle vasoconstrictor function.

6. Ionophoretic application of 5-HT in the vicinity of fifty-one sympathetic preganglionic neurones caused increases in the discharge of 53%, decreases in the firing of 12% and did not affect the discharge of 35%.

7. Sympathetic preganglionic neurones which had excitatory responses to 5-HT

showed only decreased discharge or no response to noxious stimulation of the ipsilateral hindlimb. Conversely, sympathetic preganglionic neurones which had discharge decreased by 5-HT had primarily excitatory responses to noxious inputs.

8. It is concluded that lumbar sympathetic preganglionic neurones consist of a heterogeneous population with respect to their physiological properties and their responses to ionophoretically applied 5-HT: both may be related to function.

INTRODUCTION

Sympathetic preganglionic neurones (SPNs) synapse in ganglia that are remote from the end-organs which the postganglionic neurones they make connections with innervate. As, unlike parasympathetic preganglionic neurones and somatic motoneurones, they cannot be identified functionally on the basis of their axonal projections (see Gilbey, Jordan, Richter & Spyer, 1984; Crone, Hultborn, Kiehn, Mazières & Wigström, 1988) for many years an alternative method has been sought. The physiological characteristics of an SPN may provide an insight into possible function and this approach has been pursued in the current study. This conjecture is supported by the findings of Jänig & Szulczyk (1980) who recorded from the fibres of second and third lumbar SPNs in the sympathetic chain caudal to the L4 ganglion. These fibres were examined for functional properties which typified those for postganglionic vasoconstrictor neurones supplying muscle and skin, and postganglionic sudomotor neurones. Their data indicated that under defined experimental conditions some SPNs have input profiles and activity patterns similar to those of postganglionic fibres, and therefore may subserve similar functions (see Jänig, 1985). This method of classification assumes that pre- and postganglionic neurones involved in the regulation of a particular target organ consist of separate pathways from spinal cord to end-organ (Jänig & Szulcyzk, 1981).

The fibre recording technique used by Jänig and co-workers has certain limitations regarding the analysis of central mechanisms controlling sympathetic activity: firstly, the majority of SPNs cannot be studied as they are quiescent and cannot be reflexly activated (see Jänig & Szulczyk, 1980); secondly, neuropharmacological studies cannot be carried out in which agonists and antagonists are applied by ionophoresis close to the soma and dendrites of the SPN giving rise to the fibre being recorded. For these reasons the present study has focused on examining certain characteristics of lumbar SPNs and assessing whether some of the neurones recorded in the present experimental situation have properties similar to those recorded in the study of Jänig & Szulczyk (1980).

Accordingly, sympathetic preganglionic neurones, located in L2 and L3 segments with axons projecting into the lumbar chain caudal to L4 ganglion, have been recorded extracellularly: axonal conduction velocities have been estimated in order to determine whether on the basis of this criterion a similar population of SPNs has been sampled as by Jänig & Szulczyk (1980); quiescent cells have been activated by the ionophoretic application of glutamate and input profiles and activity patterns of this population of SPNs have been studied and compared with those SPNs having on-going activity; the axonal conduction velocities, input profiles and activity patterns of SPNs observed under the present experimental conditions have been assessed in order to determine whether any of the SPNs have properties consistent

with muscle or skin vasoconstrictor function (see Jänig, 1985); finally, as the ionophoretic application of 5-HT in the vicinity of SPNs has been observed to either increase or decrease their discharge rate (Coote, Macleod, Fleetwood-Walker & Gilbey, 1981; Kadzielawa, 1983; Lewis & Coote, 1990), the possibility was investigated that the response of a SPN to ionophoresed 5-HT may be related to the SPNs' physiological characteristics.

Preliminary reports of this work have been published (Clark, Gilbey & Paton, 1988; Gilbey, Paton & Clark, 1988; Clark, Gilbey, Marks, Paton & Stein, 1989; Clark, Gilbey, Marks & Stein, 1990).

METHODS

Experiments were carried out on female cats (2–3 kg) which were anaesthetized with an I.V. injection of chloralose (80 mg/kg) plus sodium pentobarbitone (Sagatal, May and Baker; 12 mg/animal). Supplementary doses of anaesthetic were given when required (chloralose, 10 mg/kg, I.V.), as judged from recordings of heart rate, blood pressure, phrenic nerve activity and size of the pupils, and the cat's palpebral and paw-pinch reflexes. The muscle relaxant gallamine triethiodide (4 mg/kg) was administered during data collection. Within this period the animal was allowed to recover from neuromuscular block and the depth of anaesthesia was maintained at a level at which both corneal and flexor reflexes were sluggish. During neuromuscular blockade a miotic pupil, little or no response of pupillary diameter to noxious stimuli applied to the skin and stable blood pressure and heart rate were used to confirm the adequacy of anaesthesia. Additionally, it was ensured that noxious cutaneous stimulation produced only transient changes in arterial blood pressure.

In all animals the trachea was cannulated low in the neck, and catheters placed in a femoral artery and vein for monitoring arterial blood pressure and the administration of drugs, respectively. A catheter was placed in the bladder to allow for the continuous flow of urine. Animals were ventilated artificially on oxygen-enriched room air. End-tidal CO_2 was monitored continuously (P. K. Morgan Ltd, 901MK 2, CO_2 analyser) and arterial blood samples were taken periodically to check pH and blood gas tensions (Corning 158 pH/Blood Gas Analyzer). The values for pH and blood gas tensions were kept in the following range: pH, 7·35–7·45; P_{a,CO_2}, 30–40 mmHg: P_{a,O_2}, > 80 mmHg. An infusion of sodium bicarbonate was used to correct base deficits. All animals were given a pneumothorax and most animals were vagotomized (see below for details). Following the pneumothorax an end-expiratory pressure of 2–3 mmH_2O was applied to the expiratory line to prevent atelectasis. Rectal temperature was maintained at 37 ± 1 °C with a heating blanket controlled by a feedback circuit.

The left phrenic nerve was isolated by a dorsolateral approach, cut and on-going activity recorded from its desheathed central end by means of bipolar electrodes. The left lumbar sympathetic chain was exposed retroperitoneally by a left lateral incision, and stainless-steel stimulating electrodes wrapped around it between L4 and L6 ganglia for the antidromic identification of SPNs; the chain and electrodes were embedded in a vinyl polysiloxane impression material (Reprosil, light body, De Trey). The lumbar cord was stabilized by fixing the animal by pins at the iliac crests and a clamp attached to a lower thoracic vertebra; the head of the animal was fixed in a stereotaxic frame. A laminectomy was performed to expose L2 or L3 spinal cord segments. Skin flaps and muscle, at the laminectomy site, were sewn to a brass ring which added further support to the spinal cord. All exposed tissues at the laminectomy site were covered with liquid paraffin.

Extracellular recordings of the activity of SPNs were made through one barrel of a five- or seven-barrelled microelectrode assembly as described previously (Gilbey, Numao & Spyer, 1986). The recording barrel contained 4 M-NaCl; other barrels contained either 5-HT creatinine sulphate or 5-HT bimaleate dissolved in saline (0·05 M, pH 4·5), creatinine sulphate (0·05 M) in saline, or saline (pH 4·5), glutamate (0·2 M, pH 7·4) and 2% Pontamine Sky Blue in 0·5 M-sodium acetate for current balancing and marking recording sites. Glutamate was ionophoretically applied to excite quiescent SPNs. 5-Hydroxytryptamine was applied for periods of 20–250 s. A retaining current of +20 nA was applied to the glutamate solution and −10 nA to the 5-HT-containing barrel. SPNs were recorded in L2 and L3 segments.

Sympathetic preganglionic neurones were antidromically identified by collision testing using an orthodromic action potential to trigger the antidromic stimulus (see Results section). The axonal conduction velocities of SPNs were calculated from the distance between the recording site in the spinal cord and the stimulating electrode on the sympathetic chain divided by the antidromic

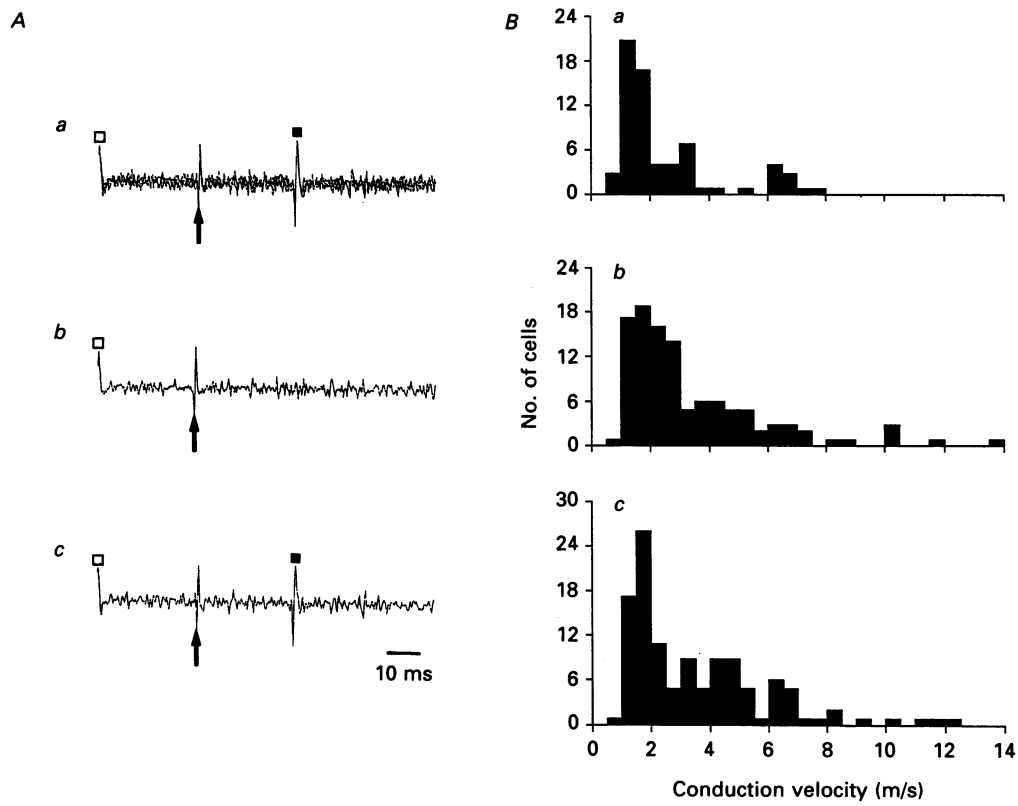

Fig. 1. *A*, antidromic identification of a lumbar sympathetic preganglionic neurone, projecting into the left lumbar sympathetic chain, by collision testing using an orthodromic action potential to trigger the antidromic stimulus. *a*, five superimposed antidromic action potentials; *b*, the delay between the orthodromic spike and the stimulus to the sympathetic chain has been reduced to 28 ms and collision has occurred; *c*, the antidromic spike reappears when the delay is increased to 29 ms. Open squares indicate orthodromic spike; filled squares indicate antidromic spike; arrows indicate stimulus artifacts. *B*, histograms of the estimated conduction velocities of sympathetic preganglionic neurones which had on-going discharge (*a*), glutamate-evoked discharge (*b*) and those which were quiescent and could not be activated by glutamate (*c*).

latency. Noxious stimuli were applied to the ipsilateral hindlimb for 10–30 s; noxious radiant heat was applied to the paw (> 50 °C halogen lamp) or toes were pinched with either toothed forceps or haemostats.

Data collection

Sympathetic preganglionic neuronal activity, phrenic nerve activity, blood pressure, tracheal pressure, ECG and trigger pulses were stored on tape (Racal 7DS) for off-line analysis.

Data analysis

All data analysis was carried out off-line using an interface and software supplied by Cambridge Electronic Design Ltd in conjunction with an IBM-compatible microcomputer. Sympathetic

preganglionic neuronal activity was delivered to a spike processor (Digitimer D130) and single-unit activity used to generate TTL pulses which were delivered to the computer via an interface. To generate phrenic-triggered histograms (analysis for respiratory-related activity), phrenic nerve activity was rectified and smoothed (time constant 100 ms) and this was led into an interface (Neurolog, NL515) which generated a TTL pulse when integrated phrenic nerve activity reached a pre-set level. This TTL pulse was used to trigger the microcomputer. To generate ECG-triggered histograms the ECG signal was passed through an interface and the R-wave used to generate a TTL pulse which was then used to trigger the computer. Responses to noxious stimuli were measured as the change in firing rate of an SPN using a rate histogram display.

Statistical comparisons between groups of neurones were made using the Wilcoxon rank-sum non-parametric test for unpaired data. The G-test of independence (similar to χ^2) was used to test for significant differences between proportions of neurones which had different combinations of physiological characteristics (Sokol & Rohlf, 1969). Differences between proportions were considered significant if $P \leqslant 0{\cdot}05$.

RESULTS

Sympathetic preganglionic neurones projecting into the lumbar sympathetic chain were identified antidromically using standard criteria as described previously (Gilbey, Coote & Peterson, 1982). Briefly these were a sharp threshold for activation, constant latency of the response evoked antidromically and most importantly the cancellation of a spontaneous or glutamate-evoked action potential with an antidromically evoked action potential (Fig. 1).

Electrode penetrations were most commonly made just medial to the dorsal root entry zone and units were located usually at depths between 1200 and 1700 μm. On occasions when Pontamine Sky Blue was deposited at a recording site the location of the dye was found to be in the lateral grey matter near, or at the interface with, the white matter.

Two hundred and ninety-seven SPNs were studied; sixty-eight (approximately 23%) had on-going discharge and the remainder were quiescent. Of the 229 (approximately 77%) quiescent SPNs, 111 were activated by glutamate with the remainder either being lost before glutamate could be applied or they were refractory to it. The axonal conduction velocity distribution of neurones in each group is shown in Fig. 1. The estimated axonal conduction velocities of SPNs were in the B and C fibre range, 0·5–13·9 m/s; 41% of SPNs had conduction velocities of less than 2 m/s. Ninety-five per cent of SPNs had conduction velocities in the range 0·5–7·5 m/s (mode class 1·5–2·0 m/s).

ECG-related activity

The influence of arterial baroreceptors on SPN activity was analysed by constructing ECG-triggered histograms. One hundred SPNs were analysed for an ECG-related pattern of discharge; thirty-five had on-going discharge and sixty-five were glutamate-activated but otherwise quiescent neurones.

For each SPN analysed ECG-triggered histograms were accumulated over 500 double cardiac cycles (10 ms bins). Attempts to measure quantitatively ECG-related activity using various methods of mathematical analysis including those used previously (e.g. Gregor, Jänig & Wilprich, 1977; Haselton & Guyenet, 1989) produced inconsistent results; as they do not take into account the pattern of activity they yielded false positives and negatives. Visual inspection of histogram plots was the most reliable method of categorizing the degree of ECG-related activity

of a SPN. The activity of a SPN was graded into category 0, 1 or 2, representing no, weak, or strong and very strong ECG-related activity, respectively. Examples of activity patterns of SPNs placed in each category are shown in Fig. 2 and the numbers of SPNS with on-going activity and those which were glutamate-activated in each group are shown in Table 1.

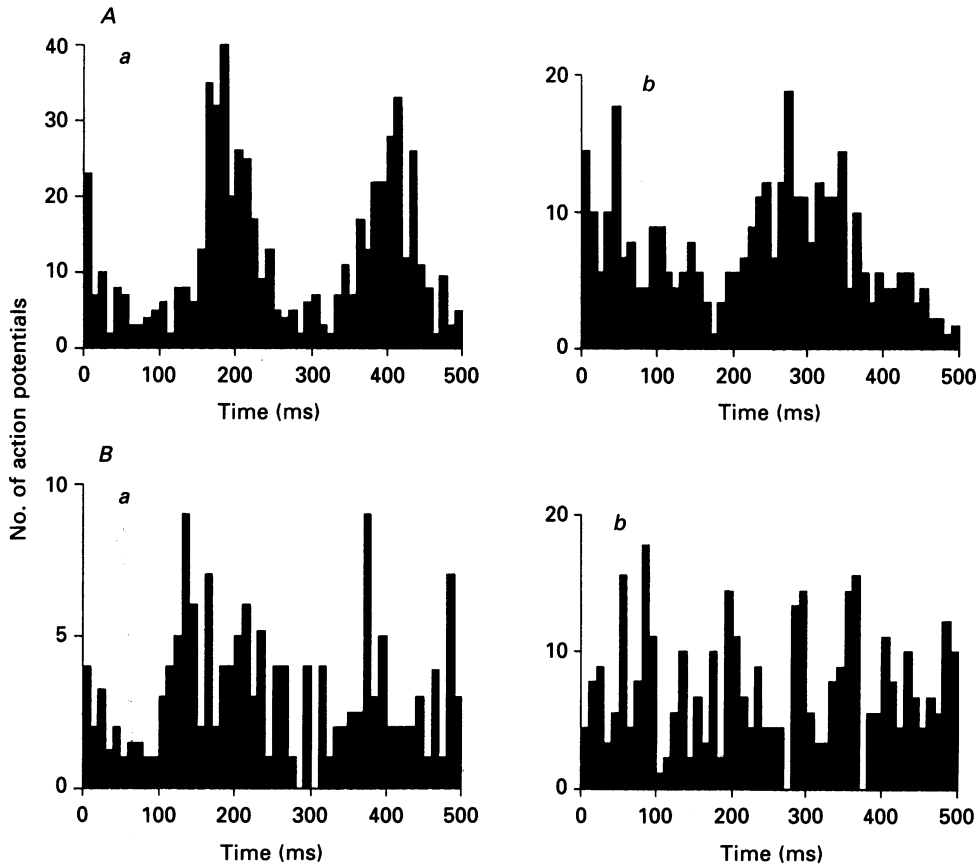

Fig. 2. ECG-triggered (R-wave) histograms of sympathetic neuronal discharge showing examples of SPNs placed in category 0, 1 and 2. *Aa* and *b* show SPNs graded as having very strong and strong ECG-related activity, respectively, and placed in category 2 (heart periods 280 and 290 ms, respectively). *Ba* and *b* show SPNs graded as having weak (category 1) or no (category 0) ECG-related activity, respectively (heart periods 230 and 240 ms, respectively). 10 ms bins, 500 cycles.

Phrenic-related activity

The influence of central respiratory drive on the discharge of fifty SPNs was analysed by constructing phrenic-triggered histograms (see Gilbey *et al.* 1986). Phrenic-triggered histograms were accumulated over 10–40 cycles (100 ms bins). Figure 3 shows examples of expiratory- and inspiratory-related activity patterns seen in two SPNs. Three SPNs had inspiratory-related activity, three had expiratory-related activity and the remainder showed no respiratory modulation (see Table 1). Forty-two of the SPNs were recorded in cats in which the vagi were cut and eight in

cats in which the vagi were intact. In vagi-intact cats seven SPNs were non-modulated and one had expiratory-related activity.

Responses to noxious cutaneous stimuli

The effect of noxious heat or pinch applied to the toes or paws of the ipsilateral hindlimb was examined on the activity of forty-one SPNs. Both SPNs with on-going

TABLE 1. Summary of physiological properties of SPNs recorded in relation to axonal conduction velocities, ECG-related activity and respiratory-related activity with respect to their response to a noxious stimulus to the ipsilateral hindlimb

Total...	↓ 10 (7)	↑ 7 (4)	0 9 (5)	N 15 (9)	NT 138 (86)	Total 179 (111)
Conduction velocities (m/s)						
Range	1·1–8·6	1·7–5·5	1·0–6·0	0·8–5·9	0·5–13·9	179 (111)
Median	2·7	2·9	4·0	1·8	1·7	
ECG category 0	6 (5)	3 (1)	6 (3)	7 (4)	27 (20)	49 (33)
ECG category 1	2 (1)	1	3 (2)	1	18 (12)	25 (15)
ECG category 2	2 (1)	3 (3)	0	7 (5)	14 (8)	26 (17)
Inspiratory related	1	0	0	1	1	3
Expiratory related	0	1 (1)	0	1 (1)	1 (1)	3 (3)
Non-modulated	4 (3)	3 (2)	5 (4)	7 (5)	25 (10)	44 (24)

↓ = decrease in activity; ↑ = increase in activity; 0 = no change in activity with increase in blood pressure; N = no change in activity with no increase in blood pressure; NT = not tested; numbers in parentheses = glutamate-activated quiescent SPNs.

activity and glutamate-activated SPNs had their firing rate influenced by this afferent input (see Figs 4, 5 and 6). Table 1 summarizes the effects of noxious stimulation on the activity of these SPNs and also indicates their conduction velocities and how their activity patterns were related to ECG and to the respiratory cycle. Cells had their activity either increased, decreased or unaltered by noxious stimuli. Those SPNs that were unaffected by a noxious stimulus were subdivided into two groups based on whether or not the noxious stimulus evoked an increase in blood pressure. A blood pressure response to a noxious stimulus was taken as confirmation that the stimulus was evoking an autonomic reflex. However, the absence of a blood pressure response was not taken to mean the converse; the latter could be due to dilatation and vasoconstriction in different vascular beds resulting in no overall change in blood pressure. Seven SPNs had their activity increased in response to a noxious stimulus. Four of these responded with a transient burst of action potentials which was followed by a period of decreased activity (Fig. 4); the latter is likely to result from activation of the baroreceptor reflex by the blood pressure increase. Ten SPNs had their activity decreased by a noxious stimulus (Figs 4 and 5), two in the absence of any change in blood pressure. Of the eight SPNs which showed a decrease in activity in the presence of an increasing blood pressure, four showed a decrease in firing before the increase in blood pressure indicating that baroreceptor inhibition was unlikely to be responsible for the decrease in activity. Two of the remaining four SPNs had category 2 ECG-related activity raising the possibility that at least a component of the inhibition elicited by noxious inputs may have been baroreceptor mediated.

Effects of iontophoresed 5-HT

The effect of 5-HT was examined on fifty-one SPNs. Seventeen of these cells had on-going activity and thirty-four were quiescent and were activated by the ionophoretic application of glutamate (0–157 nA). The ionophoretic application of 5-

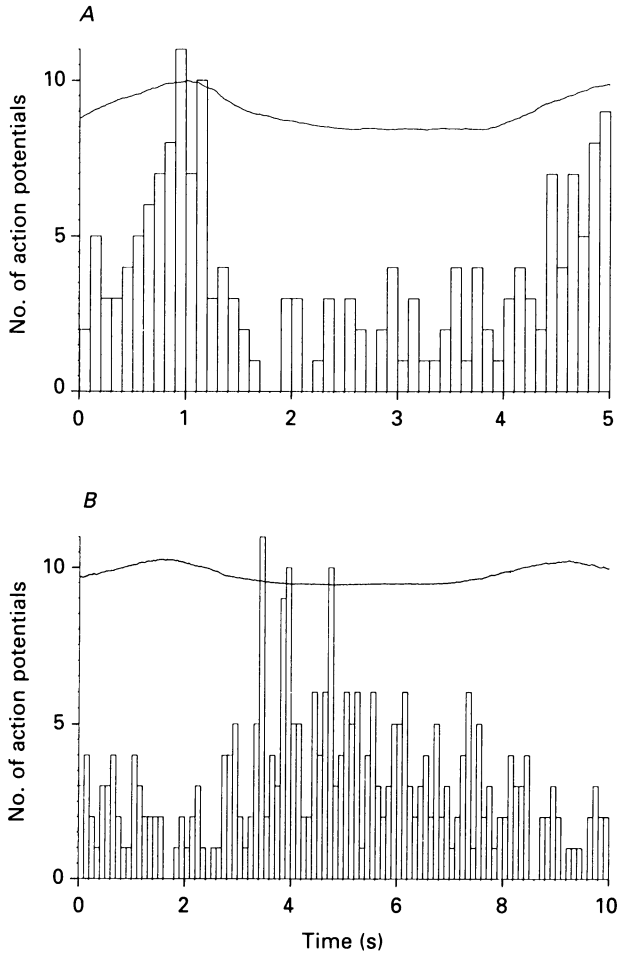

Fig. 3. *A* and *B* are phrenic-triggered histrograms of two different SPNs. *A* has an inspiratory-related discharge pattern (14 cycles, 100 ms bins) and *B* has an expiratory-related discharge pattern (20 cycles, 100 ms bins). Averaged rectified phrenic nerve activity accumulated over the period is shown above each histogram. Vagi cut.

HT in the vicinity of SPNs caused variable effects on the discharge of the neurones (currents: 5–120 nA). The effects of 5-HT creatinine sulphate did not differ from those of 5-HT bimaleate; responses to the ionophoretic application of both salts have been pooled and are presented together. Twenty-seven SPNs had their discharge increased, six neurones had their firing rate decreased, and the discharge of eighteen SPNs was not affected by 5-HT (Table 2). Three units which were not affected by 5-HT were also unaffected by ionophoresis of glutamate; these SPNs were not analysed further and are not included in Table 2.

Excitatory responses of SPNs to 5-HT were of two types. Seventeen neurones (five with on-going activity, twelve glutamate-activated) had their discharge increased in a dose-dependent manner during, and for 15–60 s after, the application of 5-HT (Fig. 5*Ba*). Ionophoresis of saline or creatinine sulphate at similar currents caused either

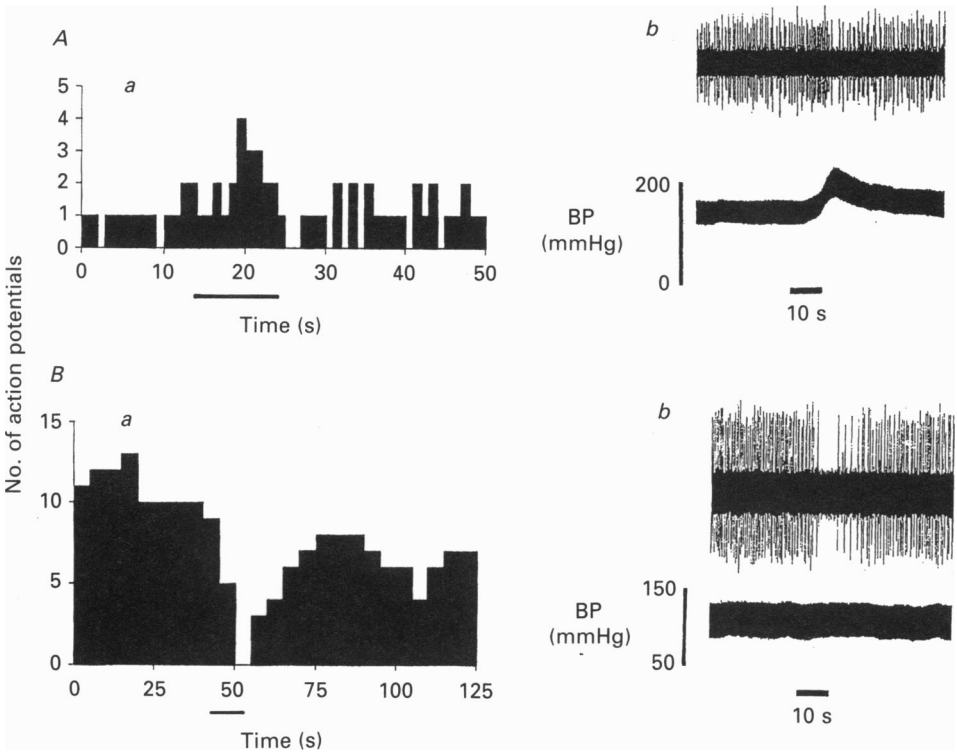

Fig. 4. Response of two SPNs to noxious heat applied to the ipsilateral hindlimb. *A*: *a* shows a rate histogram (1 ms bins) of discharge rate and the transient excitation in this SPN when the stimulus was applied as indicated by the bar (10 s duration); *b* shows the neurogram (bar indicates stimulus) and the blood pressure (BP) trace during the same period. *B*, as *A*, but this neurone had its activity decreased by the stimulus.

no effect or an inhibitory effect in this group of neurones. In ten SPNs (one spontaneously active, nine glutamate activated), immediately after termination of the application of 5-HT an increase in activity was observed (Fig. 5*Aa*). In these cases the lack of excitation or depression seen during the 5-HT application was attributed to current effects as current controls caused depression of neuronal discharge with no subsequent excitation.

The application of 5-HT caused a reduction in the firing rate of six SPNs (two with on-going activity, four glutamate activated; Fig. 6) which appeared to be dose dependent. These depressant effects of ionophoretically applied 5-HT could not be attributed to current alone as current controls had no effect on the discharge of these neurones.

Neurones which had their discharge increased by noxious stimulation of the ipsilateral hindlimb had responses to 5-HT which were significantly different ($P < 0{\cdot}05$; G-test of independence) from those of SPNs which had their firing decreased or

unaffected by hindlimb stimulation (Figs 5 and 6, Table 2). Of the four units whose activity was increased by hindlimb stimulation, three had their discharge decreased in response to ionophoretic application of 5-HT and the firing of one was unaffected by 5-HT. Rate histograms of the responses of two such units are shown in Fig. 6. In contrast, of the nine SPNs which had their firing decreased by noxious stimulation

TABLE 2. Comparison of effects of ionophoretic application of 5-HT to the physiological characteristics of SPNs

	n	↑	↓	N
Total	48	27	6	15
On-going discharge	14	8	2	4
Glutamate driven	34	19	4	11
Conduction velocities (m/s)	48	1·0–3·0 (15 SPNs) 4·2–10·0 (12 SPNs)	0·8–3·5 (5 SPNs) 5·5 (1 SPN)	0·8–3·9 (14 SPNs) 7·8 (1 SPN)
Response to noxious stimuli	20			
Increase	4	0	3	1
Decrease	9	6	1	2
No change	7	6	0	1
ECG-related activity	48			
Modulated	19	8	2	9
Non-modulated	29	19	4	6
Respiratory-related discharge	24			
Inspiratory	2	2	0	0
Expiratory	3	1	2	0
Non-modulated	19	11	3	5

n, sample size; ↑, increase in SPN discharge; ↓, decrease in SPN discharge; N, no change in SPN discharge.

of the hindlimb, six had excitatory responses to 5-HT application, one had its activity decreased, and the firing rate of two neurones was unaffected by 5-HT (Table 2). Responses of two SPNs which had their firing increased by 5-HT and decreased by noxious stimuli are shown in Fig. 5. The firing of an additional seven SPNs was not affected by noxious stimulation of the hindlimb; discharge of six of these cells was increased and that of one cell was unaffected by 5-HT.

In summary, none of the twenty SPNs tested had excitatory responses to both ionophoresis of 5-HT and noxious stimulation of the hindlimb and only one unit had its activity decreased by both 5-HT and noxious input.

Sympathetic preganglionic neurones which had an ECG-related pattern of discharges had responses to 5-HT which were not significantly different (G-test of independence) from those SPNs which did not have an ECG-related pattern of discharge. Similarly there was no significant difference in the responses of SPNs to 5-HT in relation to their respiratory modulation.

Neurones which had excitatory responses to 5-HT tended to have a broader range of axonal conduction velocities than did those which had inhibitory responses and those that were unaffected by 5-HT (Table 2). The twenty-seven SPNs which had their firing rate increased by 5-HT were divided into two groups based on the estimated conduction velocity of their axons: a slowly conducting group of fifteen SPNs (range = 1·0–3·0 m/s; median = 1·3) and a faster group of twelve SPNs

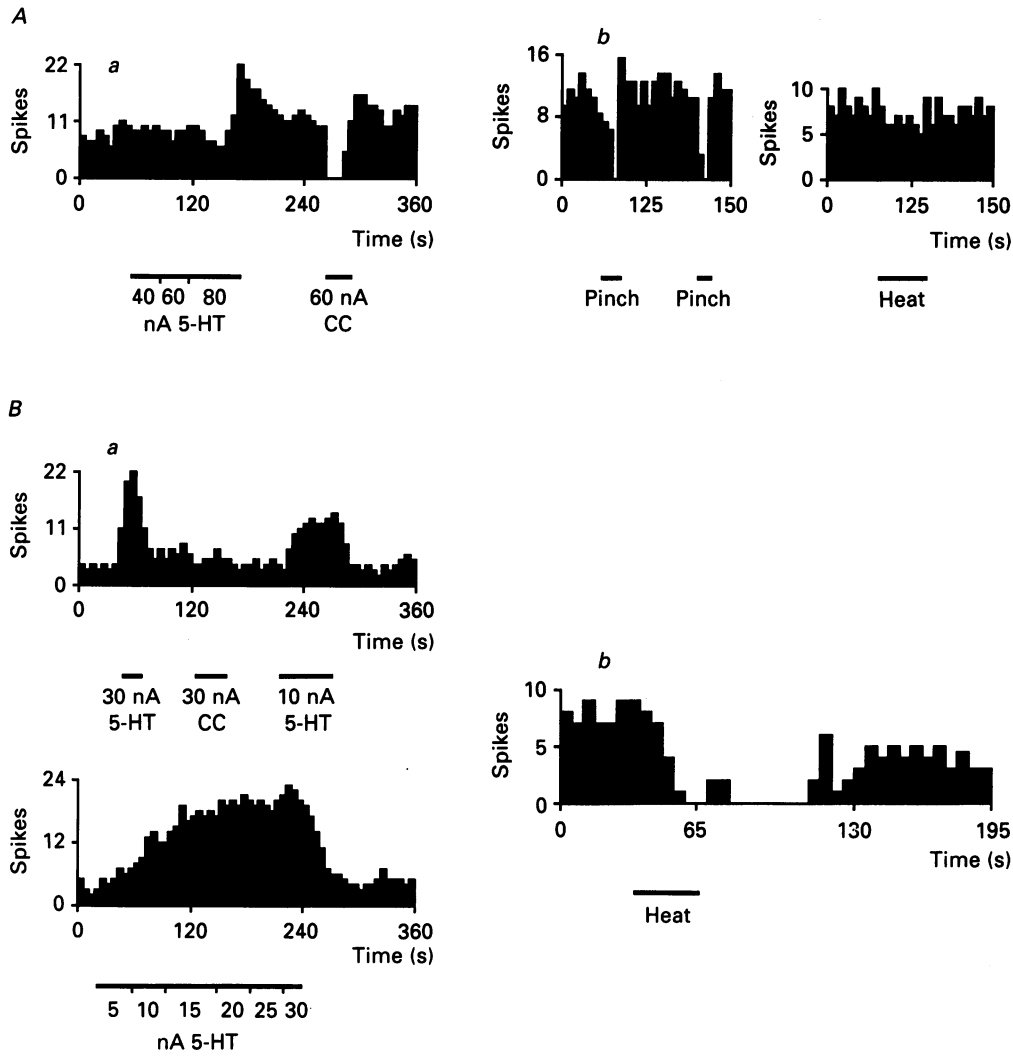

Fig. 5. Rate histograms showing responses of two SPNs to the ionophoretic application of 5-HT and to stimulation of the ipsilateral hindlimb with noxious radiant heat (5 s bins). SPN in panel *A* had on-going discharge; SPN in panel *B* was driven by glutamate. *Aa* and *Ba* illustrate two types of excitatory responses to ionophoretic application of 5-HT. Periods of 5-HT application and saline applied as a current control (CC) are indicated. *Aa* shows an example of a glutamate-activated SPN which did not respond during the ionophoretic application of 5-HT, but had an increase in firing immediately following termination of the ionophoretic current. As this SPN had a marked inhibitory response to the saline current control (CC) the response to 5-HT was interpreted as being excitatory. *Ba* shows an example of a SPN which had on-going activity and responded to increases in the ionophoretic current with dose-related increases in discharge. *Ab* and *Bb*, rate histograms showing inhibitory responses to noxious heat or pinch applied to the ipsilateral hindlimb.

(range = 4·2–10·0 m/s; median = 5·5). The estimated axonal conduction velocities of the six units that had firing which was inhibited by 5-HT ranged from 0·8 to 5·5 m/s (median = 2·6) and those of the fifteen SPNs which were not affected by 5-HT ranged

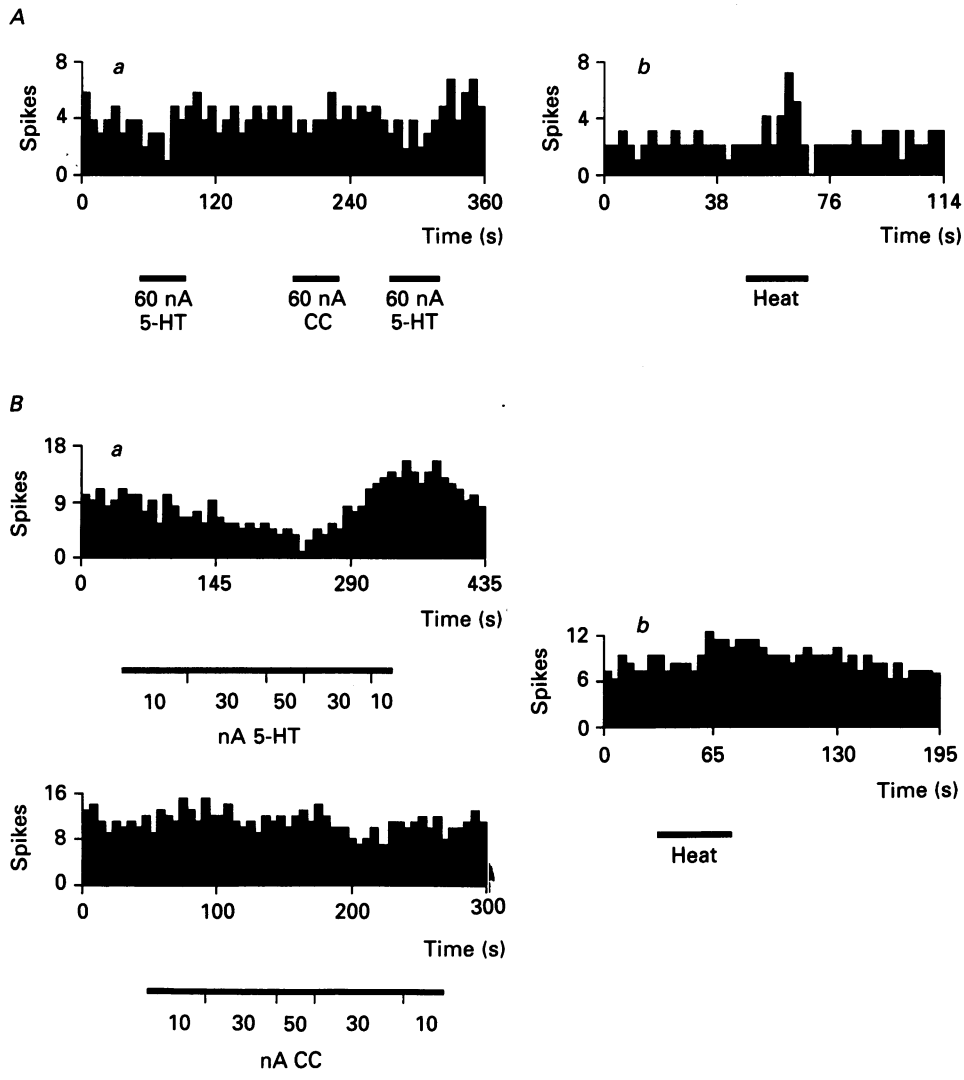

Fig. 6. Rate histograms showing responses of two SPNs to the ionophoretic application of 5-HT and to stimulation of the ipsilateral hindlimb with noxious radiant heat (5 s bins, *Aa*, *Ba*, *Bb*; 2 s bins, *Ab*). Format as in Fig. 2. Panel *A*, SPN had on-going discharge. Panel *B*, SPN was activated by glutamate. Histograms *Aa* and *Ba* illustrate two examples of inhibitory responses to ionophoretic application of 5-HT. *Ab*, noxious stimulation of the hindlimb caused a transient marked increase in neuronal discharge. *Bb*, noxious stimulation caused a moderate, but sustained, increase in neuronal discharge.

from 0·8 to 7·8 m/s (median = 2·6). The SPNs which had their activity decreased and those that were unaffected by ionophoretic application of 5-HT tended to have axons with slower conduction velocities (< 4·0 ms); however, there were no significant differences (Wilcoxon rank-sum test) in the range of conduction velocities among the three groups of SPNs.

DISCUSSION

In this study the axonal conduction velocities, activity patterns, responses to noxious somatic stimuli and responses to ionophoresed 5-HT of SPNs in L2 and L3 projecting into the sympathetic chain beyond L4 ganglion have been investigated. The extracellular recording technique was used in the current study as it has major advantages over the single-fibre recording technique used by Jänig & Szulczyk (1980) in that it permits neurochemicals to be applied in the vicinity of the SPN being recorded. Importantly, under the present experimental conditions some SPNs were recorded which had similar properties to those reported by Jänig & Szulczyk (1980). Some SPNs with on-going activity and those activated by glutamate sometimes shared common properties. The response of an SPN to ionophoresed 5-HT appeared to be related to its physiological characteristics.

The estimated axonal conduction velocities of SPNs in the present study are in the B and C fibre range with 95% of SPNs having estimated axonal conduction velocities in the range 0·5–7·5 m/s (mode class = 1·5–2·0 m/s). This distribution of axonal conduction velocities is markedly different from that seen by Jänig & Szulczyk (1980); 59% of SPNs in their sample had conduction velocities in the range 0·5–7·5 m/s (mode class = 6·5–7·0 m/s); a higher proportion of SPNs had conduction velocities above 7·5 m/s than in the present study (compare Fig. 1 of this study with Fig. 6 of Jänig & Szulczyk, 1980). This difference between studies may be explained by the probability that the fibre recording technique selects for larger axons which have higher conduction velocities. Accordingly, based on the criterion of axonal conduction velocity, the sample of SPNs in the present study differ from those of Jänig & Szulczyk (1980) not so much in their range but in their distribution of axonal conduction velocities.

The percentage of quiescent SPNs in the current study was 77% which is similar to that seen in other studies (e.g. Jänig & Szulczyk, 1980; Gilbey *et al.* 1986). An important question regarding the population of quiescent SPNs is whether some of them receive similar inputs to SPNs with on-going activity and innervate postganglionic neurones with similar functions. It may be that such a high proportion of SPNs have an absence of resting activity or reflex activity because of anaesthesia or because they are only activated under certain physiological conditions. Gilbey *et al.* (1986) demonstrated that glutamate-activated quiescent SPNs receive similar central respiratory drive-related inputs to those with on-going activity. The present investigation demonstrates that some glutamate-activated lumbar SPNs and those with on-going activity share similar characteristics and therefore the two 'populations' of SPNs may have similar functions (see Table 1 and below).

In the present study 26% of all SPNs had a strong ECG-related discharge pattern and 25% had a weak ECG-related discharge pattern. A similar proportion of SPNs with on-going activity and those which were glutamate activated had ECG-related activity. As cardiac rhythmicity is indicative of baroreceptor modulation (Blumberg, Jänig, Rieckman & Szulczyk, 1980) this observation indicates that both SPNs which are quiescent and those with on-going activity may be under similar baroreceptor control. The proportion of lumbar SPNs which have cardiac-related activity in different studies appears highly variable; Jänig & Szulczyk (1980) reported that 60%

of SPNs arising from L2 and L3 segments had a strong ECG-related pattern of discharge and Seller (1973) recorded a strong ECG-related activity from 13% of fibres in L1 and L2 white rami communicantes. These differences may be due to a disparate mix of functions of SPNs sampled.

Of fifty SPNs analysed for phrenic-related activity, three had peak activity during phrenic silence (expiratory) and three had peak activity during phrenic activity (inspiratory). In comparison Preiss, Kirchner & Polosa (1975) reported that of sixty-four single fibres running in the cervical sympathetic nerve, forty-one had inspiratory-related activity, three expiratory-related activity and twenty showed no respiratory modulation. As in the present study and that of Preiss *et al.* (1975) cats were vagotomized and end-tidal CO_2 was held at similar levels these data indicate that SPNs arising from different thoracolumbar segments may be influenced by central respiratory drive to different degrees.

Boczek-Funcke, Habler, Jänig & Michaelis (1989) reported that postganglionic sympathetic vasoconstrictor neurones to muscle have phrenic-related activity as a consistent feature, whereas skin vasoconstrictors may or may not show clear phrenic-related activity. Assuming that the interpretation is correct that SPNs in the present study that were excited by noxious stimuli and had ECG-related activity are muscle vasoconstrictor neurones (see below), it is significant that respiratory modulation was not always present at physiological levels of P_{a,CO_2} (see Table 1). This would indicate that only some SPNs with muscle vasoconstrictor function have respiratory modulation. Jänig & Szulczyk (1980) did not report on the respiratory modulation of the SPNs they classified.

The postganglionic sympathetic neurones which are innervated by the population of SPNs projecting into the lumbar sympathetic chain beyond the L4 ganglion innervate vascular beds, sweat glands and erector pili muscles of hindlimb, tail and hip, and to a minor degree colon, urinary tract and reproductive organs (Kuo, Hisamitsu & de Groat, 1984; Baron, Jänig & McLachlan, 1985*a*, *b*, *c*; Jänig, 1985; Jänig & McLachlan, 1986, 1987). Jänig & Szulczyk (1980) have reported that different populations of SPNs can be distinguished based on their responses to cutaneous noxious stimuli and patterns of on-going discharge. In the present study forty-one SPNs were examined for their response to noxious stimuli applied to the hindlimb. Ten SPNs (seven glutamate activated) had their activity decreased in response to noxious stimuli, eight of which had no or weak ECG-related activity. These properties and their axonal conduction velocities (see Table 1) are consistent with them being type 2 neurones of Jänig & Szulczyk (1980); they may synapse onto vasoconstrictor neurones influencing skin blood vessels. Seven SPNs (four glutamate activated) had their activity increased by noxious stimuli. Three of these neurones had a strong ECG-related activity pattern and had properties fully consistent with type 1 neurones of Jänig & Szulczyk (1980), possibly synapsing onto vasoconstrictor neurones influencing muscle blood vessels. The other four neurones had weak (1) or no ECG-related activity (3) and therefore differ from type 1 neurones. They are unlikely to be sudomotor in function as they had slower conduction velocities (1·7–5·5 m/s) than type 3 (4·7–18·5 m/s; possible sudomotor) neurones of Jänig & Szulczyk (1980). As some glutamate-activated SPNs and those with on-going activity responded in a similar manner to noxious stimuli and shared other

characteristics these results provide support for the idea that some quiescent SPNs may have similar functional dedications to those that have on-going activity.

5-Hydroxytryptamine was found to have primarily excitatory effects on the discharge of lumbar SPNs in general, and in this respect lumbar SPNs are similar to their counterparts in the thoracic spinal cord (de Groat & Ryall, 1967; Coote *et al.* 1981; Kadzielawa, 1983; McCall, 1983). The present study has indicated, however, that the effect of 5-HT on the discharge of a SPN is related to the SPNs' physiological characteristics. The ionophoretic application of 5-HT had different effects on the firing of neurones which were depressed or excited in response to noxious stimuli. Lumbar SPNs which had excitatory responses to noxious stimulation of the hindlimb comprised a subpopulation of neurones which had their firing decreased by 5-HT; these may subserve a 'muscle vasoconstrictor' function. Those SPNs which had excitatory responses to 5-HT and had their firing decreased by noxious stimuli may synapse onto postganglionic neurones innervating skin blood vessels (see above).

The axonal conduction velocities of SPNs which had excitatory responses and those which had their activity decreased by 5-HT in the present study are similar to those in Jänig & Szulczyk's (1980) description of preganglionic neurones which may synapse onto cutaneous and muscle vasoconstrictor neurones, respectively (Table 2). Jänig & Szulczyk (1980) reported that the conduction velocities of axons which synapse onto muscle vasoconstrictor neurones are slow and are unimodal in distribution, similar to those of the SPNs in the present study which had their activity decreased by 5-HT. In contrast, according to Jänig & Szulczyk (1980), the axonal conduction velocities of SPNs which supposedly synapse onto cutaneous vasoconstrictor neurones have a bimodal distribution, similar to that of the units in the present study which had excitatory responses to 5-HT. Thus, these observations lend further support to the idea that the action that ionophoresed 5-HT has on an SPN is related to the neurone's function.

The mechanism(s) by which 5-HT has its differential action on the activity of SPNs is unknown. The effect of ionophoretic application of 5-HT on the firing of a SPN, be it an increase or a decrease, may occur via an action on the SPN itself or via an interneurone. If both increases and decreases in SPN activity occur by 5-HT acting directly on SPNs then more than one receptor type is likely to be involved. In this respect *in vitro* autoradiographic binding studies have demonstrated the presence of 5-HT_{1A} and 5-HT_2 ligand binding sites in the intermediolateral cell column (IML) of the cat spinal cord (Dashwood, Gilbey & Ramage, 1988, 1989) and in the rat, the ionophoretic application of the selective 5-HT_{1A} agonist 5-carboxyamidotryptamine and the selective 5-HT_2 agonist α-methyl-5-hydroxytryptamine caused increases in SPN activity, whereas ionophoretic application of the 5-HT_3 agonist 2-methyl-5-hydroxytryptamine caused decreases in SPN discharge (Lewis & Coote, 1990).

If the excitations and the decreases in SPN activity caused by 5-HT are through a direct action on the SPN rather than through an action at antecedent neurones different subpopulations of SPNs may be associated with different 5-HT receptors or have different distributions and/or proportions of 5-HT receptor subtypes over their cell bodies and dendrites. Consequently, ionophoresed 5-HT may either activate different receptors or a different combination of receptors. The use of selective

agonists and antagonists of different subtypes of 5-HT receptor should enable a distinction to be made between these possibilities.

In conclusion, this is the first report of the extracellular characterization of lumbar SPNs which project into the lumbar sympathetic chain beyond the L4 ganglion. The effect that ionophoresed 5-HT had on the activity of an SPN appeared to be related to the neurone's physiological characteristics. Some of the properties of the neurones resembled those reported by Jänig & Szulczyk (1980). Glutamate-activated quiescent SPNs and those with on-going activity sometimes shared similar characteristics indicating that these SPNs may have similar functional dedication. This investigation has confirmed that lumbar SPNs consist of a heterogeneous population of SPNs with respect to their physiological properties, and under the experimental conditions of this study it was possible to tentatively identify the functional dedication of some SPNs using the database of Jänig and co-workers. This strategy should aid neurophysiological and neuropharmacological approaches to the analysis of how the central nervous system differentially controls subpopulations of SPNs.

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