Assessment of cutaneous sensory and autonomic axon reflexes in rheumatoid arthritis

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Abstract

Objective—To assess sensory function in skin overlying the joints of patients with rheumatoid arthritis, in relation to the pain and tenderness which commonly arises in structures not directly involved in the inflammatory process.

Methods—An intradermal injection of capsaicin 0·05 μg in 10 μl was made over the wrists and forearms of 40 patients with rheumatoid arthritis and 46 control subjects. Axon reflex vasodilatation was measured using laser Doppler flowmetry. Cholinergic sympathetic function was assessed by measuring axon reflex sweating induced by a single intradermal injection of nicotine 0·5 μg in 0·1 ml.

Results—Capsaicin induced axon reflex vasodilatation over the wrists was found to decrease with age in normal subjects ($r = -0.62$, $p < 0.001$). In patients with rheumatoid arthritis, capsaicin induced axon reflex vasodilatation was significantly greater over the wrists, but not the forearms, when compared with age matched normal controls ($p < 0.01$). A minimal correlation between axon reflex vasodilatation and visual analogue pain score was apparent in the rheumatoid group ($r = -0.37$, $p < 0.05$). Nicotine induced sweating responses were similar in the rheumatoid and normal groups, and both showed a linear age related decline.

Conclusions—The results show a selective increase of capsaicin induced vasodilatation in skin overlying joints in patients with rheumatoid arthritis. This suggests that the activity of a subpopulation of periarticular small sensory fibres is altered, which may explain, at least in part, some of the clinical findings in this disorder.

Recent experimental studies have provided new insights into peripheral mechanisms involved in the generation of arthritic pain.[1] Within the joint itself, inflammatory mediators appear to sensitise previously inactive sensory fibres (nociceptors) which then respond to various chemical and mechanical stimuli to signal pain.[2–4] In contrast, the origin of the pain and tenderness (hyperalgesia) which commonly arises from periarticular structures not directly involved in the articular disease process remains unclear. Both peripheral and central mechanisms have been proposed,[1] although direct evidence for either mechanism is lacking. The aim of this study was to seek evidence for altered peripheral function of small sensory fibres in periarticular tissues of patients with chronic rheumatic disease.

Detailed assessments of small sensory fibre function in rheumatoid arthritis have not been performed despite evidence of sensory neuropathy in many patients with this disorder.[5] Capsaicin, the algesic substance in red peppers, has a selective action on small sensory fibres to produce pain and axon reflex vasodilatation[6] and has been used as a tool to examine sensory function in normal and pathological states.[7–9] In degenerative disease of the spine, skin flares appear to be reduced,[10] whilst in chronic musculoskeletal pain syndromes of non-inflammatory origin a generalised increase has been reported.[11]

In the present study we have examined small sensory and autonomic fibre function in the skin of patients with rheumatoid arthritis. Intradermal capsaicin was used to compare skin flares and pain over involved rheumatoid joints with control sites and with flares in normal individuals. Cholinergic sympathetic function was also assessed in the same regions by measuring nicotine induced axon reflex sweating,[12] to exclude the presence of a generalised small fibre neuropathy.

Methods

SUBJECTS

A total of 86 subjects were involved in the study. Forty patients with rheumatoid arthritis as defined by the American Rheumatism Association criteria[13] were chosen from those attending a rheumatology outpatient department. Control data were obtained from 46 subjects who were selected from hospital staff and relatives of patients. Approval for the study was granted by the relevant ethics committee and informed consent obtained before any procedure.

A history was taken from all subjects and included details of past medical illness and current medication. Subjects taking adrenergic receptor blocking drugs and other vasoactive drugs were excluded, as were subjects with overt diabetes. Inspection was made of the skin overlying the wrists and subjects with eczema, psoriasis or surgical scars in this area were also excluded. Wrists and elbows were examined for tenderness and graded on a scale 0 = no tenderness, 1 = mild tenderness, 2 = moderate or severe tenderness accompanied by facial grimacing or withdrawal of the limb. The rheumatoid group had blood taken for

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erythrocyte sedimentation rate (ESR) as a general marker of inflammation.

SENSORY STUDIES

Subjects were rested for 30 minutes in a quiet room maintained at a constant 22°C; they had taken no recent food or drink. They were seated with the arm to be tested resting comfortably on a table, supported by a pillow. Sensation to light touch and pinprick at the sites to be tested were measured using cotton wool and a sterile pin, respectively. Cutaneous blood flow was measured using laser Doppler flowmetry.[14][15] The study utilised a Periflux PF3 (LDPF3, Perimed) laser Doppler with a 632·8 nm helium neon laser and a PF314 angle probe (small). The laser light was transmitted to the skin via a probe consisting of three silica fibres (fibre separation of 0·25 mm, core diameter 0·125 mm) with a sampling area over the skin surface of approximately 1 mm diameter and 1 mm depth. The reflected light was collected by a sensitive photoelectric detector with the voltage signal in mV (expressed as units of 'flux') being proportional to the velocity and number of the red blood cells within the intravascular space.[15] Before each study the instrument was calibrated by setting output to zero while the probe was directed away from any reflecting surface.

The Doppler probe was placed on the skin, supported by a semicircular disc and secured with a double sided adhesive ring. This enabled accurate positioning of capsaicin injection adjacent to the Doppler probe without disturbing the position of the probe. Two sites were assessed, including the dorsum of the wrist and the mid volar forearm at a point equidistant from the olecranon and ulnar styloid.

A solution of capsaicin (Sigma) 5 μg/ml was made using Tween 80 and normal saline according to a standard method,[16] and was filtered through a 0·02 μm Anatop microfilter. Before the injection, baseline cutaneous laser Doppler measurements were recorded for one minute. Capsaicin 0·05 μg in 10 μl was then injected intradermally using a U-100 0·5 ml syringe (Terumo), ensuring that a good 'bleb' was produced by each injection. The dose chosen corresponded to the midpoint of a previously obtained dose response curve and allowed for increased or decreased responses to be observed. The injection was situated 2 mm medial to the tip of the probe. Results obtained from subjects in whom no bleb was observed were discarded. The pen recorder was kept running for the duration of the test at 1 cm/min.

Before starting the study, subjects were instructed in the use of a visual analogue scale (VAS) and were requested to score the most intense pain felt from the injection of capsaicin. A purpose built instrument was used, comprising a freely moving pointer with 100 mm of travel, marked at one end by the term 'no pain' and at the other by 'worst pain ever'. Gradations of 100 mm (not seen by the subject) were marked on the underside of the slide. The VAS was placed within easy reach of the upper limb not being assessed. The reproducibility of the techniques was assessed in 10 normal volunteers by comparing results of repeat testing one week apart.

SWEATING STUDIES

Nicotine for injection was diluted with sterile saline immediately before use, from a stock solution of nicotine acid tartrate prepared by University College Hospital Pharmacy.

Conditions for this part of the study were as for the sensory tests. An intradermal injection of 0·1 ml (0·5 μg) of nicotine acid tartrate was administered using a U-100 0·5 ml syringe (Terumo). Optimum nicotine dosage was chosen after dose responses were performed on a small group of control subjects.

This test was performed on 25 rheumatoid patients and 25 control subjects over the dorsum of the wrist. Before testing, the area was cleaned using an alcohol wipe, to remove any surface grease that may have influenced the recordings of sweat rate, and the area was allowed to dry thoroughly. Peak rate of sweating was assessed using an evaporimeter resting on the skin surface over the injection site.[12] Baseline measurements were taken after the evaporimeter had been in situ for at least 30 seconds before the injection of nicotine. An intradermal injection was then performed and the probe resited immediately adjacent to the site of injection. Recordings were taken every 15 seconds for the first minute and subsequently every 30 seconds until the sweat rate returned to within 10% of baseline.

STATISTICS

Data assessment was performed using a statistical software package (Statsgraphics: version 5). All results are expressed as mean (1 SE). Comparison of two groups was performed using a Student's t test, after data were checked for normal distribution, with $p < 0.05$ considered significant. ANOVA testing was used for comparison of more than two variables.

Results

SENSORY STUDIES

Capsaicin induced vasodilatation in 10 normal volunteers tested on two occasions one week apart showed high reproducibility ($r = -0.72$, $p < 0.001$).

In normal subjects there was an age related decrease in axon reflex vasodilatation over the wrist joint ($r = -0.62$, $p < 0.001$) (fig 1). In contrast, no correlation was found between age and axon reflex vasodilatation in the rheumatoid group ($r = +0.06$, p = 0·73). There was significantly increased axon reflex vasodilatation over the wrists of patients with rheumatoid arthritis compared with normal control subjects ($t = -3.32$, p = <0·01) (fig 2).

There was no correlation between axon reflex vasodilatation and clinical signs of joint disease. Specifically, wrist tenderness in the rheumatoid group as assessed by the two point
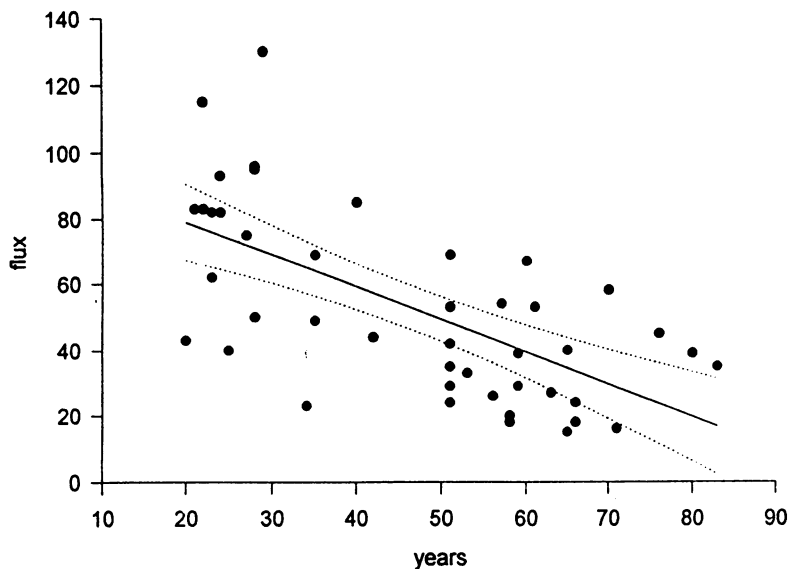
Figure 1   Capsaicin induced vasodilatation (expressed as arbitrary units of flux) compared with age in years in 46 control subjects. Solid line represents mean linear regression (r = −0·72; p < 0·001); dotted line represents 99% confidence intervals of the mean.

scale did not correlate with capsaicin induced changes in blood flux recordings (table). Similarly, there was no correlation between axon reflex vasodilatation and ESR ($r = 0·07$, $p = 0·68$).

Cutaneous axon reflex vasodilatation was compared in the wrists and forearms of a subset of 25 subjects with rheumatoid arthritis and 25 age matched (but not sex matched)
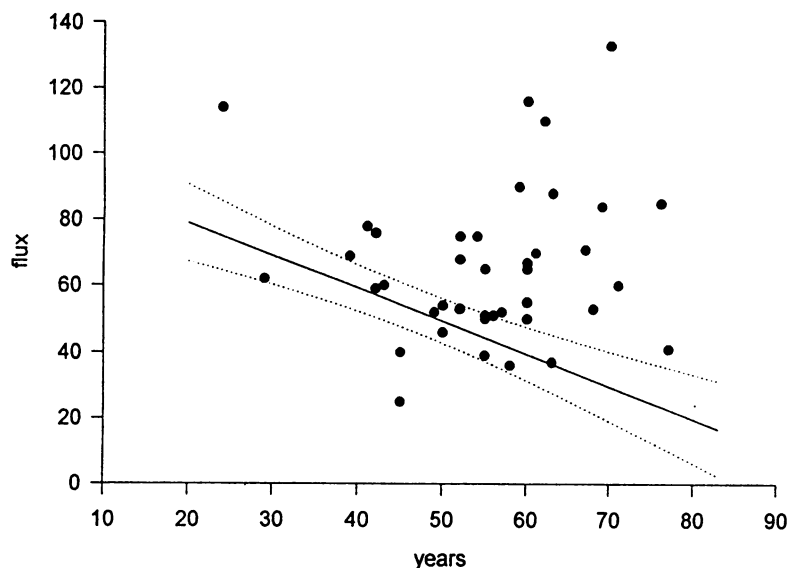

Figure 2   Capsaicin induced vasodilatation compared with age in years in 40 subjects with rheumatoid arthritis. Solid and dotted lines represent mean linear regression with 99% confidence intervals for data obtained from 46 normal subjects tested using an identical protocol (see fig 1).

Comparison of clinical parameters with capsaicin induced vasodilatation and pain in patients with rheumatoid arthritis

Grade†	Number	ESR (mm/1st h)	Capsaicin induced vasodilatation (flux) ‡	Capsaicin induced VAS pain score
0	15	36·4 (10·3)	63·6 (6·5)	52·3 (6·1)
1	12	40·5 (10·9)	61·6 (6·5)	61·9 (7·8)
2	13	57·3 (12·2)	62·3 (6·7)	51·8 (7·5)

Values are mean (SE). †Grade 0 = no joint tenderness, 1 = mild tenderness, 2 = moderate or severe tenderness. ‡Arbitrary units.

control subjects. The mean age was 57·2 (2·1) years in the rheumatoid group and 57·4 (2·1) years in the control group. Baseline blood flux recordings were alike over the forearms and wrists of the rheumatoid and control subjects. Capsaicin induced axon reflex vasodilatation was similar in the forearms of the rheumatoid and control subjects, but was substantially greater in wrists of rheumatoid subjects (71·4 (5·2) units) compared with controls (37·6 (3·3) units) ($p < 0·01$) (fig 3).

The mean VAS pain score was similar in both the rheumatoid subjects (55 (3)) and controls (54 (4)) ($p = 0·89$). There was no correlation between VAS pain score and axon reflex vasodilatation in the control group ($r = +0·24$, $p = 0·1$); however, a weak correlation was apparent in the rheumatoid group ($r = −0·37$, $p < 0·05$).

All subjects in the rheumatoid group were taking non-steroidal anti-inflammatory drugs. In order to determine if these agents might influence the response to capsaicin, a group of six normal volunteers were given ibuprofen 400 mg eight hourly for 24 hours and capsaicin responses were compared before and after the therapy period. Mean baseline blood flux recordings were similar before (5·5 (0·7) units) and after (6·0 (0·6) units) drug administration ($p = 0·91$). The magnitude of capsaicin induced vasodilatation was also similar before (61·2 (10·5) units) and after (59·3 (10·1) units) drug administration ($p = 0·9$). Ibuprofen did not significantly alter the VAS pain scores recorded by the subjects (64 (9) v 58 (7); $p = 0·66$).

SWEATING STUDIES
An age related decrease in nicotine induced sweating was found in both the normal group ($r = −0·59$, $p < 0·01$) and the rheumatoid group ($r = −0·58$, $p < 0·01$). No differences were apparent between the normal or rheumatoid subjects ($t = −0·70$, $p = 0·48$) or between males and females ($t = −0·40$, $p = 0·68$). In the rheumatoid group there was no correlation between sweating and axon reflex vasodilatation ($r = 0·11$, $p = 0·67$).

Discussion
The present study has shown increased vasodilatation in response to intradermal capsaicin over the joints of patients with rheumatoid arthritis. The increase was localised to the area overlying the joint and was not apparent at sites away from the joint, implying a regional alteration of capsaicin sensitive small fibre function in patients with inflammatory joint disease. This parallels observations made in experimental arthritis, where the majority of articular small afferent fibres have been shown to develop a long lasting 'sensitisation' after the onset of joint inflammation.[2-4]

Capsaicin is a naturally occurring substance that has direct excitatory actions on polymodal (or 'mechano-heat') classes of somatic and visceral small diameter sensory nerve fibres. These fibres serve to transmit nociceptive
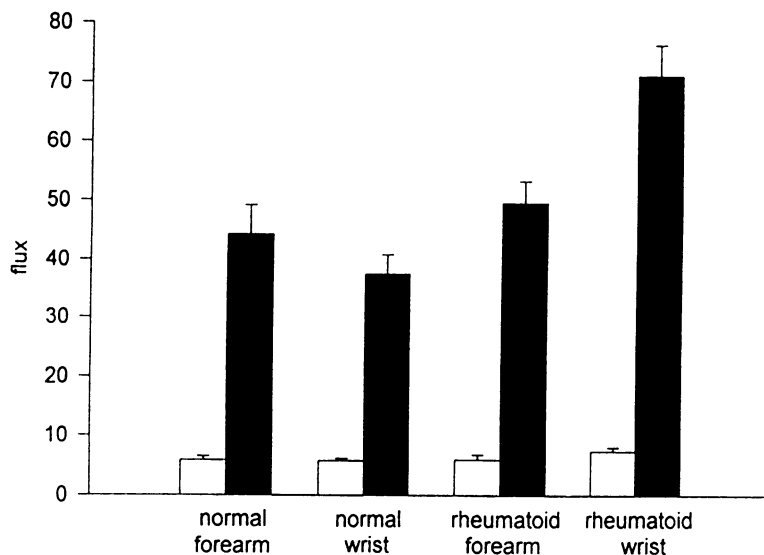
Figure 3 Mean (SEM) unstimulated (□) and capsaicin induced (■) blood flow (expressed as arbitrary units of flux) in the forearms and wrists of 24 age matched normal subjects and 24 patients with rheumatoid arthritis. Significant difference (p < 0·01) between stimulated blood flow over the wrists of rheumatoid patients and that in normal subjects.

signals related to noxious stimuli and should be distinguished from more rapidly conducting myelinated fibres which, under normal circumstances, transmit non-noxious sensory information.[6] [17] Application of capsaicin induces small fibre depolarisation and involves the opening of non-selective cation channels, causing an acute burning pain sensation together with local vasodilatation.[17] This latter phenomenon is mediated by vasoactive neuropeptides released locally via the so-called axon reflex and serves as a useful measure of peripheral small fibre activity.

Capsaicin has been applied topically to study cutaneous vasodilatation in non-inflammatory musculoskeletal disorders.[10] [11] Skin flares were reduced in patients with spinal pain secondary to degenerative disease;[10] in conjunction with the present study, this suggests quantifiable differences of small fibre function between inflammatory and non-inflammatory musculoskeletal disorders. Conversely, skin flares were increased in patients with chronic musculoskeletal pain syndromes;[11] however, this remains an ill defined group, in which the pathophysiology remains unclear.

A correlation between changes in evoked pain sensation and flare size has been reported after application of topical capsaicin, which allows for serial recordings in the same subject.[9] However, pain may be dissociated from skin flare responses after activation of nociceptors, with respect to both magnitude and timing of the onset and disappearance of flares.[18] We found only a weak correlation between capsaicin induced pain and peak axon reflex vasodilatation in the rheumatoid group, and none at all in the normal population. Further, both the rheumatoid and normal groups reported similar mean pain magnitude scores in response to the standard dose of capsaicin. These results suggest a dissociation between sensation and axon reflex vaso dilatation in chronic inflammatory disease.

In agreement with previous studies,[12] we found that nicotine induced axon reflex sweating decreased with age. Disturbances of autonomic activity have been observed in rheumatoid arthritis[19] [20] which may be associated with clinical sensory neuropathy.[19] In the present study, however, there was no clinical evidence of peripheral neuropathy among the rheumatoid patients. Decreased sweat area in females with rheumatoid arthritis has been reported,[21] but we were unable to confirm this. Alterations of sympathetic vasomotor activity have been correlated with persistence of pain in the musculoskeletal disorder frozen shoulder,[22] but no alteration of sympathetic (cholinergic) activity was observed in the present study.

The mechanism involved in the increased capsaicin induced vasodilatation in skin overlying joints in rheumatoid arthritis remains unclear. It is unlikely that this results from a generalised neuropathy or vasculopathy, as baseline and axon reflex vasodilatation was unaffected in skin not overlying joints. It is also unlikely that the increased vasodilatation was the result of generalised sympathetic dysfunction,[23] [24] as we did not observe any change in axon reflex sweating. Although all the patients with rheumatoid arthritis were taking non-steroidal anti-inflammatory drugs, these agents, at least in the normal volunteers tested with ibuprofen, did not appear to influence the magnitude of the capsaicin induced response. Finally, it is possible that the laser beam may have penetrated through the skin and detected changes in capsular blood flow, although we believe this to be unlikely in view of the relatively short penetration of laser beams at the wavelength used.

Skin flares are mediated, at least partly, by the neuropeptides substance P and calcitonin gene related peptide (CGRP),[25] and it is relevant that substance P has been reported to be altered in skin tissue in an animal model of tissue inflammation.[26] In turn, the expression of substance P and CGRP appears to be regulated by nerve growth factor (NGF),[27] [28] as does capsaicin sensitivity of sensory neurones.[29] There is a report of increased concentrations of NGF in the synovial fluid of patients with rheumatoid arthritis[30] and a similar increase in skin overlying inflamed joints would explain the findings of the present study. Such an increase might arise as a result of a purely peripheral mechanism following regional increases in mediators released from inflamed joints. There was, however, no correlation between ESR or wrist joint tenderness and capsaicin induced vasodilatation, as might have been expected if this were the case.

A second possibility is that the observed changes were secondary to a central spinal mechanism. Convergence of wrist joint afferents and wrist cutaneous afferents potentially might alter NGF expression in the latter, thereby producing sensitisation. Such synaptic connections could outlast the acute inflammatory response, leading to prolonged effects. Such a mechanism remains highly speculative, how ever, and awaits the results of further studies.

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