The relationship between cutaneous C fibre type and antidromic vasodilatation in the rabbit and the rat

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1. Skin blood flow was monitored during antidromic stimulation of identified cutaneous C fibres in fine filaments dissected from the saphenous nerve of anaesthetized rabbits and rats. The techniques used to monitor skin blood flow were laser Doppler perfusion imaging and laser Doppler flowmetry.

2. In the rabbit filaments a total of thirty-three C fibres were tested for their ability to produce antidromic vasodilatation. The only C fibres found to have vasodilator actions were of the polymodal nociceptor afferent class, and fourteen (50%) of the twenty-eight polymodal nociceptor units tested were vasoactive. The afferent receptive fields of polymodal nociceptor afferents were mapped carefully using suprathreshold mechanical stimuli, and there was a good correlation between afferent receptive field area and area of vasodilatation.

3. In the rat, eleven of the fifty-four C fibres antidromically stimulated had vasodilator actions. All eleven vasoactive C fibres were nociceptive and comprised seven polymodal nociceptor units, two heat nociceptor units and two incompletely classified nociceptor units. The area of increased blood flow was always coincident with the afferent field of the stimulated unit.

4. In the rat the vasodilator units were not evenly distributed over the saphenous nerve receptive field. Nine of the eleven vasoactive C fibres had receptive fields located on the foot or the digits, and only two were on the ankle or lower leg. Overall, the population of nociceptive C fibres was evenly distributed over the saphenous nerve receptive field.

5. In both the rabbit and the rat, a subclass of polymodal nociceptor afferents form the majority of the vasoactive units and will make the main contribution to axon reflex flare and other neurogenic inflammatory responses involving vasodilatation. The vasoactive polymodal nociceptor units tend to have relatively low mechanical sensitivity, although they have typical heat thresholds. In the rat heat nociceptor units also have vasodilator actions. However, such heat nociceptor units form a minor functional class of afferent C fibre in the rat saphenous nerve, and are not found in the rabbit saphenous nerve.

6. The findings from this study in the rabbit and the rat are compared with the situation in pig skin. The close relationship between afferent receptive field area and spread of flare across species is noted, and the way these measures increase with body size is discussed.

The inflammatory events involving the actions of afferent nerve fibres are collectively termed 'neurogenic inflammation', and comprise antidromic vasodilatation, plasma extravasation and flare (see reviews by Lynn, 1996; Szolcsányi, 1996). The phenomenon of antidromic vasodilatation has been studied since the turn of the century (Bayliss, 1901) with important work concerning the capsaicin sensitivity of the fibres involved being carried out by Janscó, Janscó-Gabor & Szolcsányi (1967, 1968). The question of which types of afferent nerve fibre produce the neurogenic component of inflammation is an important one. The unmyelinated afferent fibres (C fibres) have been shown to be involved in the production of vasodilatation and plasma extravasation (Janscó *et al.* 1967, 1968), and the inflammatory reactions are thought to be brought about by axon reflexes in these C fibres (Lisney & Bharali, 1989). In rabbit and rat skin, the high threshold mechanoreceptor Aδ units have a minor role in antidromic vasodilatation (Lynn & Shakhanbeh, 1988; Jänig & Lisney, 1989; Lewin, Lisney & Mendell, 1992; Kolston & Lisney, 1993), although Aδ fibres are not involved in the process of plasma extravasation (Jänig & Lisney, 1989). There have only been a few single unit studies to establish which functional classes of C fibre have neurogenic inflammatory actions. In

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the rat the C polymodal nociceptor afferents are the only class of C fibre with the ability to increase microvascular permeability upon antidromic stimulation (Kenins, 1981; Bharali & Lisney, 1992). In the pig, the vasoactive afferent fibres are a specialized class of C nociceptor, called heat nociceptor units, which are highly sensitive to noxious heating and irritant chemicals but have little or no mechanical sensitivity (Lynn, Faulstroh & Pierau, 1995; Lynn, Schütterle & Pierau, 1996). However, such heat nociceptor afferents are not found in rabbit cutaneous nerves (Lynn, 1979; Shea & Perl, 1985), and are rare in nerves supplying rat skin (Lynn & Carpenter, 1982). This study, therefore, was undertaken to reveal which functional class or classes of afferent C fibre are responsible for antidromic vasodilatation in the skin of the rabbit and the rat. The findings are compared with the situation in pig skin, and are discussed in relation to flare in these species. In addition, this study discusses the relationship between receptive field area of single afferent units, spread of flare in response to noxious stimuli and body size.

Some preliminary results from the study in the rat have been published in abstract form (Gee, Lynn & Cotsell, 1996).

METHODS

Anaesthesia and surgery

Experiments were carried out on adult male and female Sprague–Dawley rats ($n = 35$) and on male New Zealand White rabbits (2·5–3·5 kg; $n = 13$). Sodium pentobarbitone (Sagatal) anaesthesia was used (60 mg kg^{-1} I.P. for induction in rats, and 40 mg kg^{-1} I.V. in rabbits). In rabbits, 1·5–2 ml of 1% (w/v) lignocaine (lidocaine) was injected subcutaneously in the neck region, along both sides of the trachea to facilitate its cannulation. Body temperature was maintained close to 37 °C (rats) or 38 °C (rabbits) using a heated underblanket controlled by a rectal thermistor probe. The left jugular vein was cannulated for anaesthetic administration. In rats Sagatal was infused at a rate so that the animal was areflexic to a noxious stimulus (e.g. strong pinch to the front paw, touching the eyeball with blunt glass probe). In rabbits a mixture of Sagatal (60 mg ml^{-1}), Gelofusine (a plasma substitute, B. Braun Medical Ltd) and 0·9% saline (1 : 1 : 1) was infused intravenously at a rate sufficient to maintain areflexia. The left carotid artery was cannulated and blood pressure was monitored throughout.

The saphenous nerve was exposed in the thigh and was cut proximally. The surrounding skin was stitched to a metal ring to form a pool and this was filled with liquid paraffin oil. The foot and leg were mounted to prevent any movement from occurring in the pool.

At the end of the experiments the animals were killed with an anaesthetic overdose.

Recording and classification of afferent units

A pair of platinum stimulating electrodes was placed under the whole nerve at the distal end of the pool. A second pair of platinum electrodes were placed under the cut end of the nerve at the proximal end of the pool. Signals were recorded via an AC amplifier and were filtered (bandpass, 160–1600 Hz). The signals were displayed on an oscilloscope (Tektronix 2230), and were logged to a computer via an A/D board (Microstar Laboratories, DAP 2400).

The cut end of the nerve was placed on a mirrored platform and was desheathed using a small piece of razor blade. Filaments were dissected out using watchmaker's forceps. The filaments were repeatedly split until stimulation of the whole nerve (0·5 ms pulse width) showed there to be identifiable C fibres conducting in the filament. Although some filaments contained just one conducting C fibre, multiunit filaments were also used providing that individual C fibres could be identified from the recording. C fibres were identified by their slow resting conduction velocity; all C fibres included in the results presented here had a resting conduction velocity of less than 1·5 m s^{-1}.

Individual C fibres were characterized according to their sensitivity to pressure (von Frey hairs ranging in force up to 20 g) and to heat (contact heat probe delivering a ramp stimulus from 35 °C increasing at a rate of 1 °C s^{-1}, to a maximum of 65 °C). The stimulus for innocuous cold was a brief application of ice or a cold metal ball. The individual C fibres were classified into polymodal nociceptor units (sensitive to noxious mechanical and thermal stimuli, von Frey thresholds $> 0{\cdot}05$ g and heat thresholds > 39 °C), mechanical nociceptor units (sensitive to noxious mechanical stimuli only), heat nociceptor units (sensitive to noxious heat only), mechanoreceptor units (sensitive to innocuous mechanical stimuli, von Frey threshold < 5 mg) or inexcitable units (insensitive to the mechanical or thermal search stimuli). In a number of instances the electrical receptive fields of inexcitable units were located using electrical skin stimulation (Kress, Koltzenburg, Reeh & Handwerker, 1992). No cold thermoreceptor units (sensitive to innocuous cold only) were found in this study. A small number of units had von Frey thresholds in the noxious range but were not tested with heat. These units have been labelled incompletely classified nociceptor units or polymodal/mechanical nociceptor units.

Antidromic filament stimulation

The collision technique was used to determine the electrical thresholds of individual C fibres for antidromic filament stimulation (Andrew, Matthews & Coates, 1996; see Fig. 1). The whole nerve was given a single shock at a strength just supramaximal for the C fibre component of the compound action potential to excite all the conducting C fibres within the filament. The filament was stimulated at the same time using a constant current stimulator with high output resistance. By using a constant current stimulator it was possible to record continuously from the filament without the need to switch out the recording for filament stimulation. The intensity of filament stimulation was increased until collision occurred and the individual C fibres dropped out (Fig. 1).

Having established thresholds by collision, filaments were stimulated at 2–4 × threshold for 10–120 s at frequencies ranging from 2–10 Hz (0·5 ms pulse width). Skin blood flow was monitored before, during and after filament stimulation using laser Doppler flowmetry (MBF3 and MBF3D, Moor Instruments, UK) and/or laser Doppler perfusion imaging (Lisca Developments, Linkoping, Sweden). In experiments using laser Doppler flowmetry, two or three channels were used, with one or two probes positioned over the afferent receptive field(s) being studied and one probe monitoring skin blood flow in a control region within the saphenous nerve receptive field. With the laser Doppler perfusion imaging system the scanner head was positioned so that the area imaged included the afferent receptive field being monitored and some surrounding control skin. With filaments containing inexcitable

units the skin surrounding their electrical receptive fields was imaged. Several control scans were made until a stable baseline blood flow had been established in the area being studied. In one of the control scans the centres of afferent fields were marked with small pieces of black adhesive tape so that their positions were visible on a control image. This allowed the position of any efferent response to be compared with the location of the receptive fields of identified afferents. The percentage change in blood flow from baseline was calculated for all the C fibres tested in this study. The criterion for a vasodilator response was set at the level of two standard deviations greater than the average change in skin blood flow measured in control skin (see beginning of Results). When filament stimulation did not result in any vasodilator response, antidromic vasodilatation to whole nerve stimulation was checked using a C maximum intensity stimulus (0·5 ms pulse width) for 15–30 s at 1–2 Hz (see Fig. 2). If whole nerve stimulation failed to elevate blood flow in the test area well above background, then data from filament stimulation were not used.

It is theoretically possible that in a multifibre filament responses from two or more units could overlap, making the contribution of individual units difficult to ascertain. However, the numbers of C units studied in individual filaments were always four or less and since individual fields are small, compared with the whole innervation area of the nerve, overlap is unlikely. Where, as occasionally occurred, two afferent receptive fields did overlap these filaments were not used. It remains possible that the terminals of an inexcitable C fibre overlapped with the receptive field of a vasoactive unit, but the probability of this happening is very small.

In six experiments in the rat the entire saphenous innervation area was scanned during and after stimulating the whole nerve at maximal C fibre strength (1–2 Hz, 15–30 s). From these images the

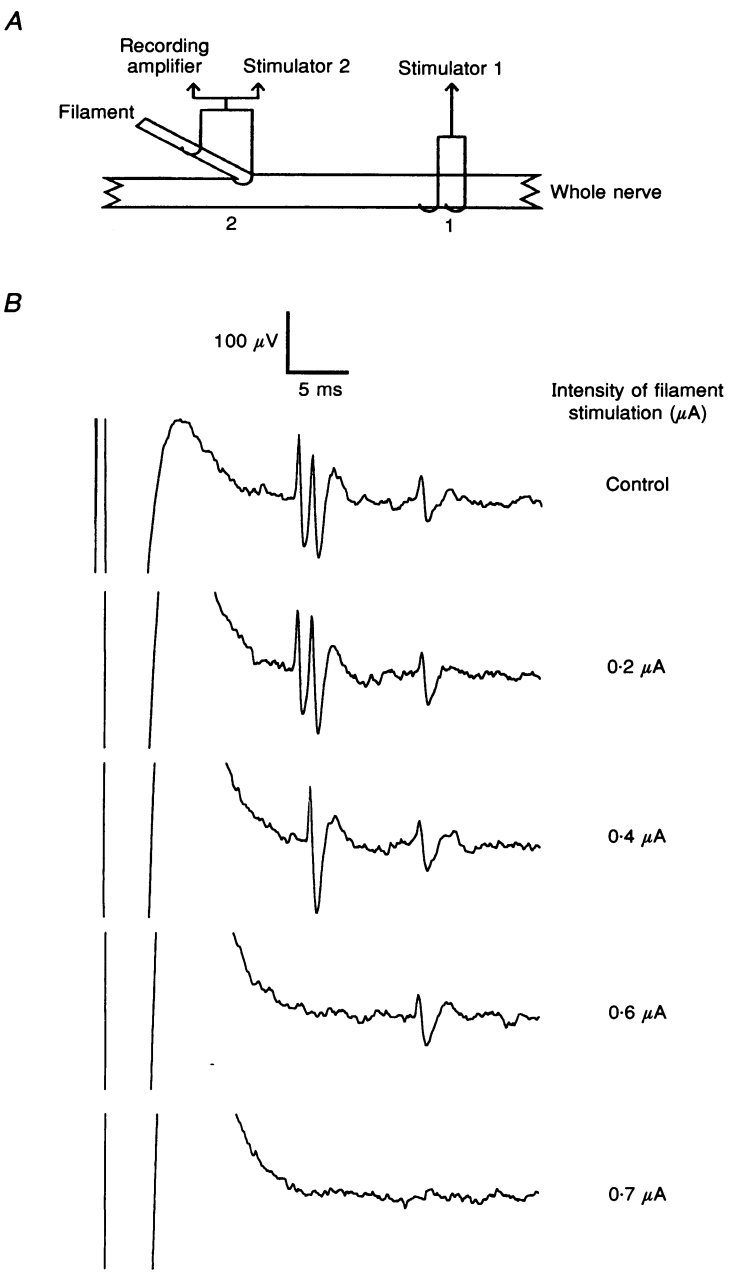

Figure 1. Use of the collision technique to establish the electrical thresholds for antidromic stimulation of individual C fibres within a filament

A, schematic diagram showing the arrangements of the electrodes, stimulators and recording amplifier during collision tests. *B*, the whole nerve is given a single C maximum intensity shock at position 1 (in Fig. 1*A*). Recording at position 2 reveals 3 conducting C fibres within the filament (top trace). The whole nerve is stimulated at position 1, and at the same time the filament is stimulated at position 2. The intensity of filament stimulation is noted in the right hand column. The intensity of filament stimulation is increased until collision occurs and the individual C fibres drop out from the recording (bottom 4 traces).

level of antidromic vasodilatation in different parts of the saphenous innervation zone could be compared.

Comparison of afferent and efferent receptive fields in the rabbit

In rabbits the extents of the afferent and efferent fields were compared. This was achieved by carefully mapping the afferent receptive fields of vasodilator units using suprathreshold (2–5 × threshold) von Frey hairs, working with a microscope and a graticule grid. For calculation of the afferent receptive field area, the number of squares of the graticule grid overlying skin where mechanical stimulation excited the unit being studied were noted. Receptive field maps were constructed from this datum and related to ink reference marks on the skin. The position of markers on images relative to the ink reference marks was also noted. It was thus possible to plot afferent receptive field outlines on scans (e.g. see Fig. 3). In rats, where the afferent receptive fields are smaller, such mapping was not carried out. Areas of efferent vasodilator responses were measured on scans. The border of the efferent response was set at 20–33 % above baseline.

Statistical methods

Data are presented as means ± S.E.M. with n the number of units, unless stated otherwise. For most comparisons Student's two-tailed t test was used. Because they appeared rather non-normally distributed, a non-parametric test (Mann–Whitney U test) was used to compare the mechanical thresholds of the vasoactive and the non-vasoactive units. A χ^2 test was performed to examine the distribution of vasoactive and non-vasoactive units throughout the saphenous receptive field of the rat. To determine the effect of antidromic stimulation on the whole rat saphenous nerve on two different areas of skin (foot *vs.* leg) a paired t test was carried out.

RESULTS

Thirty-three C fibres in the rabbit and fifty-four C fibres in the rat were tested for their ability to produce vasodilatation upon antidromic stimulation. The units did not come from a random sample and so the true proportions of the different functional classes of C fibre present in the saphenous nerves of these species are not represented. Skin blood flow was monitored using either the laser Doppler perfusion imaging system or laser Doppler flowmetry, and the percentage changes in skin blood flow that occurred within the afferent receptive fields were calculated.

Vasodilatation resulting from antidromic stimulation of rabbit cutaneous C fibres

The thirty-three C fibres from the rabbit saphenous nerve tested for their vasoactive properties comprised twenty-eight polymodal nociceptor units, one mechanical nociceptor unit and four mechanoreceptor units, and their properties are given in Table 1. Figure 3 shows images of the vasodilatation resulting from antidromic stimulation of identified polymodal nociceptive C fibres. The images have been scaled so that the colours represent the percentage increase in blood flow above the baseline.

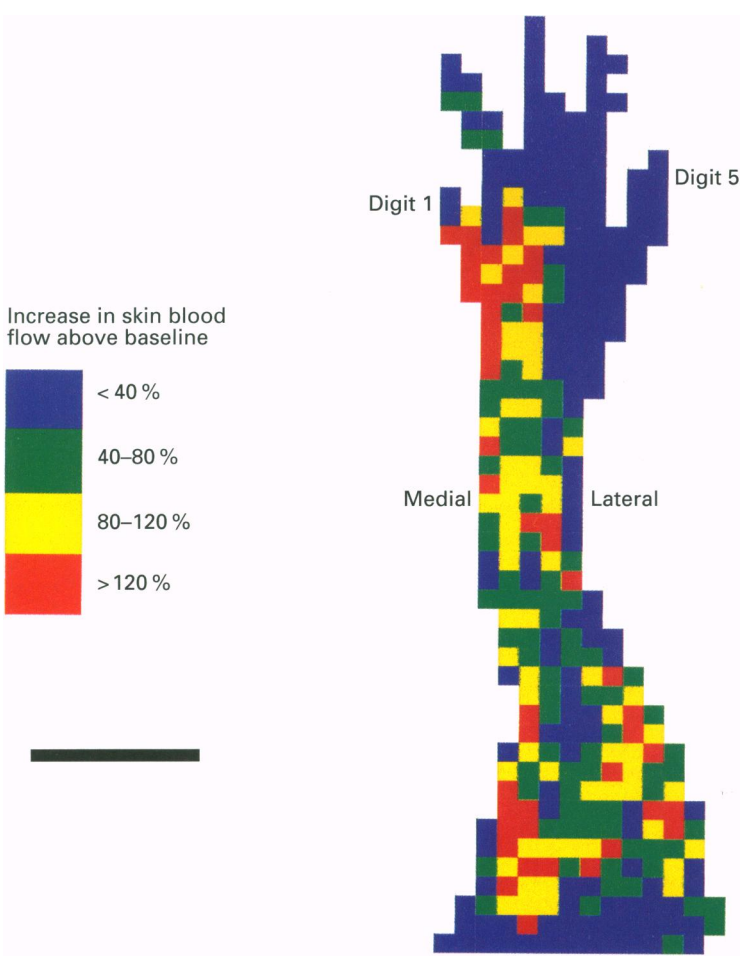

Figure 2. Antidromic vasodilatation due to stimulation of the whole saphenous nerve in the rat

Image showing cutaneous antidromic vasodilatation following electrical stimulation (1·5 mA, 15 s, 2 Hz, 0·5 ms pulse width) of the rat saphenous nerve. This image was created by subtracting the control baseline scan taken before electrical stimulation from the scan started approximately 1 min after stimulation. Skin blood flow has clearly risen in the saphenous field, and up to levels of greater than 120 % along the medial edge of the foot. Note that the view is dorso-medial and that there is a slight inward rotation at the ankle to improve the view of the dorsal surface of the foot and the medial aspect of the leg. Scale bar, 10 mm.

Figure 4*A* shows the average changes in skin blood flow in the receptive fields of the different types of afferent units and in areas of control skin on the same scans. The only class of C fibre that produced vasodilatation responses upon antidromic stimulation was that of the polymodal nociceptor afferents. However, not all of the polymodal nociceptor C fibres tested produced vasodilatation. The variation in blood flow in control areas of skin (i.e. where no afferent receptive fields were present) was measured in twenty-four pairs of scans, and the distribution is shown in the open columns in Fig. 5*A*. The average change in blood flow in control skin was $-1{\cdot}1 \pm 6{\cdot}2\%$ (mean ± S.D., $n = 24$) from the baseline. The limit for a vasodilator response was set at two standard deviations from the mean increase in control skin and therefore any change in skin blood flow that exceeded 11·3% was considered to be a positive vasodilator

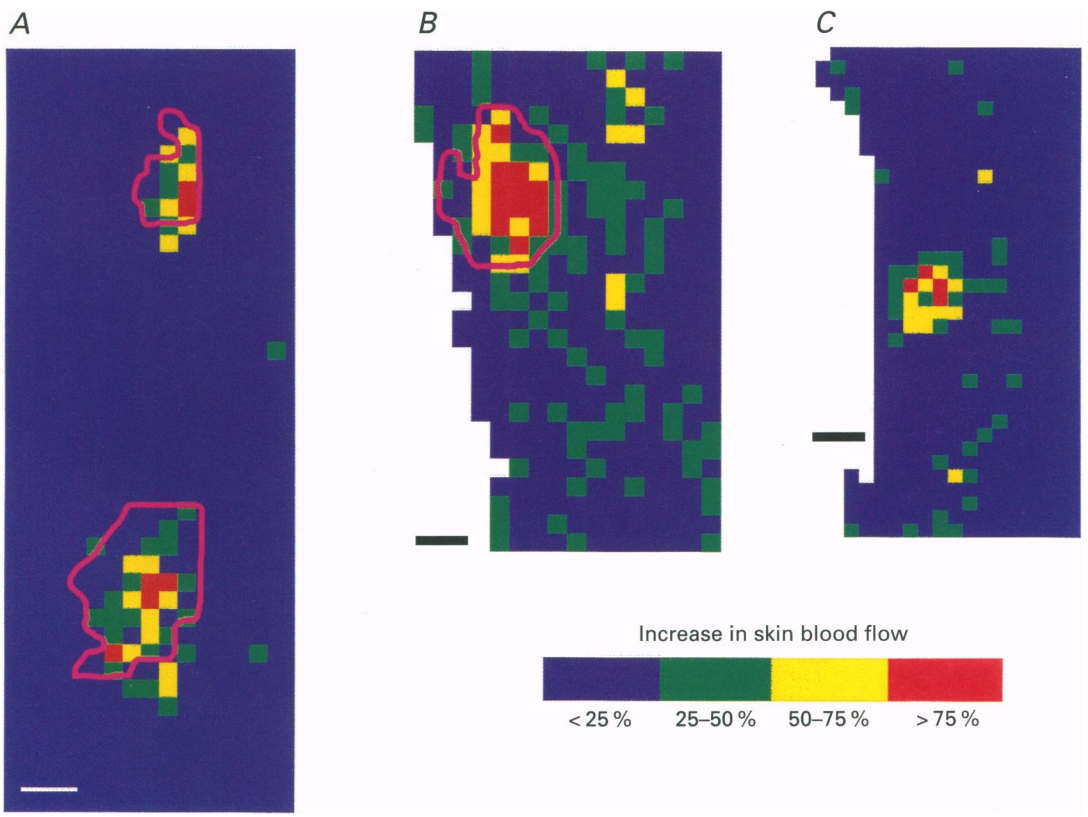

Figure 3. Cutaneous vasodilatation following antidromic stimulation of identified C polymodal nociceptor units in the rabbit saphenous nerve

To make these images, baseline scans have been subtracted from scans taken after antidromic filament stimulation to make 'difference' images. The images have all been scaled so that the colours represent percentage increase in blood flow above the baseline. The blue colour represents the smallest changes in blood flow, and includes any decreases in blood flow from the baseline. *A*, this filament contained 2 polymodal nociceptor C fibres. The upper unit had a conduction velocity of 0·89 m s^{-1}, a mechanical threshold of 0·4–0·6 g, a heat threshold of 62·5 °C and its afferent receptive field was approximately 3·9 mm × 2·3 mm. The lower unit had a conduction velocity of 0·90 m s^{-1}, a mechanical threshold of 0·4–0·6 g, a heat threshold of 56 °C and its afferent receptive field was approximately 6·2 mm × 4·7 mm. Both afferent receptive fields have been outlined on the image. The parameters for filament stimulation were 7·0 μA, 120 s, 5 Hz, 0·5 ms pulse width. In both cases, the areas of vasodilatation match well with the afferent receptive fields. Two 'difference' images have been averaged to make this image. Scale bar, 2 mm. *B*, this filament contained a C polymodal nociceptor unit conducting at 0·95 m s^{-1}, with a mechanical threshold of 0·4–0·6 g and a heat threshold of 55 °C. Its afferent receptive field was approximately 6·2 mm × 4·7 mm, and has been outlined on the image. The parameters for filament stimulation were 2·0 μA, 120 s, 5 Hz, 5 ms pulse width. Six 'difference' images have been averaged to make this image. Scale bar, 2 mm. *C*, this filament contained a C polymodal nociceptor unit conducting at 0·92 m s^{-1}, with a mechanical threshold of 0·4–0·6 g and a heat threshold of 52·5 °C. Its afferent receptive field was not mapped, but the centre of the afferent field coincided with the centre of the vasodilator response. The parameters for filament stimulation were 4·0 μA, 60 s, 5 Hz, 5 ms pulse width. Two 'difference' images have been averaged to make this image. Scale bar, 2 mm.

Table 1. Some properties of identified C fibres in the saphenous nerve of rabbits and rats

	Blood flow increase (%)				Conduction velocity ($m\ s^{-1}$)				Mechanical threshold (g)				Heat threshold (°C)			
Class	Mean	*n*	Max	Min	Mean	*n*	Max	Min	Median	*n*	Max	Min	Mean	*n*	Max	Min
Rabbit																
Vasoactive PMN	45·5	14	108	28	0·97	14	1·14	0·82	0·50	14	1·50	0·07	53·1	13	62·5	44·5
Non-vasoactive PMN	−2·6	14	9	−14	0·99	12	1·20	0·84	0·15	14	0·50	0·07	52·8	9	58·0	47·0
MN	4·4	1	—	—	1·02	1	—	—	0·07	1	—	—	—	—	—	—
Mechano	−7·7	4	−1	−12	0·91	4	1·05	0·86	<0·005	4	<0·005	<0·005	—	—	—	—
Rat																
Vasoactive PMN	65·7	9	145	17	0·82	9	1·07	0·59	1·5*	9	>20·0	0·07	49·6	7	58·0	39·0
Non-vasoactive PMN	−2·3	34	11	−16	0·76	34	0·95	0·61	0·9*	34	>20·0	0·10	51·5*	33	>60·0	40·0
HN	90·0	2	160	20	0·67	2	0·82	0·53	>20·0	2	>20·0	>20·0	56·5	1	—	—
MN	−2·5	3	0	−6	0·80	3	0·94	0·70	15·0*	3	>20·0	0·80	—	—	—	—
Mechano	7·0	2	8	5	0·76	2	0·80	0·72	<0·005	2	<0·005	<0·005	—	—	—	—
Inex	−2·2	4	4	−9	0·86	4	1·48	0·54	—	—	—	—	—	—	—	—

n, number of units; Max, maximum value; Min, minimum value; PMN, polymodal nociceptors (including some incompletely classified nociceptors in the rat); MN, mechanical nociceptors; HN, heat nociceptors; Mechano, mechanoreceptors; Inex, inexcitable units. A criterion of +2 standard deviations from the average increase in blood flow in control skin was used to define the vasoactive units. * Note that for calculation of average threshold values, mechanical thresholds of > 20 g were assigned a value of 25 g, mechanical thresholds of > 10 g were assigned a value of 15 g, and heat thresholds of > 60 °C were assigned a value of 65 °C.

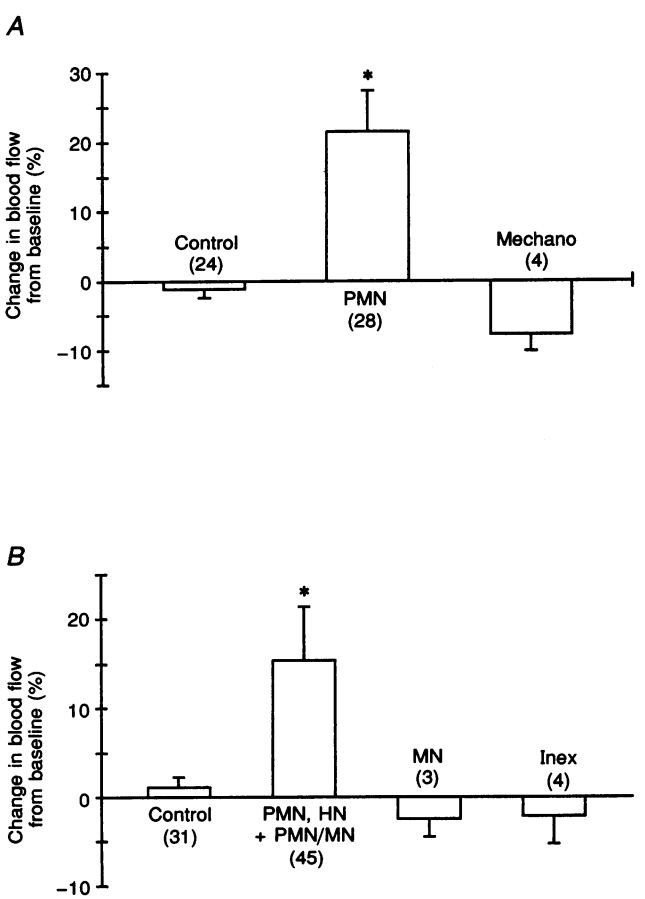

Figure 4. Changes in skin blood flow in the receptive fields of identified afferent units and in control skin areas

Data from the rabbit (*A*) and the rat (*B*). Control, control areas of skin where no afferent receptive fields were located; PMN, polymodal nociceptor units; MN, mechanical nociceptor units; HN, heat nociceptor units; PMN/MN, incompletely classified nociceptors; Mechano, mechanoreceptor units; Inex, inexcitable units. Note that only classes containing more than two units have been included. Mean (+ S.D.) is plotted with *n* in parentheses. * $P < 0·05$ for a *t* test comparing the percentage changes in skin blood flow produced by antidromic stimulation of classified C fibres with the changes measured in control skin.

response. Using this cut-off point for a vasodilator response, fourteen of the twenty-eight polymodal nociceptor afferents tested in this study were classed as vasoactive. All fourteen vasoactive polymodal nociceptor units produced clear patches of increased blood flow upon antidromic stimulation, ranging from 28 to 108% above baseline (see Table 1 and Fig. 5*A*). The areas of vasodilatation were always coincident with the afferent receptive fields of the vasoactive polymodal nociceptor units (see Fig. 3 and Results section below).

Antidromic stimulation of nineteen C fibres in the rabbit saphenous nerve (comprising 14/28 polymodal nociceptor afferents, 1/1 mechanical nociceptor afferents and 4/4 mechanoreceptor afferents) did not result in a vasodilator response, i.e. any changes in blood flow were < 11·3%. The average changes in blood flow for each functional class of non-vasodilator C fibre are given in Table 1 and Fig. 4. In all cases stimulation of the whole saphenous nerve resulted in vasodilatation in the region of the units being studied. This showed that the skin in and around the afferent receptive fields of the identified units was capable of producing a vasodilator response. Also all of the non-vasodilator units were able to be stimulated orthodromically following the period of antidromic filament stimulation, showing that conduction block had not occurred during the period of filament stimulation.

The distribution of the heat thresholds of the polymodal nociceptor units is shown in Fig. 6*A*. The average heat threshold of the vasodilator polymodal nociceptor afferents was 53·1 ± 1·4 °C ($n = 13$), and the average heat threshold of the non-vasodilator polymodal nociceptor afferents was very similar (52·8 ± 1·4 °C, $n = 9$). The distribution of the mechanical thresholds of the polymodal nociceptor units is shown in Fig. 6*B*. The average mechanical threshold of the vasodilator polymodal nociceptor units was 0·47 ± 0·10 g ($n = 14$), and the average mechanical threshold of the non-vasodilator polymodal nociceptor units was 0·18 ± 0·03 g ($n = 14$). Statistical comparison between these two groups shows that the vasodilator polymodal nociceptor C fibres had higher mechanical thresholds than the non-vasodilator polymodal C units (Mann–Whitney *U* test, $P = 0·01$).

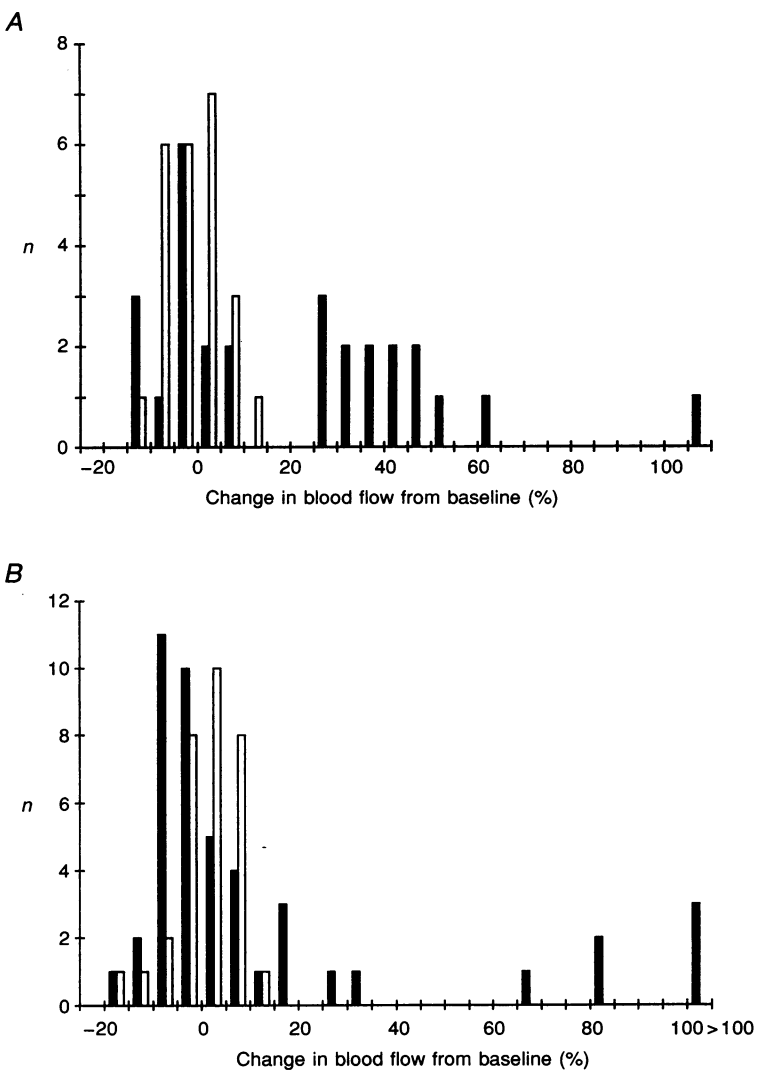

Figure 5. Distribution of the percentage changes in skin blood flow in receptive fields of polymodal nociceptor units and in control areas

Data from the rabbit (*A*) and the rat (*B*). The filled bars represent the percentage increases in skin blood flow measured in the afferent receptive fields of identified polymodal nociceptor units following their antidromic stimulation. In the rat, the 2 heat nociceptor units and the 3 incompletely classified nociceptor units have also been included. The open bars represent measurements of skin blood flow changes in control areas of skin (i.e. where no afferent receptive fields were present). Note that the control skin values average −1·1 ± 6·2% (mean ± S.D., $n = 24$) in the rabbit and +1·1 ± 6·2% (mean ± S.D., $n = 31$) in the rat.

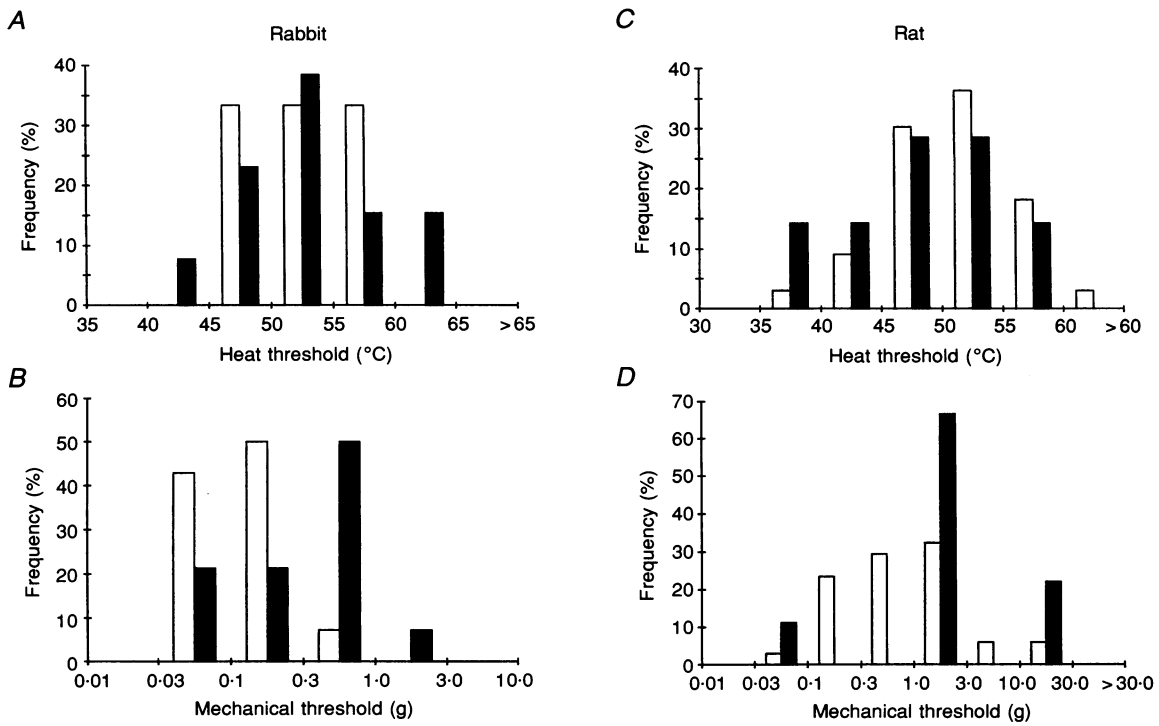

Figure 6. Normalized frequency distribution of heat thresholds and mechanical thresholds of polymodal nociceptor units

Histograms of the heat thresholds (*A* and *C*) and the mechanical thresholds (*B* and *D*) of the polymodal nociceptor units in the rabbit (*A* and *B*) and rat (*C* and *D*) saphenous nerves. The filled bars represent the vasoactive units and the open bars represent the non-vasoactive units. Note the logarithmic scale in the mechanical threshold histograms. Also note that the mechanical thresholds of 3 incompletely classified rat nociceptors are included in *D*.

Comparison between the afferent and efferent receptive fields of rabbit C polymodal nociceptor afferents

The afferent receptive fields of fourteen polymodal nociceptor units (including 11 of the 14 vasoactive polymodal units) from the rabbit saphenous nerve were mapped with suprathreshold (2–5 × threshold) von Frey filaments. The afferent receptive field dimensions ranged from 3·1 to 8·6 mm in the proximal–distal direction and from 2·0 to 5·5 mm in the medial–lateral direction. In all fourteen vasoactive polymodal nociceptor units in the rabbit the areas of vasodilatation (i.e. the efferent receptive fields) were coincident with the afferent receptive fields of the units (see Fig. 3). There was no significant difference between the sizes of the afferent receptive fields (11·6 ± 3·1 mm^2, $n = 14$) and the efferent receptive fields (i.e. the areas of vasodilatation;

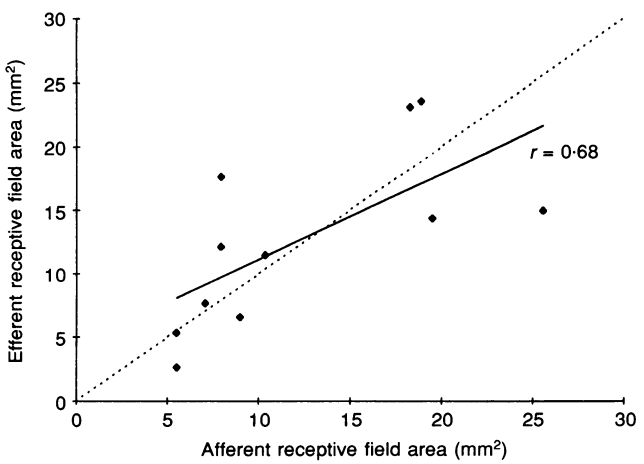

Figure 7. The relationship between the size of the afferent and efferent receptive fields of polymodal nociceptor units in the rabbit

The areas of the afferent and efferent receptive fields of 11 polymodal nociceptor units in the rabbit saphenous nerve have been plotted against each other. Dotted line, unity line; continuous line, best fit line ($r = 0·68$). Afferent receptive field areas were mapped with suprathreshold (2–5 × threshold) von Frey filaments, using a dissecting microscope with a graticule grid (see Methods). Efferent receptive fields were measured as the area of elevated blood flow on the laser Doppler images.

$11{\cdot}8 \pm 1{\cdot}7$ mm^2, $n = 14$). Figure 7 shows that there is a good correlation ($r = 0{\cdot}68$, $P < 0{\cdot}01$) between the sizes of the afferent and efferent receptive fields in the eleven rabbit polymodal nociceptor units where both of these measurements were made.

Vasodilatation resulting from antidromic stimulation of rat cutaneous C fibres

Fifty-four C fibres in the rat saphenous nerve were studied for their ability to produce antidromic vasodilatation, and they comprised forty polymodal nociceptor units, three polymodal/mechanical nociceptor units, three mechanical nociceptor units, two heat nociceptor units, two mechanoreceptor units and four inexcitable C units. Figure 8 shows images of the vasodilatation resulting from antidromic stimulation of nociceptive C fibres of the rat saphenous nerve. In each case elevations of blood flow are seen at the same place as a nociceptor receptive field. Figure 9 shows laser Doppler flowmeter traces illustrating the skin blood flow at three sites before, during and after stimulation of a fine filament. The filament contained two identified C fibres (both classified as polymodal/mechanical nociceptor units), and only one of these two C fibres demonstrated detectable vasodilatation.

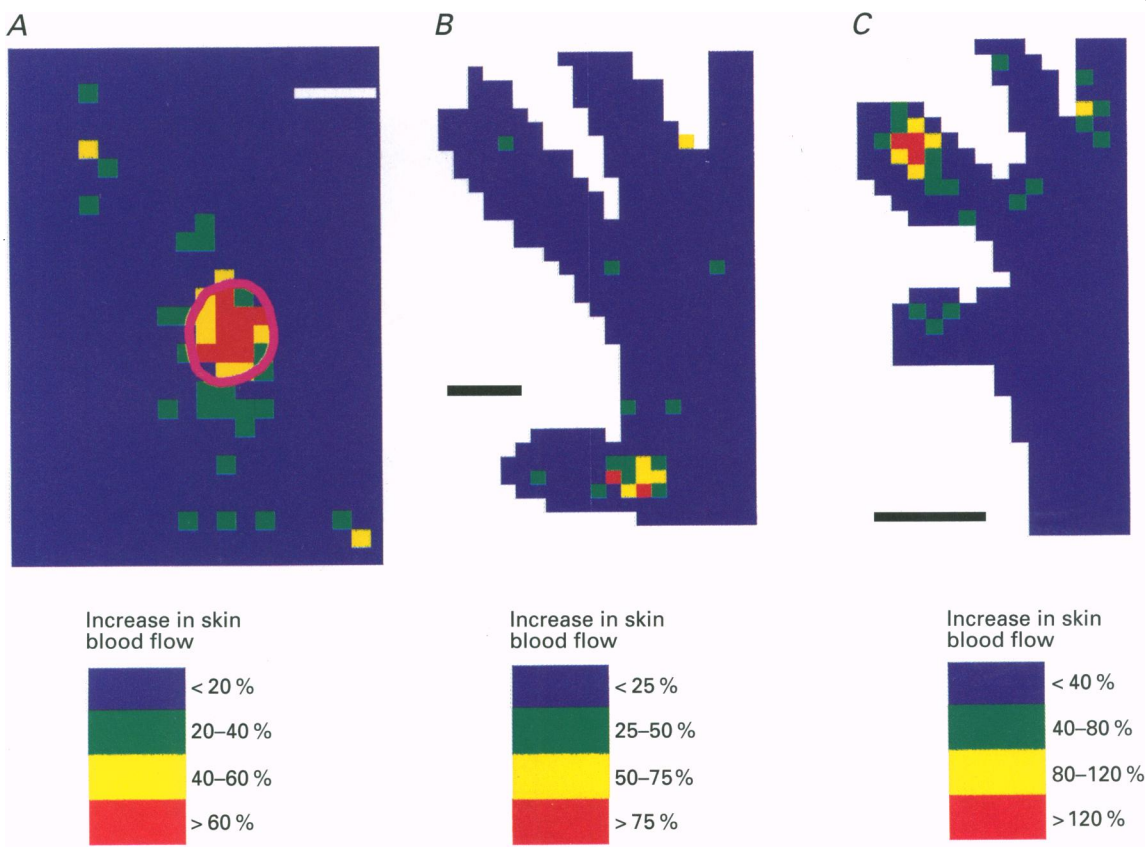

Figure 8. Cutaneous vasodilatation following antidromic stimulation of identified C polymodal nociceptor units in the rat

These images have been made in the same way as the rabbit images in Fig. 3, with the colours scaled to represent percentage increase in blood flow above the baseline and with the blue colour including any decreases in blood flow. *A*, this filament contained a single C polymodal nociceptor unit conducting at 0·79 m s^{-1}, with a mechanical threshold of 1–2 g and a heat threshold of 53 °C. Its afferent receptive field was located on the anterior foot near the ankle, and was approximately 2·5 mm × 2 mm, as shown by the red outline. The parameters for filament stimulation were 0·9 μA, 60 s, 5 Hz, 0·5 ms pulse width. Three 'difference' images have been averaged to make this image. Scale bar, 2 mm. *B*, this filament contained a C polymodal nociceptor unit conducting at 0·91 m s^{-1}, with a mechanical threshold of 1–2 g and a heat threshold of 52·5 °C. Its afferent receptive field was a small zone on the base of digit 1, coincident with the zone of vasodilatation. The parameters for filament stimulation were 1·0 μA, 120 s, 5 Hz, 0·5 ms pulse width. Five 'difference' images have been averaged to make this image. Scale bar, 2 mm. *C*, this filament contained a C heat nociceptor unit conducting at 0·53 m s^{-1} with a heat threshold of 53 °C. Its afferent receptive field was located on the anterior surface of digit 2, again coinciding with the area of elevated blood flow. The parameters for filament stimulation were 1·4 μA, 30 s, 5 Hz, 0·5 ms pulse width. Three 'difference' images have been averaged to make this image. Scale bar, 5 mm.

Figure 4*B* shows the average changes in skin blood flow in the receptive fields of the different types of afferent units and in areas of control skin on the same scans. The only classes of C fibre that produced antidromic vasodilatation were the polymodal and heat nociceptor units (including the polymodal/mechanical nociceptor units). The variation in blood flow in control areas of skin (i.e. where no afferent receptive fields were present) was measured in thirty-one pairs of scans, and the distribution is shown in the open columns in Fig. 5*B*. The average change in blood flow in control skin was +1·1 ± 6·2% (mean ± S.D., $n = 31$) from the baseline. The limit for a vasodilator response was set at two standard deviations from the mean increase in control skin. Any change in skin blood flow that exceeded 13·5% was therefore considered to be a positive vasodilator response. Using this value as the lower limit for a vasodilator response, just eleven of the fifty-four units tested were classed as vasoactive. All eleven vasoactive units were nociceptors (7 polymodal, 2 heat and 2 polymodal/mechanical; see Fig. 4) and their properties are given in Table 1. They produced increased blood flow ranging from 17 to 180% above baseline (see Fig. 5*B*) and the areas of vasodilatation were always coincident with the afferent receptive field of the units. Nine of the eleven vasodilator units had receptive fields on the foot or digits. The vasodilator units were significantly more likely to be on the foot (including the digits) where nine out of twenty-two nociceptors caused vasodilatation, than on the ankle or leg where this occurred with only two out of twenty-four nociceptor units ($r^2 = 5{\cdot}02$, $P = 0{\cdot}025$).

Despite having similar baseline blood flow levels, the skin on the foot and the skin on the leg of the rat had significantly different vasodilatation responses to whole nerve stimulation (e.g. see Fig. 2). The increase in blood flow following antidromic stimulation of the whole rat saphenous nerve was on average 91·3% on the foot and 65·6% on the leg, a difference that is statistically significant ($P = 0{\cdot}007$, paired t test, $n = 6$ pairs).

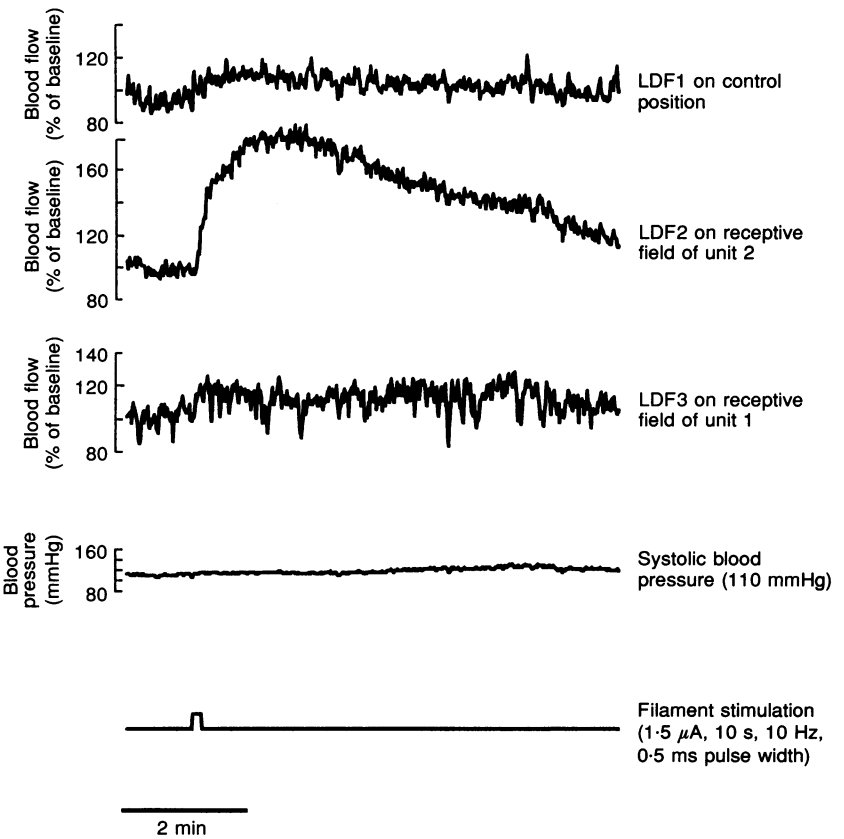

Figure 9. Single unit vasodilatation measured using laser Doppler flowmetry

Laser Doppler flowmeter traces during antidromic stimulation (1·5 μA, 10 s, 10 Hz, 0·5 ms pulse width) of a filament from the rat saphenous nerve containing 2 nociceptive C fibres. Neither C fibre was tested with heat stimuli, and so these units were classed as polymodal/mechanical nociceptor units, although their mechanical thresholds were typical of polymodal nociceptor C fibres. Unit 1 (3rd trace from the top) was located on the medial lower leg near the ankle; no vasodilatation was detected at this position. Unit 2 was located on the top of digit 1. Antidromic filament stimulation resulted in a great increase of skin blood flow (up to 145% increase above baseline) at the receptive field of unit 2 (2nd trace from the top). A laser Doppler flowmeter probe placed in a control position on the anterior mid-foot did not record any vasodilatation (top trace). The blood pressure remained constant at approximately 110/80 mmHg.

Antidromic stimulation of forty-three C fibres in the rat saphenous nerve (comprising 33/40 polymodal nociceptor afferents, 1/3 polymodal/mechanical nociceptor afferents, 3/3 mechanical nociceptor afferents, 2/2 mechanoreceptor units and 4/4 inexcitable units) did not result in a vasodilator response, i.e. any changes in blood flow were $< 13{\cdot}5\%$ and, when the imager was used, no consistent zone of elevated blood flow could be seen. The average changes in blood flow for each functional class of non-vasodilator C fibre are given in Table 1 and Fig. 4. Note that for the inexcitable units the blood flow changes within the electrical receptive field (determined by mapping with electrical skin stimulation as described in the Methods) were calculated. As in the rabbit, stimulation of the whole saphenous nerve resulted in vasodilatation in the region of the non-vasoactive units and all of the non-vasodilator units could be stimulated orthodromically following the period of antidromic filament stimulation.

The distribution of the heat thresholds of the polymodal nociceptor C fibres is shown in Fig. 6*C*. Those units that produced vasodilatation had a mean heat threshold of $49{\cdot}6 \pm 2{\cdot}4$ °C ($n = 7$) and the non-vasodilator polymodal units had similar mean heat thresholds of $51{\cdot}5 \pm 0{\cdot}9$ °C ($n = 33$). The distribution of the mechanical thresholds of the polymodal nociceptor units is shown in Fig. 6*D*. With the exception of one unit, the polymodal nociceptor afferents that produced antidromic vasodilatation had mechanical thresholds of greater than 1 g. The vasoactive polymodal and incompletely classified nociceptor units had significantly higher mechanical thresholds than the non-vasodilator units (Mann–Whitney U test, $P = 0{\cdot}04$). The rat polymodal nociceptor units had higher mechanical thresholds than the rabbit polymodal nociceptor units (see Table 1), and this is a consistent finding in the saphenous nerves of these two species (Lynn & Baranowski, 1987).

DISCUSSION

Vasodilator units in the rabbit and the rat

In both the rabbit and the rat the only type of C fibres shown to have vasodilator actions when individually antidromically stimulated were nociceptors (see Fig. 4). In the rabbit all of the vasodilator nociceptive C fibres found were of the polymodal type, although no inexcitable units were tested. However, none of the four inexcitable C fibres from the rat saphenous nerve produced vasodilatation upon antidromic stimulation. In the rat the majority of the vasoactive C fibres were polymodal nociceptor units (7 of 11, i.e. 64%), and the other vasoactive units were heat nociceptor units ($n = 2$) and polymodal/mechanical nociceptor units (i.e. incompletely classified nociceptor afferents, $n = 2$). It is likely that the two incompletely classified nociceptor units were polymodal nociceptor units, since they had mechanical thresholds within the polymodal range and because, in the rat, mechanical nociceptor afferents constitute only a minor class of cutaneous C fibres (Lynn, 1994).

In the rabbit it seems that the vasoactive units constitute a clear sub-population of polymodal nociceptors (see distribution on Fig. 5*A*), and they have relatively low mechanical sensitivity. Fifty per cent of the rabbit polymodal nociceptors tested in this study were vasoactive. In the rat, however, the situation is less clear-cut. Although the majority of the vasoactive C fibres were polymodal nociceptor afferents, the matter is complicated by the presence of vasoactive heat nociceptor units. In addition just 23% of the nociceptors tested were vasoactive. This low proportion of vasoactive C fibres found in the rat when compared with the vasodilatation response to whole nerve stimulation raises the question of whether all of the vasoactive units were positively identified in this study. In the rat the distribution of vasoactive units is not as clearly separated from the non-vasoactive units as in the rabbit (compare Fig. 5*A* and *B*). It is therefore possible that there are some vasoactive units whose single contribution is not great enough to be classed as vasoactive by the methods used in this study. In other words, the level of a 13·5% increase in blood flow which has been used as the lower limit for a true vasodilator response in the rat may conceal some genuine vasodilator responses of small size. If this is so, the proportion of vasoactive units found in the rat will be an underestimate.

The finding that just 23% of the rat nociceptor units tested produced antidromic vasodilatation contrasts with the results of single C fibre plasma extravasation studies in this species which found that between 67 and 100% of polymodal nociceptor units produced plasma extravasation when antidromically stimulated (Kenins, 1981; Bharali & Lisney, 1992). This discrepancy could be due to the proportion of vasoactive C fibres being underestimated, as discussed above. However, C fibres that contain only substance P would produce plasma extravasation but not antidromic vasodilatation because substance P is not an effective vasodilator in rat skin (Brain & Williams, 1988; Garret *et al.* 1991). Therefore, if C fibres containing just substance P, or another mediator that causes just plasma extravasation, form a large enough part of the innervation of rat skin, this would explain the discrepancy between the single unit results on vasodilatation and plasma extravasation.

In both the rabbit and the rat the efferent vasodilator response was always coincident with the afferent receptive field. Furthermore, in the rabbit, a comparison between afferent and efferent receptive field areas was possible and they were found to be very similar. The close similarity of afferent and efferent receptive field size is consistent with the theory that individual terminals of cutaneous C fibres have dual afferent and efferent functions, rather than there being separate afferent and efferent terminals (Lisney & Bharali, 1989; Szolcsányi, 1996).

In the rat the vasodilator units were not distributed evenly throughout the saphenous nerve receptive field. The majority (9 of 11) of the vasodilator units were located on the foot,

with the remaining two units being located on the ankle or leg. The finding that there were fewer vasodilator units on the leg than on the foot and digits provides a partial explanation for the relatively poor neurogenic vascular effects that are seen on the leg, both as shown here for antidromic vasodilatation (see Results and Fig. 2) and in plasma extravasation studies (Lisney, 1987; Bharali & Lisney, 1988).

Relationship with neuropeptide content

Neurogenic inflammatory responses are brought about by the release of neuropeptides from nociceptive neurones. In the skin, neurogenic vasodilatation is thought to be mostly due to the action of calcitonin gene-related peptide (CGRP) on arterioles (e.g. see review by Lynn, 1996). Immunocytochemical studies carried out in the rat show that 50% of the C fibres retrogradely labelled from the skin contained CGRP (O'Brien, Woolf, Fitzgerald, Lindsay & Molander, 1989). In the study in the rat, 24% of the C fibres classified as polymodal nociceptor units, polymodal/mechanical nociceptor units or heat nociceptor units were found to have vasoactive properties. Polymodal nociceptor units account for between 48 and 75% of the total C fibre population in the rat saphenous nerve (Lynn & Carpenter, 1982; Fleischer, Handwerker & Joukhadar, 1983; Kress *et al.* 1992). This means that between 12 and 18% of cutaneous C fibres can produce detectable vasodilatation, and should therefore contain the potent vasodilator CGRP. This is a much lower proportion than the 50% which are said to contain CGRP (O'Brien *et al.* 1989). This difference between the percentage of C fibres shown to have vasodilator properties and the percentage containing CGRP may be because, as discussed above, there are some C fibres with weak vasodilator actions that remained undetected in this study, but still contained enough peptide to show as positive in the immunohistological studies. On the other hand, the much lower proportion of vasoactive C fibres compared with the proportion of cutaneous C fibres containing CGRP may reflect the possibility that CGRP has other roles apart from causing vasodilatation. For example, it is known that the terminals of some CGRP-containing nerve fibres are located in the epidermis, which is an avascular structure (Dalsgaard *et al.* 1989; Garcia-Caballero, Gallego, Roson, Fraga & Beiras, 1989). Also CGRP receptors have been found at the surface of some Langerhans cells and CGRP has been shown to inhibit antigen presentation (Hosoi *et al.* 1993; Asahina, Hosoi, Grabbe & Granstein, 1995).

Species differences

These findings in the rabbit and the rat differ from the results of a recent study in pig skin which found that neurogenic vasodilatation was brought about by the action of heat nociceptor afferents, and that none of the nine polymodal nociceptor units tested had vasoactive properties (Lynn *et al.* 1996). However, such heat nociceptor C fibres are not found in rabbit cutaneous nerves (Lynn, 1979; Shea & Perl, 1985). Instead, in rabbit skin, a proportion of the polymodal nociceptor afferents are responsible for producing antidromic vasodilatation. It is interesting to note that not all the polymodal nociceptor C fibres are involved, and the subpopulation of vasoactive polymodal units have low mechanical sensitivity. There was no significant difference in the sensitivity to heat of the vasodilator and non-vasodilator polymodal nociceptor units of the rabbit saphenous nerve.

The situation in rat skin also differs from that in the pig. In the rat the majority of vasoactive C fibres belong to the polymodal nociceptor class, although the two heat nociceptor units tested were also vasoactive. However, since such heat nociceptor units are relatively uncommon in the rat saphenous nerve compared with the pig saphenous nerve, it is difficult to critically assess the role that this small class of C fibres play in neurogenic inflammation in rat skin. As in the rabbit, the vasoactive nociceptive units in the rat had relatively low mechanical sensitivity. This therefore provides an explanation as to why the kinds of pressures that excite most rat cutaneous polymodal nociceptor units do not result in flare (Lynn & Cotsell, 1992). There was no difference between the vasodilator and non-vasodilator polymodal units in the rat in terms of their heat sensitivity.

Heat nociceptor units are reported to be present in significant numbers in primate cutaneous nerves. Microneurography studies in the peroneal nerve of man have shown that 6% of C fibre afferents are heat nociceptor units (Schmidt, Schmelz, Forster, Ringkamp, Torebjörk & Handwerker, 1995). In monkey hairy skin it is reported that heat nociceptor afferents make up 7% of C fibres (Baumann, Simone, Shain & LaMotte, 1991), and in monkey glabrous skin they make up 5·5% of nociceptive C fibres (Georgopoulos, 1976). It will be of interest to see the relative roles that the heat and polymodal nociceptor units play in generating neurogenic inflammation in primate skin.

Scaling of afferent receptive field area and spread of flare with body size

In the rabbit there was a close association between the afferent receptive field size of polymodal nociceptor units and the area of vasodilatation produced by antidromic stimulation of vasoactive polymodal nociceptor units. Afferent receptive fields of a sample of eleven rat polymodal nociceptor units to punctate pressure range in maximum extent from 1·3 to 5·7 mm, with most units measuring about 2·5 mm in maximum extent (Lynn & Cotsell, 1993), and this corresponds well with the small, localized increases in skin blood flow seen in the rat following antidromic stimulation of vasoactive nociceptors (see Fig. 8). The flare response to noxious stimuli or injury is thought to be brought about via an axon reflex mechanism in vasoactive afferent fibres (Lewis, 1927). By comparing the size of the afferent and efferent receptive fields of individual units with the spread of the flare response it is possible to check the validity of the axon reflex hypothesis. This has already

been carried out in the pig, where heat nociceptor units have receptive fields of up to 13 mm in maximum extent which are very similar in size to the maximum spread of flare following intradermal injection of capsaicin (15 mm) (Lynn *et al.* 1996). The spread of flare in rabbit and rat skin is currently under investigation in our laboratory and preliminary data support a close relationship between afferent receptive field area, efferent vasodilatation area, extent of flare and body size.

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