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NOCICEPTIVE INPUTS INTO ROSTRAL VENTROLATERAL MEDULLA–SPINAL VASOMOTOR NEURONES IN RATS

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SUMMARY

1. In anaesthetized rats recordings were made from thirty-eight neurones in the rostral ventrolateral medulla (RVL) with spinal-projecting axons. Their responses to mechanical, thermal and/or electrical stimulation were examined as were the accompanying changes in arterial pressure.

2. Mechanical, thermal and electrical stimulation of either hindpaw at a strength that can be regarded as noxious produced a consistent rise in arterial pressure. RVL–spinal-projecting 'vasomotor' neurones were excited by the noxious mechanical and thermal (52 °C) stimulation at a latency that was shorter than that of the evoked pressor response.

3. Percutaneous electrical stimulation of either hindlimb extremity resulted in an early peak of excitation (fourteen out of fourteen), an early trough of inhibition (twelve out fourteen), and a later peak of excitation (two out of fourteen). This response pattern to stimulation of either limb was independent of which limb was activated, but contralateral hindpaw stimulation elicited excitation at a shorter latency. The differences in latency of responses to stimulating two locations along the tail suggested that the early excitation and inhibition of RVL–spinal 'vasomotor' neurones were evoked by activation of peripheral fibres with a mean conduction velocity in the Aδ range.

4. Short-latency excitatory and inhibitory responses in RVL–spinal 'vasomotor' neurones were observed also when single-pulse stimuli were delivered within the lateral part of the spinal cord.

5. Ionophoretic application of bicuculline, a $GABA_A$ receptor antagonist, blocked the evoked inhibition of these neurones on electrical stimulation of the hindpaw without attenuating the excitatory input from the same stimulus.

6. These results indicate that RVL–spinal 'vasomotor' neurones receive an input from cutaneous nociceptive afferents. This suggests that these neurones mediate, at least partly, the cardiovascular responses related to nociceptor stimulation.

INTRODUCTION

The rostral ventrolateral nucleus (RVL) of the medulla oblongata, and particularly the RVL–spinal-projecting neurones that receive a powerful baroreflex inhibitory input (Caverson, Ciriello & Calaresu, 1983), are believed to play an important role in

vasomotor control. Any disruption of their functional integrity by cooling, pharmacological inhibition or lesioning results in a drastic reduction of sympathetic vasomotor tone and a fall in arterial pressure to that observed in spinal preparations after spinal cord transection at cervical level (Feldberg, 1976; Dampney & Moon, 1980; Granata, Ruggiero, Park, Joh & Reis, 1985). These neurones have also been shown to be involved in various sympathetic reflexes, including somato-sympathetic reflexes (Brown & Guyenet, 1985; Sun & Guyenet, 1985, 1987; Sun, Hackett & Guyenet, 1988*a*; Sun & Spyer, 1990), the arterial chemoreceptor reflex (Sun & Spyer, 1991), and the integration of outputs from the hypothalamus, cerebellum and the central respiratory generator (Sun & Guyenet, 1986*a*; Haselton & Guyenet, 1989; Silva-Carvalho, Paton, Sun & Spyer, 1990). Electrophysiologically, the activity of this restricted group of 'vasomotor' neurones is coupled with sympathetic nerve discharge (Brown & Guyenet, 1985; Sun & Guyenet, 1985, 1986*b*; Sun *et al.* 1988*a*). Furthermore, under conditions of low synaptic input, these neurones exhibited activity that is indicative of intrinsic pacemaker activity (Sun *et al.* 1988*a*; Sun, Young, Hackett & Guyenet, 1988*b*, *c*). Whilst it is clear that regulation of the discharge of the RVL–spinal 'vasomotor' neurones by afferent inputs underlies many of the reflex adjustments of the circulation in response to peripheral and central stimuli, little is known of the role played by these neurones in mediating the cardiovascular responses related to nociception.

The aim of the present study was to determine whether RVL–spinal 'vasomotor' neurones respond to noxious stimuli that produce cardiovascular effects and, if so, which afferent fibres are responsible for the evoked responses.

METHODS

General procedures

Experiments were performed on twenty-eight male adult Sprague–Dawley rats weighing 350–400 g. The animals were anaesthetized with a mixture of urethane–sodium pentobarbitone (20 and 0·24 %, respectively), given via a tail vein at doses adjusted to eliminate the paw-pinch reflex (usually 1·3–1·5 ml). Additional doses were given as I.V. injections of pentobarbitone to maintain a stable level of anaesthesia. A jugular vein and left brachial artery were cannulated for I.V. injection of drugs and arterial pressure recordings, respectively. A tracheal cannula was inserted. The rats were artificially ventilated with O_2 and paralysed with gallamine (4–5 mg/kg, I.V.). The end-tidal CO_2 and rectal temperature were kept within 3·5–4·5 % and 36–38 °C, respectively. A remotely inflatable snare was placed around the descending aorta below the diaphragm and was used to gradually increase total peripheral resistance, thereby increasing arterial pressure and baroreceptor activity as described previously (Sun & Guyenet, 1985, 1986*b*).

After surgery, anaesthesia was maintained, by I.V. administration of additional doses of pentobarbitone, at a level at which only intense noxious stimuli were able to elicit a small and transient elevation of arterial pressure. This level of anaesthesia is considered as adequate for ethical purposes, since a stable level of arterial pressure (mean arterial pressure < 130 mmHg) was maintained and no obvious withdrawal reflex to paw pinch was observed in the absence of gallamine. An additional I.V. administration of a small amount (< 1 mg) of sodium pentobarbitone usually eliminated the cardiovascular and neuronal responses to noxious stimuli.

Stimulation

Two concentric bipolar electrodes (Clark Electromedical Instruments, NE100, tip separation 0·5 mm) were implanted, one in the fascia surrounding the right marginal mandibular branch of the facial nerve, and the other in the spinal cord at the T2–T3 level. The former was used to locate the facial motor nucleus by means of recording the antidromic field potential as guidance for the

location of the RVL–spinal vasomotor neurones as described previously (Brown & Guyenet, 1985; Sun & Guyenet, 1985). The latter was placed on the right side (ipsilateral to the recording side) with its tip 1·0 mm below the dorsolateral sulcus and used for antidromic stimulation of the RVL–spinal vasomotor neurones and activation of the lateral part of the spinal cord.

The responses of RVL–spinal 'vasomotor' neurones and arterial blood pressure to nociceptive (natural and electrical) stimulation were determined. Two types of natural stimuli were employed: thermal stimuli and mechanical stimuli. The noxious thermal stimuli were provided by immersing the metatarsus of the ipsilateral hindpaw in a water-bath at a temperature of approximately 52 °C for 4 s. The noxious mechanical stimuli were given by applying an intense hindpaw pinch for less than 1 s. Noxious electrical stimuli were delivered through pairs of stainless-steel stimulating electrodes inserted subcutaneously into the extremities of the hindpaws (toes 2 and 4). The effects of repeated application of single rectangular wave stimuli (1 Hz, 2 ms duration, 50 V) were analysed by constructing peristimulus time histograms. This intensity of stimulation would be expected to activate all fibre groups in afferent nerves, including C fibres. In some experiments, two additional pairs of stimulating electrodes were inserted subcutaneously into the tip and the base of the tail. The stimulation sites on the tip and the base of the tail were separated by 100 mm. These were used to determine the conduction velocities of primary afferent fibres contributing to the response.

When different types of noxious stimuli were tested, in each individual cell these stimuli were applied in a random order and not more frequently than once every 10 min.

Electrical recording and ionophoresis

Unitary extracellular recordings were made with glass microelectrodes filled with 3 M-NaCl (4–8 MΩ resistance, measured DC) by means of a dorsal transcerebellar approach, amplified, counted and recorded on a chart recorder and on tape. Peristimulus time histograms and ECG-triggered time histograms of the neuronal discharge were generated using a microcomputer (CED). These histograms were analysed to determine the response ratio (evoked spikes per stimulus) of the RVL–spinal 'vasomotor' neurones.

Six-barrelled electrodes were used for ionophoresis. The recording pipette protruded 25–35 μm from the other barrels. One barrel was filled with 3 M-NaCl for current balancing and the others were filled with one of the following solutions: 0·8 M-GABA (Sigma) in 160 mM-NaCl, pH 4·5; 0·2 M-L-glutamate (Sigma) in 160 mM-NaCl, pH 8·5; 0·2 M-kynurenic acid (Sigma) in 160 mM-NaCl, pH 8·5; 20 mM-bicuculline methiodide (Sigma) in water, pH 4·0. A retaining current of 10 nA was applied between periods of drug ejection.

Statistics

Statistical comparisons were performed by the use of Student's paired *t*-test. P values $< 0{\cdot}05$ were considered significant. Mean values are expressed with their standard errors (S.E.M.).

RESULTS

General properties of the recorded rostral ventrolateral medulla–spinal vasomotor neurones

The activity of thirty-eight RVL–spinal 'vasomotor' neurones was recorded following conventional identification. As described previously (Brown & Guyenet, 1985; Sun & Guyenet, 1985), all these units displayed on-going activity ($19{\cdot}5 \pm 1{\cdot}4$ spikes/s) and were excited by ionophoretic application of glutamate and inhibited by ionophoretic application of GABA. Their activity was sensitive to baroreflex inhibition (Fig. 1*A*) and exhibited a pulse-synchronous discharge (Fig. 1*C* and *D*). Their spinal projections were determined by means of antidromic activation from a spinally placed electrode. Figure 1*B* shows the antidromic response of a RVL–spinal 'vasomotor' neurone to spinal cord stimulation and its collision when the spinal stimuli were delivered within the critical period following the on-going spikes. The

antidromic latencies of the evoked spikes ranged from 8 to 80 ms, which correspond to a mean conduction velocity of 3·0 m/s (±0·2 m/s, $n = 38$).

Responses to mechanical stimulation

A brief intense pinch (duration < 1 s) of either hindpaw produced a transient rise in arterial pressure in all the rats tested (Fig. 2*Ab*, *Ba* and *Bc*). The resting mean arterial

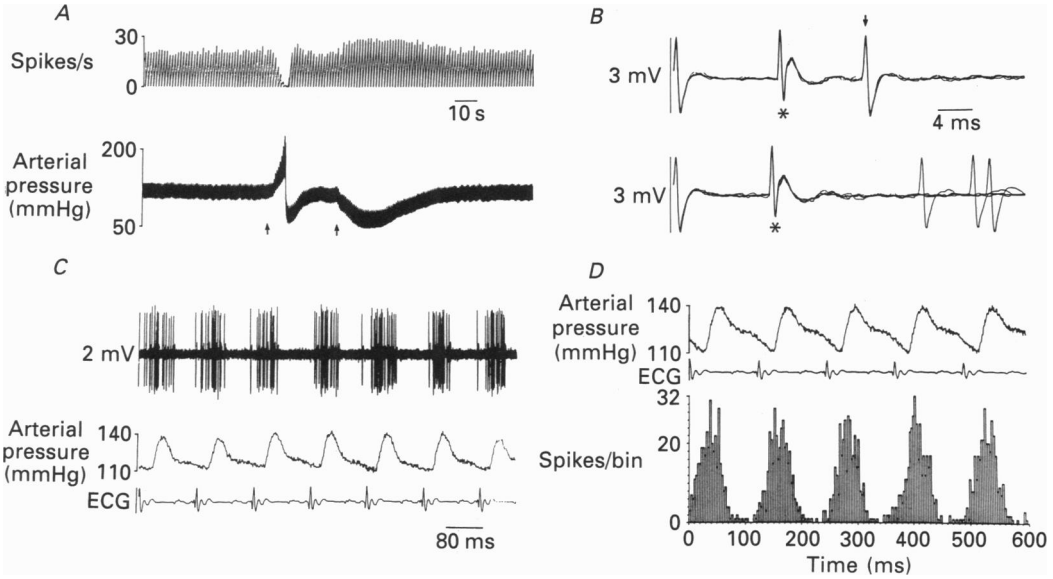

Fig. 1. Characteristics of the identified RVL–spinal 'vasomotor' neurones. *A*, effect of change in arterial pressure on the neuronal discharge rate. Neuronal activity is represented in the form of integrated rate histogram. Arterial pressure was elevated via descending aortic constriction (started at the first arrow and stopped when the neurone became silent) and reduced by I.V. injection of 0·1–0·2 mg sodium nitroprusside (at the second arrow). *B*, spinal projection of the neurone. The evoked antidromic spikes (arrow) by the spinal cord stimulation (asterisks) collided with spontaneously occurring spikes and failed to occur at recording site when the stimulation was applied within a critical period after spontaneously occurring spikes (bottom trace). *C*, pulse-synchronous discharge of the RVL–spinal 'vasomotor' neurone. The arterial pressure trace (middle) and ECG signal (bottom) represent a single sweep, whilst the trace of neuronal discharge represents twelve consecutive sweeps, all triggered on ECG signals. *D*, ECG-triggered time histograms of the neuronal activity (300 sweeps, 3 ms/bin). The top and middle traces represent averaged arterial pressure and ECG signals, respectively (fifty sweeps each).

pressure was 112 mmHg (±1·5 mmHg, $n = 7$) before stimulation, which evoked a maximal increase in mean arterial pressure of 24 mmHg ($n = 7$, $P < 0·05$), with an average peak mean arterial pressure of 137 mmHg (±5·0 mmHg, $n = 7$). In ten RVL–spinal 'vasomotor' neurones subjected to a similar intensity of stimulation, a resting discharge of 15±3 spikes/s ($n = 10$) before the stimulation was followed by a consistent pattern of discharge. Following the pinch, there was an increase in discharge with a latency of 200–600 ms (Fig. 2*Ab*, *Ba* and *Bc*). This was followed by a reduction in discharge coincident with the evoked increase of arterial pressure. The rise in arterial pressure began 340–500 ms after the initial increase in the neuronal

discharge. The maximal firing rate elicited by stimulation was 22·9 ± 3·9 spikes/s, an increase of 7·4 ± 1·3 spikes/s ($n = 10$, $P < 0·05$). Similar responses were observed in both the faster-conducting neurones (Fig. 2*Ab* and *Ba*) and the slower-conducting neurones (Fig. 2*Bc*). The maximal rise of arterial pressure in these ten tests was 19 ± 2·5 mmHg. These pressor responses were always transient.

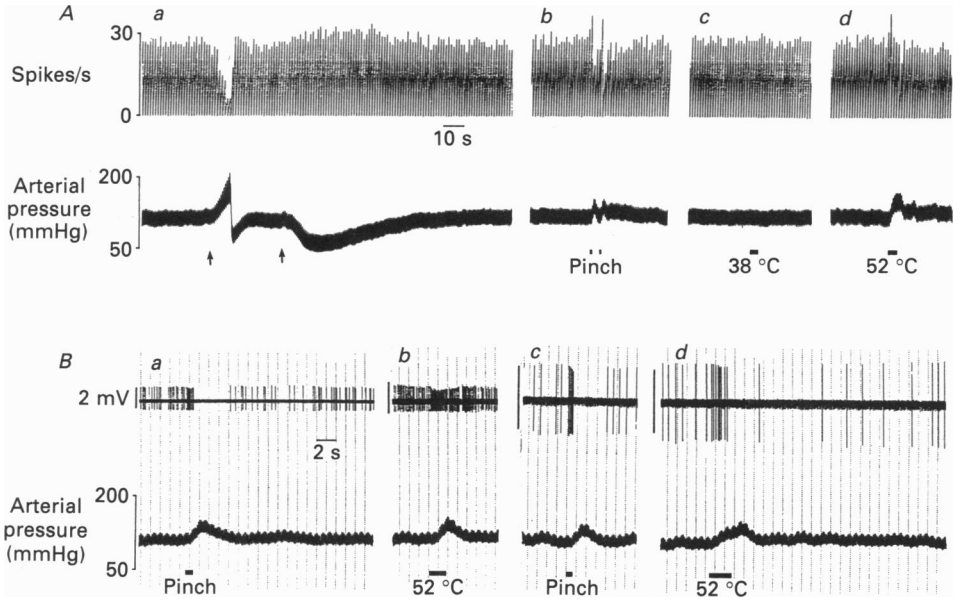

Fig. 2. Examples of responses of the RVL–spinal 'vasomotor' neurones to hindpaw stimulation. *A*, effect of change in arterial pressure (*Aa*: the 1st arrow, aortic constriction; the 2nd arrow, I.V. injection of 0·1 mg sodium nitroprusside), ipsilateral hindpaw pinch (*Ab*), and immersion of the ipsilateral hindpaw in 38 °C (*Ac*) and 52 °C (*Ad*) water-baths on the neuronal activity. The neurone had a spinal axon with conduction velocity of 3·2 m/s. *B*, effect of noxious hindpaw stimuli on activity of the RVL–spinal 'vasomotor' neurones with fast conduction velocity (*Ba* and *Bb*) and with slow conduction velocity (*Bc* and *Bd*), showing the neuronal responses in relation to arterial pressure.

The effects of moderate pressure or brushing the hindpaw were investigated. Neither neuronal activity nor arterial pressure was affected by these stimuli ($n = 10$).

Responses to thermal stimulation

The effects of noxious thermal stimulation on arterial pressure were determined in seven rats by immersing the distal limbs up to the metatarsus in water at 52 °C for approximately 4 s. This evoked a significant increase in mean arterial pressure of 135 ± 4·0 mmHg ($n = 7$).

The effects of thermal stimuli on the activity of RVL–spinal 'vasomotor' neurones were determined in eight identified neurones. The discharge increased during noxious thermal heating to the hindpaw (52 °C) by 10·3 ± 1·8 spikes/s ($n = 8$, $P < 0·05$), from a resting discharge of 19 ± 3·3 spikes/s ($n = 8$). The peak firing rate was 30 ± 3·6 spikes/s ($n = 8$). Examples of the responses to noxious heat are illustrated in Fig. 2*Ad*, *Bb* and *Bd*. The responses had rapid onsets and preceded the accompanying

changes in arterial pressure. The evoked excitation of the neurones was then overcome by baroreflex inhibition as the arterial pressure increased. Both faster-conducting RVL–spinal 'vasomotor' neurones (Fig. 2*Ad* and *Bb*) and slower-conducting neurones (e.g. Fig. 2*Bd*) gave similar patterns of response to the thermal

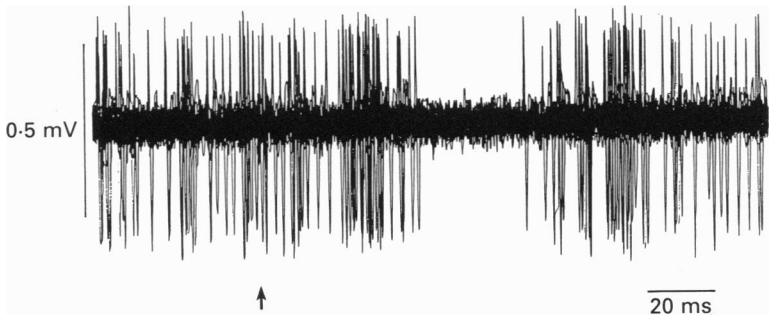

Fig. 3. An example of the response of a RVL–spinal 'vasomotor' neurone to ipsilateral hindpaw stimulation (1 Hz, 50 V, 2 ms pulses, at arrow); twenty sweeps superimposed.

stimulation. The changes in arterial pressure accompanying these tests evoked an averaged maximal increase in mean arterial pressure of $19{\cdot}1 \pm 4{\cdot}0$ mmHg ($n = 8$, $P < 0{\cdot}05$), from 111 ± 2 mmHg ($n = 8$) to a peak of $130 \pm 3{\cdot}9$ mmHg ($n = 8$).

The effects of innocuous warming of the hindpaw (38 °C, 4 s) were also determined on the activity of RVL–spinal 'vasomotor' neurones. Immersion of the hindpaw in water at 38 °C neither induced cardiovascular responses nor affected the neuronal activity ($n = 6$). Figure 2 shows the contrasting responses of a RVL–spinal vasomotor neurone to noxious thermal stimulation (Fig. 2*Ac*) and to innocuous warming (Fig. 2*Ad*).

Responses to percutaneous and spinal cord stimulation

To determine the characteristics of the afferents responsible for the changes noted, RVL–spinal 'vasomotor' neurones were examined for their responses to noxious electrical stimuli (2 ms pulses, 1 Hz, 50 V) delivered to the hindpaw. Such stimuli, when delivered at higher frequencies, resulted in rises of arterial pressure. An averaged maximal increase in arterial pressure of $20 \pm 2{\cdot}3$ mmHg ($n = 5$, $P < 0{\cdot}05$) was obtained at 10 Hz stimulation, from a resting mean arterial pressure of $117 \pm 3{\cdot}9$ mmHg ($n = 5$) which rose to $137{\cdot}4 \pm 5{\cdot}2$ mmHg ($n = 5$).

The responses were determined of fourteen identified RVL–spinal 'vasomotor' neurones to noxious ipsilateral percutaneous electrical stimuli. All these neurones had on-going activity with a mean firing rate of $19{\cdot}1 \pm 4{\cdot}4$ spikes/s ($n = 14$) under resting conditions. The stimulus intensity to the hindpaw was set at a level (50 V) that produced a vasopressor response on 10 Hz stimulation. Most of the neurones (twelve out of fourteen, 86%) tested to single-pulse stimulation at 1 Hz showed an early excitatory peak followed by a period of decreased firing (Fig. 3) as illustrated in peristimulus time histograms (see Fig. 4*A* for a typical response), while the remaining two (two out of fourteen, 14%) responded with an early excitatory peak followed later by single or double excitatory peaks, without an obvious early

inhibitory trough in the histograms (Fig. 4*B*). The means of the number of spikes evoked per stimulus for the early peak and of the latencies of the early excitatory peak were $0{\cdot}96 \pm 0{\cdot}29$ ms ($n = 14, P < 0{\cdot}05$) and $24{\cdot}9 \pm 0{\cdot}9$ ms ($n = 14, P < 0{\cdot}05$). The early excitation lasted $22{\cdot}6 \pm 2{\cdot}2$ ms ($n = 14, P < 0{\cdot}05$), which represents an increased

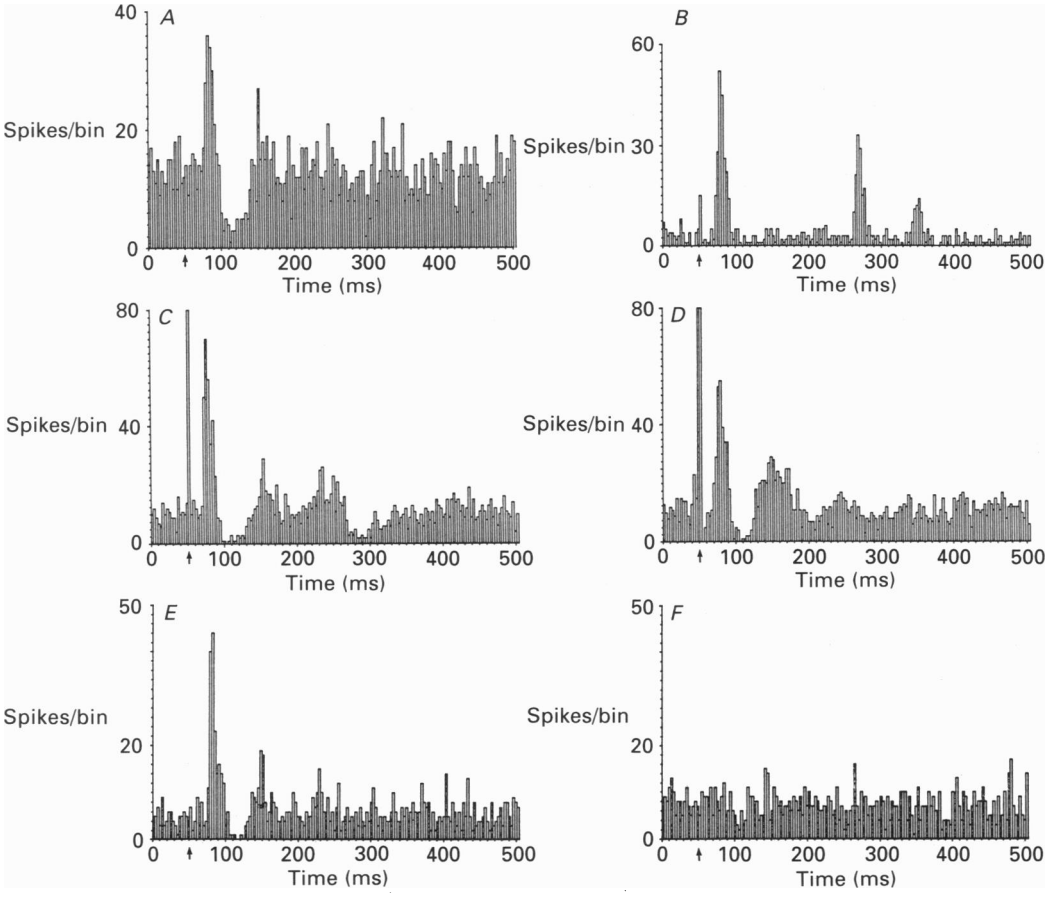

Fig. 4. Effect of single-pulse percutaneous stimulation on the RVL–spinal 'vasomotor' neurones. *A*, a typical example of peristimulus time histograms (180 sweeps, 3 ms/bin) of activity of these neurones (86%, twelve out of fourteen) in response to ipsilateral hindpaw electrical stimulation (50 V, 2 ms pulses). *B*, an example of peristimulus time histograms (180 sweeps, 3 ms/bin) of activity of these neurones (14%, two out of fourteen), in response to the same stimulation as in *A*. *C–F*, an example of an RVL–spinal 'vasomotor' neurone (axon conduction velocity: 3 m/s) in response to ipsilateral hindpaw stimulation (*C*, 180 sweeps, 3 ms/bin), contralateral hindpaw stimulation (*D*, 180 sweeps, 3 ms/bin) and tail-base stimulation (*E*, 120 sweeps, 3 ms/bin, 65 V, 2 ms pulses; *F*, 160 sweeps, 3 ms/bin, 4 V, 2 ms pulses). All the stimuli were delivered at arrows.

firing of $572 \pm 206\%$ ($n = 14$, $P < 0{\cdot}05$) over the resting firing rate. The early inhibitory trough in the peristimulus time histogram had a latency of $46{\cdot}6 \pm 2{\cdot}3$ ms ($n = 12$, $P < 0{\cdot}05$) and a reduction in discharge per stimulus of $0{\cdot}46 \pm 0{\cdot}09$ spikes ($n = 12, P < 0{\cdot}05$). The inhibition lasted $39{\cdot}8 \pm 4{\cdot}0$ ms ($n = 12$) with a minimal firing

rate of 11·2±4·7% ($n = 12$) of the resting firing rate. The inhibitory phase was usually followed by some rebound excitation. A late excitatory peak was observed in the peristimulus time histograms of two neurones (of fourteen neurones tested, 14%). This excitatory peak had a latency of 200±2 ms ($n = 2$) and a maximal firing of 7·6% above the resting activity. It lasted 27 ms with 0·575±0·085 evoked spikes per stimulus. Another feature of these neurones in response to noxious ispilateral percutaneous hindpaw electrical stimulation was the lack of an obvious inhibitory trough in their peristimulus time histograms (Fig. 4*B*).

Seven of the fourteen identified RVL–spinal 'vasomotor' neurones were examined for their response to noxious percutaneous stimulation of the contralateral hindpaw (Fig. 4*D* as compared with Fig. 4*C*). The same intensity of electrical stimulation elicited an equivalent early excitatory peak (evoked number of spikes per stimulus: 0·95±0·24; $n = 7$, $P < 0·05$) as to ipsilateral stimulation, which was followed by an early phase of inhibition (reduced number of spikes per stimulus: 0·45±0·09; $n = 6$, $P < 0·05$, 86%, seven neurones). The remaining cell lacked an inhibitory trough in the peristimulus time histogram. The latencies of the early excitation and inhibition to contralateral hindpaw stimulation were 23±1·6 ($n = 7$) and 45·3±1·4 ms ($n = 6$), respectively, and the latency of the early excitation was significantly smaller than that to ipsilateral stimulation (24·9±0·9 ms; $n = 7$) by 2±0·9 ms ($n = 7$, $P < 0·05$). The latency of subsequent inhibition was similar to that evoked by ipsilateral stimulation (46·6±2·3 ms; $n = 6$), with an insignificant difference of 0·17±2·0 ms ($n = 6$, $P < 0·05$).

RVL–spinal 'vasomotor' neurones showed an identical response pattern when equivalent stimuli were delivered to the tail. Figure 4*E* and *F* illustrates the peristimulus time histograms in response to tail-base stimulation of the same neurone as shown in Fig. 4*C* and *D*. A three-phase response comprising excitation–inhibition and some rebound excitation was observed at a stimulation intensity of 65 V (Fig. 4*E*) but not at 4 V (Fig. 4*F*). Five identified RVL–spinal 'vasomotor' neurones were examined for their responses to noxious percutaneous stimuli (65 V) of the tail tip (Fig. 5*B*) and base (Fig. 5*A*). The distance between the tip and base of the tail was always set at 100 mm. The evoked spikes per stimulus and latencies of the early excitation at the tail base were 0·86±0·23 ($n = 5, P < 0.05$) and 24·2±3·0 ms ($n = 5$), respectively, whilst the values for the tail-tip stimulation were 0·48±0·1 ($n = 5, P < 0·05$) and 32±2·4 ms ($n = 5$). The early excitation elicited from the tail-base stimulation was significantly shorter than that evoked by tail-tip stimulation (+0·38±0·16 evoked spikes/stimulus on average; $n = 5$, $P < 0·05$). The difference in latency of the early excitatory peak was 7·4±0·7 ms ($n = 5$, $P < 0·05$). Assuming that this difference was due solely to afferent conduction between the sets of electrodes spaced 100 mm apart, the peripheral conduction velocity averaged 13·8±1·4 m/s ($n = 5$). An early inhibitory trough was observed in the peristimulus time histograms when either tail base or tail tip were stimulated. The reduced number of spikes per stimulus at the tail base and the tail tip were 0·41±0·11 ($n = 5$, $P < 0·05$) and 0·28±0·07 ($n = 5$, $P < 0·05$), respectively, with the tail-tip stimulation eliciting less-marked inhibition (0·13±0·04, $n = 5$, $P < 0·05$). Similarly, the latency of the inhibition evoked by the tail-base stimulation (50±2·5 ms, $n = 5$) was significantly shorter than that (58·2±1·7 ms, $n = 5$) from the tail tip, with a

difference of $8{\cdot}2 \pm 1{\cdot}2$ ms ($n = 5$, $P < 0{\cdot}05$). The calculated peripheral conduction velocity was $13{\cdot}2 \pm 2{\cdot}0$ m/s ($n = 5$). The early excitation and inhibition thus appear to be due to activation of peripheral afferents with conduction velocity in the range of Aδ fibres. The conduction velocity of the fibres responsible for the early inhibition

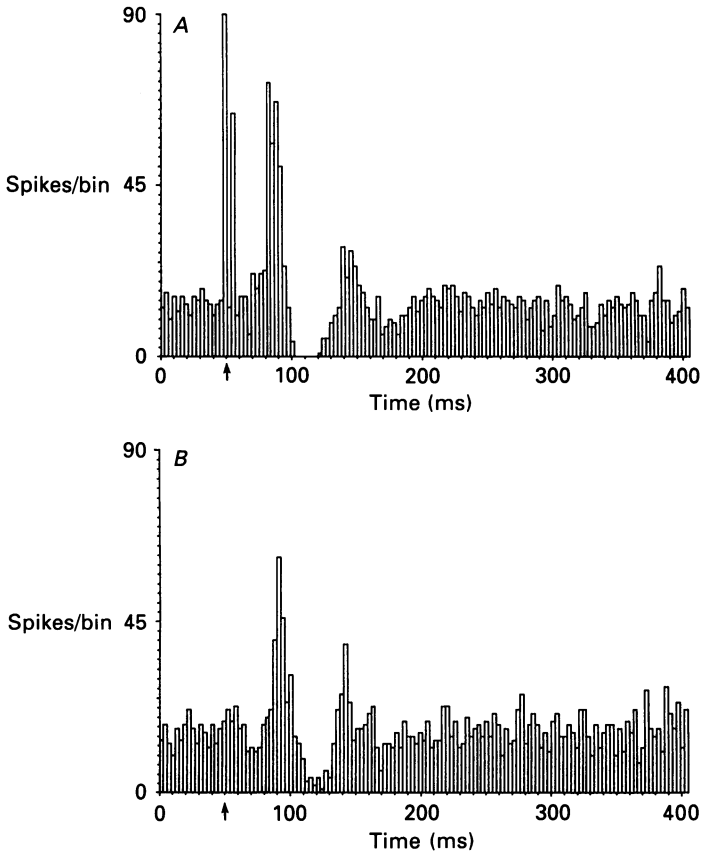

Fig. 5. Peristimulus time histograms of activity of a RVL–spinal 'vasomotor' neurone in response to percutaneous electrical stimulation (65 V, 2 ms pulses) of the tail base (*A*) and tail tip (*B*). The two pairs of stimulating electrodes were set 100 mm apart (180 sweeps, 3 ms/bin). The stimuli were delivered at arrows.

of the RVL–spinal 'vasomotor' neurones was similar to that for evoking the early excitation, with a mean difference of $-0{\cdot}52 \pm 1{\cdot}5$ m/s ($n = 5$, $P < 0{\cdot}05$).

The Aδ responses of the RVL–spinal 'vasomotor' neurones to tail and hindpaw stimulation were also observed when the lateral part of the spinal cord was stimulated. The protocol used to avoid recording the effects of antidromic stimulation at the recording site was to deliver each stimulus at a latency after a spontaneously occurring spike that was within the critical period that had been determined for each individual neurone. Figure 6 illustrates one such example. This neurone had an antidromic spike latency of 15 ms from the spinal cord stimulation. The stimuli to the lateral cord were applied 6 ms after a spontaneously occurring spike. In the

fourteen neurones tested, such stimulation always evoked an early excitation and inhibition of the RVL–spinal 'vasomotor' neurones (Fig. 6*C* and *D*, as compared with Fig. 6*B*) and this was followed by a later inhibition and excitation.

Seven units located in the vicinity of the RVL–spinal 'vasomotor' neurones but insensitive to brief increases in arterial pressure were examined for their responses to

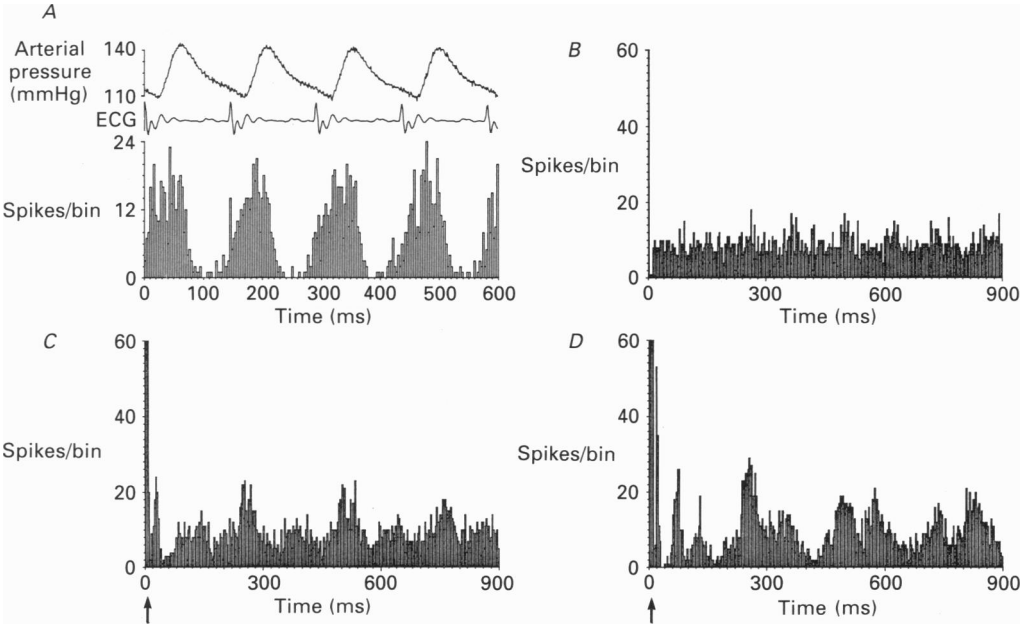

Fig. 6. Examples of an RVL–spinal 'vasomotor' neurone in response to spinal cord stimulation. *A*, ECG-triggered time histogram of the neuronal activity showing its pulse-synchronous discharge (bottom, 200 sweeps, 3 ms/bin; top and middle, averaged traces of fifty sweeps each). *B–D*, peristimulus time histograms of the neuronal activity during spinal cord sham (to control for spurious correlation) (*B*) and true (*C* and *D*) stimulation. The antidromic spikes from the stimulating site were determined and had a latency of 15 ms. All the stimuli (arrows) were applied 6 ms after spontaneously occurring spikes. Note that at 10 V (2 ms) of stimulation, the histogram (*C*) displays an early excitatory peak and inhibitory trough followed by three major late, long-lasting excitatory peaks, whilst at high intensity (30 V, 2 ms) not only the early excitatory peak and inhibitory trough and the late excitatory peaks increased, but additional excitatory and inhibitory influences became apparent.

noxious stimuli. These units discharged with respiratory rhythm (lung inflation–synchronous activity) but continued to fire with apparently the same rhythm when inflation was stopped for one to two cycles (Fig. 7*Aa* and *Ca* at bars). These units were divisible into two groups according to their responses to noxious stimuli. Four (57%) of the units tested responded as shown in Fig. 7*Ab* and *B*, with a dominant excitation to foot pinch and electrical stimulation. The early excitation and inhibition were identical to the Aδ response of RVL–spinal 'vasomotor' neurones in terms of latencies and magnitude. The remaining three units (43%) exhibited an inhibitory response to foot pinch, thermal stimulation and electrical stimulation (see Fig. 7*Ca*, *Cc* and *D*).

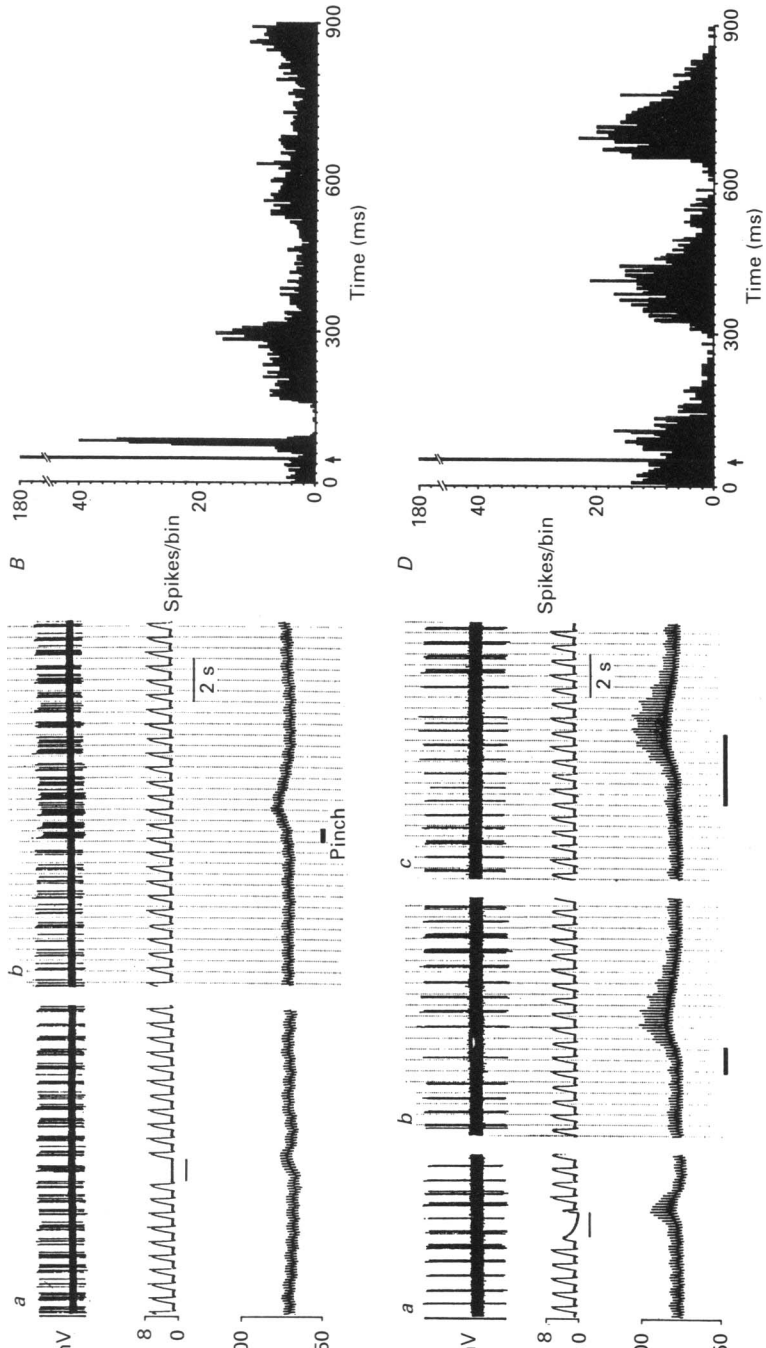

Fig. 7. Effects of noxious percutaneous stimuli on neurones with respiratory rhythm. *A* and *B* are responses of one neurone to a short period of stopping the respiration (*Aa*), to hindpaw pinch (*Ab*) and to hindpaw electrical stimulation shown as peristimulus time histograms of its activity (*B*, 180 sweeps, 3 ms/bin). *C* and *D* represent another neurone in response to these stimuli and thermal stimulation (*Cc*). In *B* and *D* the stimuli were delivered at arrows.

Effects of bicuculline

Ionophoretic application of bicuculline (60 nA), a $GABA_A$ receptor antagonist, for 5 min produced a reduction of baroreflex inhibition of the RVL–spinal neurones by 82·5±7·5% ($n = 6$, $P < 0·05$; Fig. 8*C* as compared with Fig. 8*A*). At this dose, the

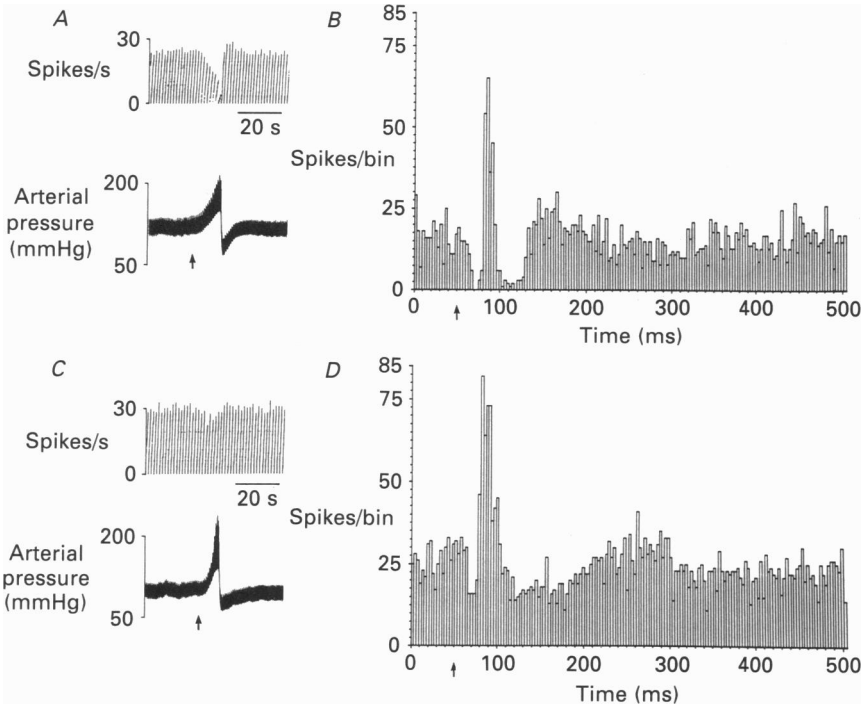

Fig. 8. Effect of bicuculline mediodide on the responses of the RVL–spinal neurone to noxious percutaneous electrical stimulation. Ionophoretic application of bicuculline (60 nA) almost totally blocked the baroreflex inhibition of the neurone (*C* as compared with *A*, aortic constriction started at arrows). The early inhibitory trough in the peristimulus time histogram of the neuronal activity in response to hindpaw stimuli (at arrows) was largely attenuated (*D* as compared with *B*, 180 sweeps, 3 ms/bin) by the drug application.

resting firing rate of the RVL–spinal neurones increased only 40±3% ($n = 6$, $P < 0·05$) from 16·2±4·9 spikes/s ($n = 6$) before the application to 23·2±7·4 spikes/s ($n = 6$) in the presence of bicuculline. The application of bicuculline largely blocked the early inhibitory trough evoked by hindpaw stimulation (Fig. 8*D* as compared with Fig. 8*B*) by 82·5±7·5% ($n = 6$, $P < 0·05$) with the fall in number of spikes per stimulus before the application of 0·45±0·14 ($n = 6$) falling to 0·12±0·07 ($n = 6$) in the presence of bicuculline. A significant increase in the evoked spikes per stimulus of the early excitatory peak due to bicuculline application was observed with a difference of 0·78±0·09 spikes/stimulus ($n = 6$, $P < 0·05$).

DISCUSSION

It is well known that cardiovascular reactions commonly accompany the somatic motor responses to noxious stimulation, particularly when the pain provoked is severe and of sudden and unexpected onset. Generally, cardio-acceleration, peripheral vasoconstriction, a rise in arterial pressure, dilatation of the pupils, and secretion from sweat glands and the adrenal medulla are elicited, all signs of intense activity in sympathetic efferent nerve fibres (Koizumi & Brooks, 1972). These are typical features of the defence reaction (see Hilton, 1966). Whilst early studies of sympathetic reflex responses to nociceptive stimuli indicate that two pathways are involved, one supraspinal and one spinal (Sato, Tsushima & Fujimori, 1965; Koizumi, Sato, Kaufman & Brooks, 1968), little is known of the neuronal circuitry responsible for the supraspinal reflex.

The present study has demonstrated that RVL–spinal 'vasomotor' neurones respond to both electrical and natural cutaneous stimulation of the hindpaws and tail at intensities that would be considered to be noxious. This indicates that these neurones may play an important role in the sympathetic reflexes related to noxious inputs. This follows from the observations that the responses of these neurones to noxious stimuli mirror the cardiovascular response evoked. An increase in neural activity of RVL neurones did not occur with a moderate intensity of stimulation or when the animals were deeply anaesthetized.

The latencies of the responses of RVL–spinal 'vasomotor' neurones to noxious electrical stimuli from the tail and hindpaws and of the early components from the spinal cord were consistent with the activation of Aδ afferent fibres. This is based on the assumption that the number of synapses involved were the same when one or other sites were stimulated. Thus, the difference in latencies of the responses to tail-tip and tail-base stimulation allows for the determination of conduction velocities of peripheral fibres activated, since an identical response pattern was observed whether the stimulus was applied to the ipsilateral or contralateral hindpaw, to the tail tip or the tail base, suggesting that they may share a symmetrical neuronal network. Interestingly, the early excitatory peak to stimulation of the contralateral hindpaw occurred earlier by some 2–3 ms than that obtained when the ipsilateral hindpaw was stimulated. One possible explanation of this difference is that the ipsilateral Aδ signals travel by a complex double-crossed pathway (Bing, Villanueva & Le Bars, 1990) before reaching the RVL–spinal 'vasomotor' neurones. The early response components, including the excitatory peak and inhibitory trough, were also present in the neuronal responses to stimulation of the lateral part of the spinal cord, a quadrant containing ascending nociceptive pathways (Vierck, Greenspan, Ritz & Yeomans, 1986). These latencies correspond closely to the estimated spinal and medullary conduction times of the Aδ afferents. This finding suggests that the early peak and trough in the neuronal responses to spinal cord stimulation arise from the activation of the Aδ afferents.

It is well established that low-intensity stimulation, activating large-diameter Aα and Aβ fibres, produces tactile sensation, and it is only when fine myelinated Aδ fibres are excited that a sensation of pain appears that builds up and becomes intolerable when unmyelinated C fibres are recruited (Besson & Chaouch, 1987). In

this respect, it is important to stress that the RVL–spinal 'vasomotor' neurones did not respond either to a skin temperature of 38 °C or to moderate pressure on the hindpaws. Thus, in the case of thermal and mechanical stimulations, it is most probable that the RVL–spinal 'vasomotor' neurones were activated from inputs emanating from peripheral Aδ and possibly C fibre polymodal nociceptors. Koizumi, Collin, Kaufman & Brooks (1970) described that an unmyelinated (C fibre) afferent, when activated, produced sympathetic reflex discharge with a very long latency. This reflex has supraspinal and spinal components (Horeyseck & Jänig, 1974). Such long-lasting, long-latency evoked responses, which might indicate a C fibre input to the RVL–spinal vasomotor neurones, were not seen after intense percutaneous electrical stimulation. Such late components did exist in the responses to spinal cord stimulation and, occasionally, in the responses to noxious hindpaw stimulation. However, it is not clear whether they represent a C fibre-evoked response since their peripheral conduction velocities were not determined because of their absence in response to tail stimulation under the present experimental conditions. Under the present experimental conditions, the anaesthetics (urethane plus pentobarbitone) administered to the rat could depress activity evoked by C fibres, whilst the faster, more synchronized responses to Aδ fibre activation may be more resistant to anaesthetics.

On the basis of anatomical and electrophysiological data obtained in several species, the main pathways responsible for the transmission of the nociceptive information in the spinal cord are the ventrolateral (Torvik, 1956; Zemlan, Leonard, Kow & Pfaff, 1978) and possible dorsolateral pathways (McMahon & Wall, 1983; Hylden, Anton & Nahin, 1989), terminating within the brain stem reticular formation and the thalamus. The RVL–spinal 'vasomotor' neurones could receive a collateral input from the lateral spinothalamic tract and/or a direct input from the spinoreticular tract (Torvik, 1956; Zemlan *et al.* 1978; Kevetter & Willis, 1983), though this remains to be investigated.

Noxious electrical stimulation of the hindpaws and tail, as well as the spinal cord, not only evoked an Aδ excitatory response but also an inhibitory response. The calculated peripheral conduction velocity of the Aδ afferent fibres responsible for this inhibition is not different from that of the fibres responsible for the excitation, but the inhibitory trough is usually present later than the excitatory peak, presumably as a consequence of an increased complexity of the central pathway involving inhibitory interneurones. The early inhibition in the peristimulus time histograms of the neuronal activity following hindpaw stimulation was blocked by ionophoresis of bicuculline, a $GABA_A$ receptor antagonist, at a dose at which the baroreflex inhibition of the same neurones was not totally eliminated. These data suggest that the stimulus-evoked inhibition is mediated by a GABA-like transmitter activating a $GABA_A$ receptor on the RVL–spinal 'vasomotor' neurone membrane. This GABAergic inhibition of the RVL–spinal vasomotor neurones may be involved in nociceptive-evoked vasodepressor responses (Mountcastle, 1980).

The convergence of nociceptive inputs onto the RVL–spinal 'vasomotor' neurones may reflect an involvement of these neurones in autonomic integration related to pain. Our present data, together with the results previously described (Brown & Guyenet, 1985; Sun & Guyenet, 1985, 1986*a*,*b*, 1987), reinforce the idea of an

important role for these neurones in central-evoked cardiovascular responses. The functional specificity of these neurones allows them to generate different patterns of sympathetic discharge and hence to effect fine adjustments in response to the continuously changing needs of the cardiovascular system under different behavioural and environmental conditions. Presumably, the highly differentiated pattern of sympathetic discharge seen in the cat to noxious stimuli (Jänig, 1985) is a consequence of the interplay of supraspinal and spinal reflex circuits. Their most intriguing feature, revealed in the present study, is their ability to encode the intensity of noxious stimuli to induce cardiovascular responses. The integrated output from these cells to the spinal cord constitutes a major command signal which determines the level of activity in the sympathetic neurones as well as setting the level of their responsiveness to noxious afferent inputs over other pathways. In conclusion, our data provide evidence that the RVL–spinal 'vasomotor' neurones have response characteristics that are suitable for the encoding of autonomic information coming from cutaneous nociceptors, suggesting that these neurones mediate, at least in part, the supraspinal sympathetic reflexes related to noxious inputs.

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