

Quantitative objective assessment of peripheral nociceptive C fibre function

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SUMMARY A technique is described for the quantitative assessment of peripheral nociceptive C fibre function by measurement of the axon reflex flare. Acetylcholine, introduced by electrophoresis, is used to stimulate a ring of nociceptive C fibre endings at the centre of which the increase in blood flow is measured with a laser Doppler flowmeter. This flare (neurogenic vasodilatation) has been compared with mechanically or chemically stimulated non-neurogenic cutaneous vasodilatation. The flare is abolished by local anaesthetic and is absent in denervated skin. The flare has been measured on the sole of the foot of 96 healthy subjects; its size decreases with age in males, but not in females.

In recent years quantitative measurement of various modalities of peripheral sensation has come to be increasingly important in both diagnosis and management of peripheral neuropathy.^{1,2} Although sensitive methods are available for measuring mechanoreceptor and thermal function, techniques suitable for clinical measurement of nociceptor function are less satisfactory. In early classical work on pain, Hardy *et al*³ used radiant heat as a stimulus and more recently heat-pain threshold has been measured using the more precisely controlled stimulus produced by a Peltier thermode.⁴ A device for measuring mechanically induced pain was produced by Lynn and Perl⁵ and used in the measurement of pain threshold in diabetics by Le Quesne and Fowler.⁶ The value of all these techniques is limited by the particular problems of subjective pain assessment.

During a study of sweating in diabetics using the technique developed by Low *et al*⁷ whereby axon reflex sweating was induced by electrophoresis of acetylcholine, Ahmed and Le Quesne⁸ noted that in control and some diabetic subjects a spreading flare developed, whereas in others the flare was absent. Lewis⁹ demonstrated that the spreading vasodilatation of the triple response was an axon reflex depending on the integrity of nociceptor afferent nerves. Recently there has been a resurgence of inter-

est in neurogenic inflammation and the vasoactive peptides, particularly substance P, responsible for the axon reflex flare.¹⁰ Lembeck¹¹ has re-emphasised the dual role of the small C fibres forming Lewis's nociceptor system, whereby stimulation of cutaneous nociceptor endings produces impulses which travel both to the CNS to produce the sensation of pain and to axon collaterals to initiate neurogenic inflammation. By means of a technique for quantifying the flare response,¹² we have used the linking of the two functions in one fibre to provide an objective measurement of peripheral pain pathways. In addition, we can measure neurogenic inflammation, whose impairment may be important in some diseases.

The flare depends on a cutaneous vascular reaction. This assumes that the vasculature is capable of responding to the vasodilator peptides released by the axon reflex. To assess the capacity of the vessels to dilate, use has been made of another component of Lewis's⁹ triple response, the direct local red reaction, which is independent of nerves. This reaction, which can be produced in a variety of ways and is confined to the area stimulated, will reveal any intrinsic vascular abnormality due for example to microangiopathy or occlusive vascular disease.

Methods

Apparatus and technique

Capsule Stimulation and measurement were performed concurrently using a small capsule applied to the skin (fig 1). This capsule is a modification of the one described by Low

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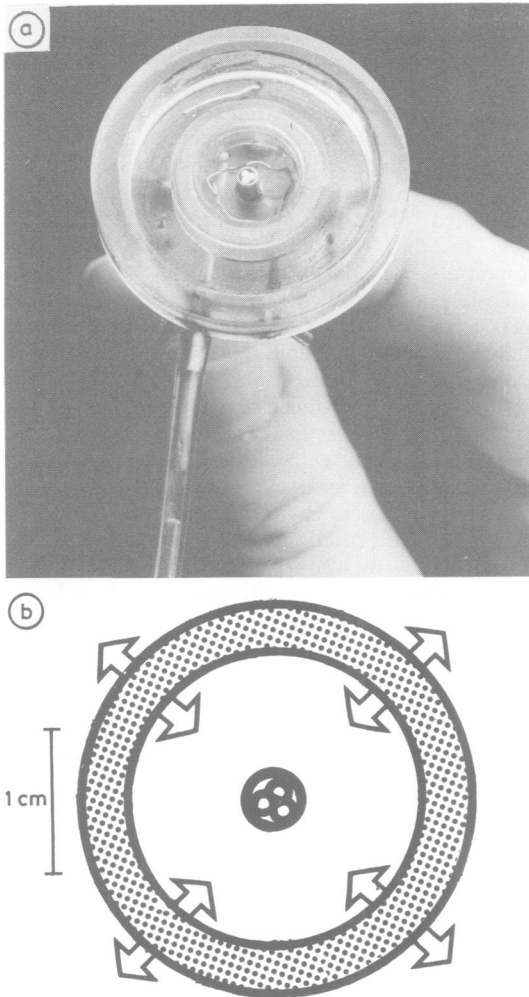


Fig 1 (a) Photograph of capsule; (b) diagram of capsule showing spread of flare both outwards and inwards from the ring of stimulation.

*et al*⁷ to measure axon reflex sweating. It has two concentric chambers, each fitted with an inlet and outlet vent, and with a platinum wire electrode, which is used as the anode for electrophoresis. The walls of the chambers are thick and the base smooth so that double sided adhesive rings (3M) produce a water tight seal with the skin, which was prepared by gentle cleansing. The probe of the laser Doppler flowmeter used for measuring blood flow is mounted vertically at the centre of the capsule with the probe inserted to a distance of 1 mm from the skin. The cathode for electrophoresis is a lead plate, 4 × 6 cm, wrapped in saline soaked gauze and attached to the skin, previously cleansed with spirit, with micropore tape. A constant current generator designed and built at The Mayo Clinic was used to provide a 1 mA stimulus.

The laser Doppler flowmeter A PF2 laser Doppler flowmeter linked to a BBC chart recorder was used to measure the changes in superficial cutaneous blood flow. A narrow laser light beam is transmitted through a fibre optic cable to the probe head and penetrates the skin to a depth of 1.5 mm. The coherent light is scattered in the tissues; the rays scattered by moving red blood cells undergo a frequency shift according to the Doppler principle. The backscattered light travels back through a separate fibre optic channel to a photodetector which produces an output linearly related to the flux of red cells i.e. the number of cells times their velocity. This output signal is fed to the pen recorder. The signal, expressed in mV rather than conventional flow units, is an indirect measure of blood flow but a close correlation has been obtained between laser Doppler flux and red cell velocity determined by direct capillary microscopy.¹³

The laser Doppler signal is sensitive to small changes in flow; sympathetic arousal stimuli cause clearly detectable changes. Movement of the subject or the probe causes an abrupt artefact which is easily distinguishable from physiologically induced changes. Measurements have generally been made on the 12 kHz scale with a gain of × 3 and a time constant of 1.5 s.

Procedure Measurements were made after 30 minutes acclimatisation in a warm room maintained at 26 °C in order to reduce sympathetic vasoconstrictor tone and to enable comparison with data from neuropathic patients in whom limb temperature is often higher than in healthy subjects. The skin temperature was 34–35 °C throughout the test.

To produce the axon reflex flare, the outer ring was filled with 10% acetylcholine (ACh) and, when the Doppler signal was stable, a current of 1 mA was passed for 5 min through the outer chamber. The flare produced spreads both outwards, where it is visible, and inwards where it is measured by the probe (fig 1b).

For the direct, chemically induced non-neurogenic reaction, the central well was filled with either 10% ACh or 1% pilocarpine, and a stimulus of 1 mA applied for 5 min through the central chamber.

A direct, mechanically induced red reaction was produced by making a firm stroke with a spring loaded dermatograph, which produces a pressure of 2.5 N. The flux was measured with the probe at the same site before and after stimulation.

Results

Characteristics of the vascular reactions

Typical findings in a control subject are shown in fig 2. The increasing laser Doppler flux recorded from the centre of the capsule during electrophoresis of ACh from the outer ring is shown in fig 2a. This is the axon reflex flare. Pilocarpine was then electrophoresed from the central wall under the laser Doppler probe and the further rise in flux during development of the non-neurogenic direct reaction is seen. This non-neurogenic reaction is the same following electrophoresis of either pilocarpine or ACh from the central well, although pilocarpine, unlike ACh, does not in addition produce a flare. The direct

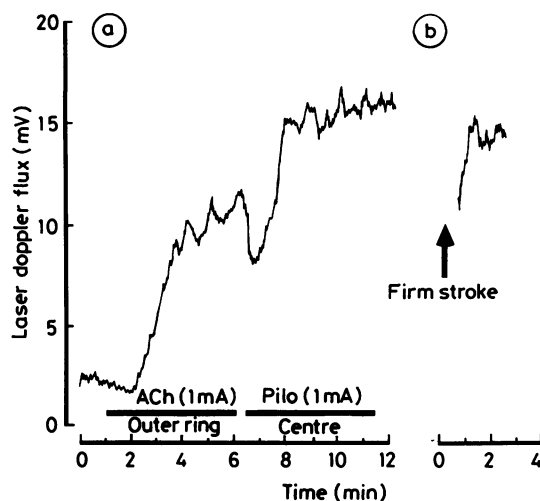


Fig 2 Laser Doppler flux (mV): (a) and (b) recorded from sole of foot of J.H. (F, aged 44). (a) during electrophoresis of acetylcholine (ACh) from outer ring, followed by pilocarpine from central well; (b) a firm stroke was then made with a dermograph at an adjacent site and the probe placed over the area of visible vasodilatation.

red reaction following a firm stroke with a dermograph is shown for the same patient in fig 2b. The direct mechanically induced red reaction is usually smaller than the pilocarpine reaction, indicating that maximal vasodilatation is not produced by this procedure.

The classical stimulus for the flare is intradermal histamine. The flux recorded from a histamine-induced flare is shown in fig 3b. It is similar in magnitude to the ACh-induced flare recorded from an adjacent site of the forearm of the same subject (fig 3a).

The specificity of ACh as a stimulus for the flare was demonstrated by comparing it with saline. The lack of reaction to electrophoresis of saline compared with the rapid increase in flux during electrophoresis of ACh can be seen in fig 4a.

The neurogenic origin of the axon reflex flare was demonstrated by examining locally anaesthetised skin. Five ml 1% lignocaine were injected deeply into the subcutaneous tissue to minimise local trauma, which would itself produce a flare. The test was carried out after an area of approx 4 sq cm had become insensitive. The results are shown in fig 4b. There was no increase in flux when ACh was electrophoresed from the outer ring, but non-neurogenic chemically-induced vasodilatation occurred when pilocarpine was electrophoresed from the central well.

Further confirmation of the neurogenic nature of

the axon reflex flare was obtained by examining totally denervated skin. Surgically transferred free flaps, consisting of skin and subcutaneous tissue on a vascular pedicle, were examined at a time when complete nerve degeneration must have occurred. The axon reflex flare to ACh was absent in the skin of such flaps, along with all other sensory modalities. The direct red reaction to mechanical stimulation was unaffected. There was, however, some slight reduction in the non-neurogenic direct pilocarpine response.

Passage of 1 mA current produces, in most subjects, a prickling sensation. Increasing the current to a painful intensity (usually 2 mA) did not increase the magnitude of the vasodilatation (fig 4c).

In order to investigate the influence of chronic ischaemia on the vascular reactions on the sole of the foot, five subjects with clinical evidence of severe major arterial disease in the lower limbs were studied. The flare was absent in two and reduced in three. The non-neurogenic reaction to both pilocarpine and a firm stroke were also reduced. All reactions were absent in the patient with the most severely ischaemic leg.

Control data

Data have been obtained on the two types of vascular reaction, the flare and the direct response, by examining 45 male and 54 female healthy subjects, aged 20–72 years. The sole of the foot was examined since this site is most commonly affected in patients with peripheral neuropathy. In each case the probe was situated on the supple skin immediately posterior to the first metatarsal head.

The size of the flare can be expressed either as the absolute magnitude of the flux recorded or as an

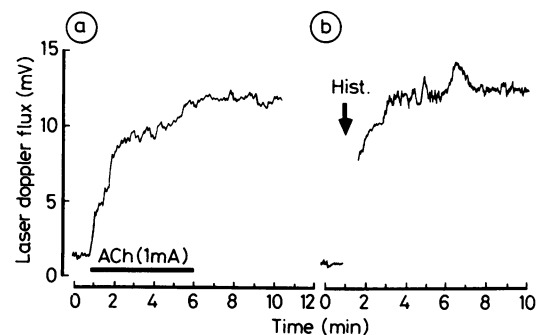


Fig 3 Laser Doppler flux (mV): (a) and (b) recorded from forearm of P.L. (a) during electrophoresis of (ACh) from outer ring; (b) intradermal histamine was then injected at an adjacent site and the probe placed over the flare which developed.

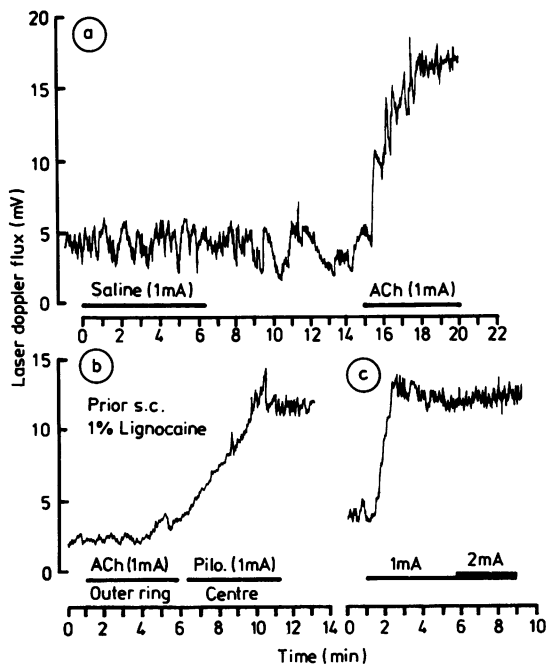


Fig 4 Laser Doppler flux (mV): (a) recorded from the sole of foot of P.L. during electrophoresis of saline followed by acetylcholine (ACh), both from outer ring; (b) and (c) from forearm of P.L. (b) during electrophoresis of ACh from outer ring followed by pilocarpine from central well 5 min after sc. injection of 1% lignocaine; (c) during electrophoresis of ACh from outer ring with 1 mA current followed by 2 mA current.

increase from the resting value. It was shown that the higher the resting flux, the higher the figure after induction of the flare ($R = 0.225$, $p < 0.02$), but the

Table Flux values for various vascular reactions recorded from the sole of the foot of 90 healthy subjects

	Resting	Flare	Stroke	Pilocarpine	Index
Mean	3.2	11.7	15.9	21.9	70.5%
SD	1.8	3.7	4.1	6.6	28.6%
p	NS	<0.001	NS	NS	<0.001

Units in mV unless otherwise stated. Index is ratio of increase in flux for flare to increase for stroke reaction. p values are for significance of regression line relating vascular reaction to age.

increase in flux diminished with increasing resting flux ($R = 0.244$, $p < 0.02$). Thus resting flux had some effect both on the absolute value and, in the opposite direction, on the change in flux after development of the flare. Absolute values are shown in the table and fig 5.

The mean flux after induction of a flare for all control subjects was 11.7 mV (SD 3.6) (table). Its size decreased with age, the diminution being statistically significant in males ($R = 0.67$, $p < 0.001$), but not in females ($R = 0.145$, NS) (fig 5). The difference in the slopes of the regression line of flux on age for males and females over the age of 29 was significant ($t = 2.739$ $p > 0.01$). Neither the resting flux, nor the direct local response to pilocarpine or a firm stroke were significantly related to age (table).

The test was performed twice, on different days, on 21 feet. The mean absolute difference in the size of the flare was 2.3 mV (SD 1.8). For each subject the difference was expressed as a proportion of the mean of the two readings. The mean of these values was 17% (SD 10.5).

In order to compare neurogenic vasodilatation with the non-neurogenic capacity to vasodilate, an index has been calculated in which the increase in flux for the flare was expressed as a proportion of the increase in flux for the direct red reaction. The mean

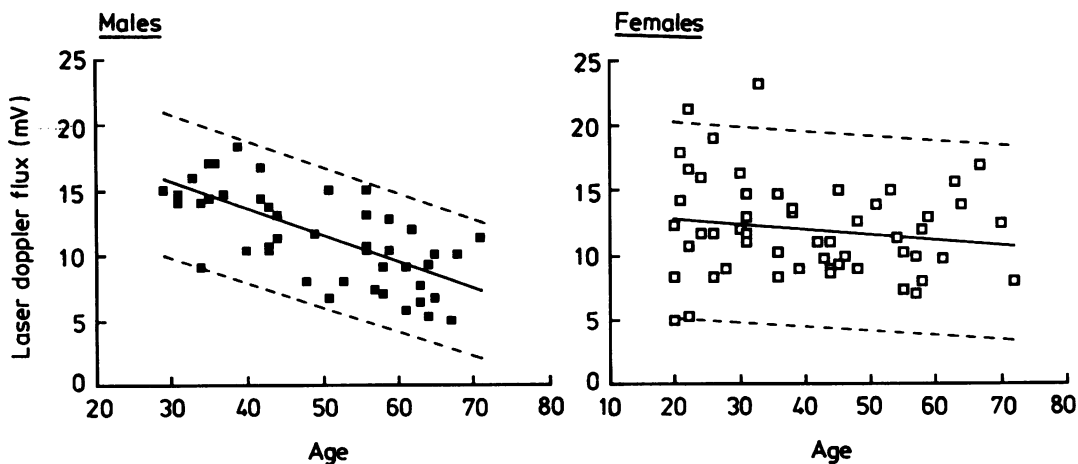


Fig 5 Laser Doppler flux (mV) after stimulation of axon reflex flare in 45 male and 54 female control subjects. Solid line is calculated linear regression and dotted lines are ± 2 SD from mean.

index was 70.5% and like the absolute size of the flare, it decreased significantly with age ($p < 0.001$) (table). The direct red reaction, although not maximal vasodilatation, has been used for this index, because of the slight reduction in pilocarpine or ACh-vasodilatation in totally denervated skin.

Discussion

We have used the electrophoresis of ACh as a standard, reproducible, atraumatic stimulus which will produce a maximal flare within a few minutes. Douglas and Ritchie¹⁴ demonstrated that ACh has a direct excitatory action on non-myelinated C fibres in the saphenous nerve of the cat, this being abolished by hexamethonium but not by atropine. Pilocarpine produces direct vasodilation identical to that produced by ACh but no flare; these two observations both suggest that stimulation of the flare depends on the nicotinic action of ACh. Other substances which produce a flare are less convenient for clinical use and for producing a quantifiable response. Histamine must be injected intracutaneously. A single application of capsaicin produces a flare, but it is abolished by repeated applications probably due to depletion of substance P.¹⁵ The flare produced by mustard oil is so variable that it is only possible to determine whether a flare is present or absent.¹⁶

Previous attempts at quantification of the flare have depended on measurement of the area of visible vasodilatation. This can be difficult, not only because of variability due to lack of standardisation of the stimulus, but also because the flare has an irregular outline and crenated edge⁹ due to anatomical variations in the distribution of the responsible nerves. We have overcome these problems by measuring the change in superficial cutaneous blood flow at one fixed point at the centre of a ring stimulus. Laser Doppler measurement has the additional advantage that an objective measurement may be made when erythema is not visible to the naked eye, such as where the skin is thickened, as on the sole of the foot, and on pigmented skin.

Electrophoresis of ACh over the area of the ring of the capsule produces, in most people, a "prickling" sensation, which is not actually painful. Prickling has in the past been ascribed to the small cutaneous nerve fibres responsible for pain sensation.¹⁷ Pain is produced by the simultaneous stimulation of many neurons, the pattern of impulse discharge probably being of importance. Microneurographic studies have shown that low frequency firing of nociceptive fibres does not produce pain, whereas pain does occur at a higher firing frequency of the same fibres.¹⁸ It is therefore quite understandable that maximum reflex vasodilatation is evoked by a stimulus which is not per-

ceived as painful. We have shown that increasing the strength of the electrophoresis current to a level which produces pain does not increase the vasodilatation.

Chronic ischaemia impairs non-neurogenic vasodilatation, so that little significance can be attached to an absent flare in an ischaemic limb. It is therefore important that the flare should always be compared with directly stimulated vasodilatation. Intradermal nitroprusside is probably the best agent for producing maximal vasodilatation.¹⁹ We did not use this stimulus because we sought to make the whole technique non-invasive. It was hoped that pilocarpine or ACh would provide an adequate direct stimulus but it was found that in totally denervated skin this reaction was slightly reduced. In keeping with this finding, recent experimental observations have suggested that ACh-vasodilatation is less in totally denervated vessels in the rabbit's ear.²⁰ For these reasons we have used direct mechanical stimulation with the dermograph as a stimulus totally independent of neural influence, even though this dilatation is not maximal.

Many neurological functions decrease with age. For example, in the lower limb threshold for vibration perception²¹⁻²³ and for cooling and warming^{24,25} have been found to increase with age. Sensory nerve action potential amplitude²⁶ and the density of nerve fibres in peripheral nerve trunks²⁷ both decrease with age in the lower limb. In a previous study of the flare produced by topical capsaicin the area of the flare over the trapezoid ridge was found to decrease with age, as was the substance P content of skin from the cubital fossae and ankle.²⁸ When sensory thresholds have been estimated separately for males and females the decrease in age has been more marked in males, for example for vibration²⁹⁻³⁰ and for temperature.³¹ Thus, the present findings of an age decrease in the flare, which is more marked in males, is similar to the findings for other peripheral nerve functions. To be able to detect this effect gives an indication of the quantitative sensitivity of the technique.

This technique provides an indirect method of measuring the integrity of peripheral pain pathways, providing an objective, atraumatic alternative to psychophysical techniques for measuring pain. Quantification of pain pathways will be valuable in assessment of peripheral neuropathies; for example, in analysing the multiple defects contributing to the formation of diabetic neuropathic foot complications. The presence or absence of an axon reflex has been diagnostically useful in the past, but a quantitative test is more valuable. The flare may be absent in diabetics,³²⁻³⁴ and we have now been able to study it quantitatively in diabetics with neuropathic ulceration and Charcot arthropathy.³⁵ Following traction injuries to the brachial plexus, the presence of a flare

in an area of sensory loss indicates a pre-ganglionic lesion.^{36,37} We have found its quantification to be useful in the diagnosis of mixed pre- and post-ganglionic lesions. A histamine flare test is valuable in the diagnosis of familial dysautonomia (the Riley-Day syndrome),³⁸ since non-myelinated nociceptive fibres are lost as well as autonomic nerves. It is possible that a quantitative deficit might be found in obligate heterozygotes which would permit accurate genetic counselling in affected families.

It is increasingly apparent that neurogenic inflammation plays an essential part in the body's defence mechanisms. The quantitative test now described allows us to explore the importance of a deficit in this mechanism. In diseases such as congenital insensitivity to pain, where the flare is absent,³⁹ and in diabetes, loss of the neurogenic inflammatory response may be as important as loss of pain sensation in the manifestations of the disease.

In conclusion, the dual role of the nociceptive system allows the flare to be used to study deficits both of pain and of neurogenic inflammation. It has been added to our battery of other quantitative tests to produce a profile of abnormalities of the different types of fibre making up a peripheral nerve.

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