Does sympathetic nerve discharge affect the firing of polymodal C-fibre afferents in humans?

Mikael Elam,1 Bengt Olausson,1 Jon O. Skarphedinsson1,2 and B. Gunnar Wallin1

Summary
Experimental and clinical studies in animals and humans have indicated that nociceptive nerve fibres can acquire sensitivity to norepinephrine after injury or chemical sensitization. To evaluate the functional relevance of such sensitization, we recorded the activity of single polymodal C-fibre afferents in healthy human volunteers and investigated whether intense physiological sympathoexcitation could affect their firing properties. This was studied before and after chemical sensitization of receptive fields by topical application of mustard oil. All afferent C fibres investigated (11 units in 10 subjects) were mechano-heat-sensitive, and four of seven fibres subjected to mustard oil were also chemosensitive. Putative sensitivity to sympathetic stimulation was investigated during low-frequency (0.25 Hz) electrical stimulation of the unit receptive field at a threshold intensity sufficient to evoke an action potential in the afferent fibre after every second stimulus. Following a prolonged period of silent rest, sympathoexcitation was elicited by forced mental arithmetic for 60 s, again followed by a long silent rest period. During stress, sympathetic nerve traffic increased to $25\pm146\%$ of the control level, while firing of the afferent units remained unchanged. There was no sign of sympathetically mediated direct activation of afferent units and no change in the relative amounts of afferent activations caused by the background electrical stimulation. Results were similar for all units, both before (seven units in six subjects) and after (seven units in seven subjects) chemical sensitization of their cutaneous receptive field. The results suggest that if chemical sensitization of nociceptive C afferent neurons with mustard oil does induce sensitivity to noradrenaline in humans, it is not sufficient to make C nociceptive fibres responsive to short-lasting physiological variations in sympathetic outflow.

Keywords: sympathetic; C afferent; single-unit; microneurography; mustard oil

Abbreviation: GSR = galvanic skin response

Introduction
The question of whether sympathetic nerve activity may affect the firing of unmyelinated nociceptive nerve fibres remains controversial, despite much attention over the years. In animals, most studies indicate that the activity of polymodal C afferent fibres is unaffected by sympathetic stimulation under normal conditions (Roberts and Elardo, 1985; Shea and Perl, 1985; Barasi and Lynn, 1986), but neural injury may induce sensitivity to sympathetic/adrenergic stimulation. Thus, both electrical stimulation of the sympathetic trunk and intravenous or intraarterial administration of adrenergic agonists have been shown to elicit discharge from C nociceptors in several nerve injury models, including partial and complete nerve lesions (Blumberg and Jänig, 1984; Häbler et al., 1987; Sato and Perl, 1991; O’Halloran and Perl, 1997). Chemical sensitization of afferent C fibres may induce similar adrenergic/sympathetic sensitivity (Hu and Zu, 1989; Sato et al., 1993). The *in vitro* finding that the discharge pattern of chemically sensitized C fibre afferents is unchanged after sympathectomy may, however, question the physiological relevance of this adrenergic sensitization (Koltzenburg et al., 1992).

In man, injection of noradrenaline around the stump neuroma of an amputated limb has been found to cause intense pain (Chabal et al., 1992). Intraoperative stimulation of the sympathetic chain has been reported to elicit immediate augmentation of pain in patients with causalgia (Walker and Nulsen, 1948; White and Sweet, 1969), and intracutaneous administration of noradrenaline in a causalgic area has been demonstrated to rekindle pain (Wallin et al., 1976; Torebjörk et al., 1995), supporting the concept of adrenergic sensitization of afferent nerve fibres after nerve injury. On the other hand, simultaneous bilateral recordings of
sympathetic nerve outflow to affected and unaffected limbs showed normal and symmetrical sympathetic activity in causalgia patients (Elam, 1997) without an obvious relationship between the degree of sympathetic discharge and the level of perceived pain. This lack of relationship is in agreement with previous unilateral sympathetic nerve recordings in causalgia patients (Wallin et al., 1976; Casale and Elam, 1992). Furthermore, a human case of painful neuropathy with sensitized nociceptors that did not modify their activity during reflex sympathetic activation has been reported (Ochoa et al., 1996). Thus, in humans also it remains unclear whether physiological variations in sympathetic outflow may modulate clinical pain transmission/perception.

Experimental topical administration of chemical irritants such as capsaicin has been shown to induce adrenergic sensitization of nociceptor afferents also in humans (Drummond, 1995). Furthermore, pain elicited by capsaicin has been demonstrated to decrease after subcutaneous (Kinnman et al., 1997) or systemic intravenous (Liu et al., 1996) α-receptor blockade.

The aim of the present study was to investigate whether the activity of single cutaneous C polymodal afferents in man can be modulated by physiological variations in sympathetic nerve discharge, both afferent and efferent nerve activity being recorded from the same nerve fascicle. This was tested in healthy volunteers, under normal conditions and/or after acute chemical sensitization of the sensory units. Sensitization was achieved by topical application of mustard oil in the receptive field of the units, a procedure known to directly activate C mechano-heat-sensitive afferents (Handwerker et al., 1991) and alter their afferent properties (Olausson, 1998a). Preliminary observations have been reported (Elam et al., 1997).

Methods

Subjects
Data were obtained from 11 experiments in 10 healthy volunteers (mean age 28 years, range 22–45 years; six males, four females) recruited among students and hospital staff. Each subject gave informed written consent to the procedures, which were approved by the human ethics committee of the University of Göteborg.

General procedures
Experiments were performed in the morning, ~2 h after a light breakfast. The ECG was monitored via standard Ag/AgCl chest electrodes and respiratory movements (to identify inadvertent sympathetic stimulations due to Valsalva manoeuvres or deep inspiratory gasps) were monitored with a strain-gauge transducer attached to a strap around the chest. With the subjects in a comfortable, supine position, the thigh was supported by a vacuum cast and the common peroneal nerve was located behind the fibular head by palpation and electrical stimulation via a surface probe. A low-impedance electrode (i.e. one with a large uninsulated tip) was placed subcutaneously a few centimetres from the estimated position of the nerve, to serve as the reference electrode. A high-impedance tungsten microelectrode (type 25-10-1; Frederick Haer Co., Brunswick, Me., USA) was then inserted into the peroneal nerve, initially guided by low-voltage electrical stimulation through the electrode. Cutaneous nerve fascicles were identified by three criteria: (i) electrical stimuli through the electrode elicited skin paraesthesias in the receptive field of the impaled fascicle; (ii) neural activity was evoked by touch stimuli within the receptive field; and (iii) spontaneous nerve activity with a firing pattern characteristic of skin sympathetic nerve fibres was often recorded. In fact, the strategy commonly used to find single C afferent fibres was to manipulate the intraneural microelectrode until well-defined bursts of sympathetic efferent C-fibre activity were recorded, and then to search for single C afferent fibres at this recording site. Afferent units were detected by searching in the receptive field of the impaled nerve fascicle with an electrode giving transcutaneous electrical stimuli (0.3 Hz, 0.5 ms, 50–150 V). When an afferent unit with stable response latency (see below) was found, transcutaneous stimulation with the same electrode was used to define its receptive field. Subsequently, two tungsten electrodes (0.2 mm diameter) with large uninsulated tips were placed intracutaneously in the centre of the receptive field (5–10 mm apart) and stimulated (0.25 Hz, 0.5 ms) with a constant-current stimulator (Grass S88, Quincy, Mass, USA).

Identification and characterization of afferent units
Although the SC/ZOOM software used for analysis of unit activity (see below) incorporates a spike recognition facility for off-line analysis of the morphology of every spike of a candidate unit, the low signal-to-noise ratio in recordings from single C-fibre afferents in human nerves may not allow the unequivocal identification of single sensory units. Therefore, a method described by Torebjörk and Hallin was adopted to identify single units and establish their afferent characteristics (Torebjörk and Hallin, 1974). Briefly, the receptive field of the recorded sensory unit was stimulated by low-amplitude, low-frequency electrical pulses (0.25 Hz) and the arrival latency of the action potentials at the intraneural electrode at the fibular head was established (Fig. 1). Since all our receptive fields were situated on the dorsum of the foot, latencies of 400–500 ms for all units included in the study indicated conduction velocities in the C-fibre range (~1 m/s). C afferent units show a stable latency to low-frequency intradermal electrical stimulation, whereas sympathetic C efferent units show a highly variable latency due to their spontaneous activity (Hallin and Torebjörk, 1974). This enables the identification of single C afferent units on strict latency criteria and also provides a basis for
Fig. 1 Illustration of experimental set-up with intraneural recording of single C afferent units and multi-unit sympathetic activity from a cutaneous fascicle of the peroneal nerve at the level of the fibular head. Within the innervation territory of the impaled nerve fascicle, electrical stimulation of recorded afferents and recording of sympathetic effector function was performed. (A) An afferent C fibre unit with typical low signal-to-noise ratio, the identification of which was based on its stable response latency to an electrical stimulus within the receptive field delivered every 4 s (each trace showing a 30 ms period starting 450 ms after the stimulus; successive traces are arranged from the top downwards). The upper left panel shows that latency stabilizes after a characteristic initial lengthening, and then serves as a reference point for afferent characterization. Any natural stimulus activating the recorded unit (in this case repeated mechanical stimuli with von Frey hairs of increasing stiffness) will be marked by a prolonged response latency to the background electrical stimulus. The upper right panel illustrates that, after afferent characterization, the intensity of the background electrical stimulus was reduced to near-threshold level, so that the afferent C fibre was activated only after every second to third stimulus. After 15 min of such near-threshold stimulation, 60 s of forced mental arithmetic was used to evaluate the putative effects of a marked sympatho-excitation on the response incidence and the latency of the recorded afferent fibre. Note the increased baseline shifts due to movement artefacts during/after noxious heat stimuli and mental stress. (B) Effects of mental stress (indicated by the horizontal bar) on a mean-voltage neurogram of multi-unit skin sympathetic nerve activity (SSA), heart rate (ECG) and galvanic skin responses (GSR) in untreated skin within the innervation territory of the recorded nerve fascicle.
the characterization of afferents. Thus, while a stable latency to ongoing low-frequency electrical stimulation is being recorded, a natural stimulus (mechanical, thermal or chemical) is added in the receptive field to test whether it can activate the unit. If so, the extra discharges lead to a slowing of conduction velocity, resulting in a transient prolongation of the latency of electrically induced potentials (Fig. 1A), thus marking or disclosing the sensitivity of the afferent unit to that particular type of natural stimulus. Mechanical stimuli were delivered with von Frey hairs, thermal stimuli with an argon laser heat source (Olausson, 1998) and chemical stimulation by the topical application of mustard oil (Handwerker et al., 1991).

**Sensitivity of afferent units to sympathetic stimulation**

After the thermal and mechanical afferent characterization, the intensity of the low-frequency electrical stimulation of the receptive field was reduced until only every second or third stimulus activated the afferent unit, the remainder of stimuli thus being subthreshold (Fig. 1A). The stimulation was maintained at this near-threshold level for 10–15 min, the subject relaxing without any communication with the experimenters. Subsequently, in order to achieve a temporally defined and intense physiological sympatho-excitation, a 1 min period of mental stress (in which the subject was required to carry out repeated mental subtraction of the number 17 or 13, starting at 1000, while being verbally harassed) was introduced, followed by another 15–20 min of complete relaxation. Sympathetic multifibre activity to the receptive field under investigation was quantitated from the mean voltage neurogram (Fig. 1B; see below). A putative sympathetic modulation of afferent unit activity was evaluated by (i) comparing the latencies of electrically evoked potentials during stress with those during relaxation (a prolonged latency indicating direct activation of the sensory unit) and (ii) comparing the relative proportion of potentials elicited by the near-threshold electrical stimulation (spike incidence) at rest and during stress (increased incidence suggesting lowered threshold for activation).

**Data recording and analysis**

Neural activity was amplified (5 × 10⁴ to 25 × 10⁶), filtered (0.1–8.0 Hz), audio-monitored, digitized at 12.8 kHz (12 bits) and stored on disk by means of the SC/ZOOM data acquisition and analysis system (Department of Physiology, University of Umeå, Sweden), for off-line analysis of the firing of single C-fibre afferents. The amplified (5 × 10³) and filtered (0.7–2.0 kHz) nerve signal was also led through a resistance-capacitance circuit (time constant 100 ms). The output of this circuit (mean voltage nerve signal) was dominated by multifibre sympathetic efferent activity whereas the contribution from the sensory afferents was negligible. This signal was digitized at 800 Hz, stored as 8 bits and recorded on a VHS tape recorder (V-Store; Racal, Southampton, UK) together with all other physiological variables (see above and below). The sympathetic nerve discharge in the investigated fascicle was estimated from the mean voltage neurogram (area under the curve) using analysis software developed in our laboratory (Wallin and Elam, 1997). Sympathetic activity was expressed in arbitrary units/min and presented as the percentage change from control. For the recording of sympathetic effector function within the receptive field, skin perfusion was measured by laser Doppler flowmetry (Periflux; Perimed AB, Järfälla, Sweden). Sudomotor function was evaluated by recording changes in skin resistance [galvanic skin responses (GSR)], with a modified van Gough GSR module (type IGSR/7A) using Ag/AgCl electrodes (Medicotest A/S, Ølstykke, Denmark).

**Statistics**

All values are expressed as means and standard error of the mean unless otherwise stated. Spike incidence was analysed with ANOVA (analysis of variance) for repeated measurements and Huynh-Feldt adjustment of degrees of freedom (Ludbrook, 1994), using STATISTICA for Windows v. 5.1 (StatSoft Inc., Tulsa, Okla., USA). P values <0.05 were considered significant.

**Results**

**Number of units and their afferent characteristics**

The effect of sympatho-excitation on sensory C-fibre firing was tested before application of mustard oil on seven afferent units in six subjects (one subject was used in two recording sessions on separate days). In three of these subjects, the sympatho-excitation protocol was repeated on the same C afferent fibre after induction of neurogenic inflammation in its receptive field. In the remaining four subjects, the afferent unit was lost before application of mustard oil. In four other subjects, the mustard oil was applied immediately after the mechanical and thermal afferent characterization of the unit, and then sympathetic activation was initiated. Thus, a total of seven afferent units in seven subjects were tested for sympathetic modulation after neurogenic inflammation of their receptive fields. All 11 C afferent units included in this study responded to both mechanical and thermal stimuli (see example in Fig. 1A). Three out of seven units responded to mustard oil application with robust activation, whereas three units showed no overt chemosensitivity. The remaining unit showed a short and weak activation, rendering impossible the unequivocal distinction between chemosensitivity and mechanosensitivity (mild tactile stimulation during topical application).
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latency of potentials elicited by the near-threshold low-frequency electrical stimuli remained unaffected, as did the relative spike incidence (Fig. 2).

Effect of chemical sensitization

After application of mustard oil within the receptive field of the recorded afferent unit, the mental stress period was not initiated until the mustard oil-induced excitation of C afferent units had subsided and the afferent unit responded to electrical stimuli with a relative spike incidence of ~30–40%. Regardless of whether mustard oil elicited pain or only a mild sense of irritation in the treated area, sympathetic nerve activity rapidly returned to a low level at rest. During the subsequent mental stress period, sympathetic outflow increased to 669 ± 316% of the control level and subsided within 2 min, i.e. there was no difference compared with the effect before sensitization. Also, the increases in GSR (from 1–2/min to >7/min), and average heart rate (from 60 to 69 beats/min) were similar to those before sensitization. A tendency for all three autonomic variables to remain slightly elevated after stress in the mustard oil protocol was not statistically significant. Just as without inflammation of their receptive fields, the firing properties of C afferent units remained unaffected during marked physiological shifts in sympathetic outflow, as shown by consistently unaltered response latencies and no significant changes in relative spike incidences (Fig. 2).

Discussion

The main findings of this study are that (i) no evidence of sympathetic modulation of polymodal C-fibre afferent unit firing characteristics was found, and (ii) the absence of sympathetic modulation of the afferent C-fibre activity persisted after induction of acute neurogenic inflammation in the cutaneous receptive fields with mustard oil. If anything, there was a weak tendency towards a lower spike incidence of the afferent C fibres during sympatho-excitation after mustard oil application. Thus, although chemically sensitized C afferent fibres may be equipped with α receptors (see Introduction), the present results suggest that in humans these receptors may not respond to physiological variation in neurally derived norepinephrine.

Although the effect of nerve injury on afferent nerve fibre properties may differ from that of chemical sensitization, our findings agree with the limited number of intraneural recordings of sympathetic nerve traffic performed in patients with injury-related neuropathic pain (Wallin et al., 1976; Casale and Elam, 1992; Elam, 1997). In these studies there was no clear relationship between the strength of the receptive fields was usually under the detection limit of laser Doppler flowmetry, precluding general assessment of cutaneous vasomotor function. As a further indication of the autonomic nervous system response to stress, average heart rate increased from 58 beats/min at rest to 75 beats/min during stress. Throughout the period of sympathetic excitation, the latency of potentials elicited by the near-threshold low-frequency electrical stimuli remained unaffected, as did the relative spike incidence (Fig. 2).

Effects of sympatho-excitation

Before application of mustard oil, the resting level of sympathetic nerve activity was consistently low. During the mental stress period the sympathetic activity (one subject shown in Fig. 1B) increased to 625 ± 146% of the control level and then subsided within 2 min after termination of stress (Fig. 2). Concomitantly, the number of GSR increased from <1/min at rest to >9/min during stress, and then returned to the resting level within 2 min. In a few experiments the sympatho-excitation during stress was also associated with a reduction in skin perfusion, illustrating the well-known fact that mental stress activates both vaso- and sudomotor sympathetic fibres (Wallin and Elam, 1997). In most experiments, however, baseline skin perfusion in the receptive fields was usually under the detection limit of laser Doppler flowmetry, precluding general assessment of cutaneous vasomotor function. As a further indication of the autonomic nervous system response to stress, average heart rate increased from 58 beats/min at rest to 75 beats/min during stress. Throughout the period of sympathetic excitation, the

Fig. 2 Averaged data from all subjects (n = 11) on the effects of 60 s of mental stress on heart rate (HR), skin sympathetic nerve activity (SSA) and the number of skin resistance responses (GSR). The bottom trace shows that afferent C-fibre spike incidence remained unchanged during sympatho-excitation, both before (open squares) and after (filled circles) sensitization of the afferent receptive field with mustard oil.
this finding raises the possibility that adrenoceptor antagonists, such as phentolamine (Olsson et al., 1990; Arnér, 1991; Raja et al., 1991), may also have analgesic properties when sympathetic nerve blocks are inefficient. This adds another level of complexity to the interpretation of studies of the analgesic properties of sympathetic blocks.

The clinical experience from various sympato-inhibitory procedures has fuelled rather than resolved the controversy concerning sympathetic involvement in pain. Although many pain clinicians and researchers maintain the traditional view (Nathan, 1947; Richards, 1967; Roberts, 1986) that sympathetic blockade may be beneficial in neuropathic syndromes involving an element of sympathetically dependent pain (Treede et al., 1991; cf. Jänig and Schmidt, 1992; Jänig and Stanton-Hicks, 1996), the analgesic efficacy or specificity of sympathetic blocks has been seriously questioned recently (Verdugo and Ochoa, 1994; Schott, 1995; Max and Gilron, 1999). Earlier reports on strong placebo effects after sympathetic nerve blocks (Brena et al., 1980) also illustrate the difficulty of evaluating physiological mechanisms based on clinical therapeutic results, and raise serious doubts regarding the interpretation of a large number of studies on the analgesic effects of sympathetic blocks (Kingery, 1997). The present results, as well as previous sympathetic nerve recordings in causalgic patients (Wallin et al., 1976; Casale and Elam, 1993; Elam, 1997), suggest that, even though an analgesic effect of a postsynaptic sympathetic block (i.e. an α-adrenoceptor antagonist such as phentolamine) has been verified in a placebo-controlled protocol, this does not necessarily mean that a sympathetic nerve blockade or a surgical sympathectomy would be beneficial. In fact, destructive procedures must be seriously questioned since sympathectomy alone, without concomitant injury of afferent nerve fibres, can sensitize C nociceptive fibres to locally administered norepinephrine (Bossut et al., 1996). The clinical relevance of this experimental finding is illustrated by several reports of neuralgia after sympathectomy (Tracy and Cockett, 1957; Litwin, 1962; Raskin et al., 1974; Churcher, 1984).

**Limitations of the study**

In the present study, intracutaneous electrical stimulation via needle electrodes placed within the receptive field was used to activate the afferent C fibres. With this mode of stimulation, some nerve fibres may have been activated at the end and others a short distance from the end. We cannot exclude the possibility that natural stimulation of only the ends of the fibres would give different results. However, sympathetic stimulation has been found to facilitate electrically evoked C afferent nerve activity in the rabbit peroneal nerve, both before (Shyu et al., 1989a, b) and after experimental nerve compression (Shyu et al., 1990), suggesting that α adrenoceptors are present on unmyelinated axons. Therefore, it seems likely that changes in excitability due to noradrenaline-induced changes of membrane properties would be detectable regardless of whether or not the stimulation activated the end of the fibre or the distal axon.

Mustard oil activated only half of the mechano-heat-sensitive C afferent units that were tested, in agreement with previous studies (Beck and Handwerker, 1974; Lang et al., 1990; Koltzenburg et al., 1992). It may be argued that those afferent C fibres that were not overtly activated by mustard oil were not sensitized by the procedure. This possibility cannot be excluded, but all subjects reported a local burning sensation within 1–2 min of application, and topical mustard oil has been found to alter the sensory characteristics of afferent units independently of whether they are directly activated (Olausson, 1998a). Therefore, we chose to include units regardless of whether or not they were overtly affected by mustard oil.

In the light of our finding that acute chemical irritation of their receptive fields with mustard oil did not sensitize C nociceptors in healthy humans to sympathetic modulation, a possible extension of the present experiments would have been to study patients with neuropathic pain and evidence for chronic sensitization. However, given the fact that these pain syndromes are often initiated by a rather limited trauma, and that the present type of single-unit approach of microneurography often involves a prolonged intraneural search procedure, we decided against including pain patients.

Although our physiological sympatho-excitation was vigorous compared with the normal physiological variations in sympathetic traffic seen in non-stressed subjects, it could be argued that the duration of our stress protocol (1 min) and the resulting sympatho-excitation (2 min) was too short to reveal sympathetic modulation. However, the recent finding that prolonged, cooling-induced cutaneous sympathetic vasoconstriction does not influence capsaicin-induced pain in humans (Baron et al., 1999) suggests that the duration of the sympatho-excitation may not be a critical factor.

In conclusion, the activity of single cutaneous polymodal C-fibre afferents in healthy humans is not affected by marked physiological variation in sympathetic nerve activity. This absence of sympathetic modulation remains after mustard oil-induced acute neurogenic inflammation of sensory unit receptive fields, indicating that this widely used model for chemical sensitization of C nociceptors does not involve acquired sensitivity to neurally released catecholamines.

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