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Nerve injury alters the effects of interleukin-6 on nociceptive transmission in peripheral afferents

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Abstract

Interleukin-6 (IL-6) is markedly upregulated in the peripheral and central nervous systems following nerve injury; however, the functional effects of this are unclear. This study investigates the effect of peripheral interleukin-6 on nociceptive transmission in naive and neuropathic states. Using an in vitro rat skin-nerve preparation, 50 ng interleukin-6 inhibited responses of single nociceptive fibers to noxious heat. A 20-ng sample of interleukin-6 only inhibited heat responses in the presence of soluble interleukin-6 receptors. To examine in vivo effects of peripheral interleukin-6, extracellular recordings from dorsal horn neurons were made in anaesthetised naive, sham-operated and neuropathic (spinal nerve ligated) rats. Peripheral interleukin-6 (40–100 ng) markedly inhibited all naturally evoked neuronal responses in naive rats, yet only neuronal responses to heat in neuropathic rats. Behaviourally, intraplantar administration of interleukin-6 (0.01–1 μg) elicited ipsilateral thermal hypoalgesia in naive rats. Thus, interleukin-6 inhibits normal peripheral nociceptive transmission, yet such anti-nociceptive effects are attenuated following nerve injury in a modality-specific manner.

Keywords: Cytokine; C-fibre; Pain; Electrophysiology; Interleukin-6; Dorsal horn

1. Introduction

Interleukin-6 (IL-6) is a multifunctional cytokine belonging to the neuropoietic cytokine family, which includes leukemia inhibitory factor (LIF), oncostatin M and ciliary neurotrophic factor (CNTF). Interleukin-6 is involved in many immunological processes such as B-cell differentiation, T-cell activation, hematopoiesis and the acute phase response to injury (Van Snick, 1990). In addition, interleukin-6 has a variety of functions in the nervous system such as neuronal differentiation, enhancing neuronal survival and neuroprotection (for review, see Gadient and Otten, 1997). Clinically, interleukin-6 may have a role in nerve injury and pain as patients with persistent sciatic pain display elevated blood levels of interleukin-6 (Geiss et al., 1997) and following traumatic brain injury increased interleukin-6 levels are found in the brain (Kossmann et al., 1996).

In humans, interleukin-6-like immunoreactivity has been found in juvenile and foetal dorsal root ganglion (Nordlind et al., 1996), and nerve explants secrete interleukin-6 from Schwann cells (Rutkowski et al., 1999). Peripheral nerve-like structures also exhibit interleukin-6-like immunoreactivity in normal human skin and to a greater extent in inflamed skin (Nordlind at al., 1996). Animal studies report extremely low expression of interleukin-6 in naive rats with dramatic increases in expression, both peripherally and centrally following nerve injury. Specifically, nerve injury results in increased levels of interleukin-6 mRNA or interleukin-6 positive cells in rat and mouse sciatic nerves (Bourde et al., 1996; Cui et al., 2000; Reichert et al., 1996; Zhong and Heumann, 1995) and in rat Schwann cells at the site of injury (Bolin et al., 1995; Grothe et al., 2000). In dorsal root ganglia neurones, interleukin-6 mRNA upregulation occurs following axotomy (Murphy et al., 1995), chronic constriction injury (Murphy et al., 1999b), nerve crush and spinal nerve root transection (Murphy et al., 1999a). In addition, increases in spinal interleukin-6 mRNA and protein levels are observed following sciatic cryoneurolysis or trauma to the L5 spinal nerve (Arruda et al., 1998; DeLeo et al., 1996; Winkelstein et al., 2001). Systemic

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upregulation of interleukin-6 is also reported following peripheral nerve injury with increased plasma levels of interleukin-6 (Wells et al., 1992).

Interleukin-6 exerts its effects by binding to a specific cell surface (cytokine type 1) receptor and for signal transduction to occur, the interleukin-6/receptor complex then associates with two gp130 molecules (Hirano et al., 1994; Mullberg et al., 1999). Interleukin-6 receptor mRNA is found in vascular endothelial cells and Schwann cells of the intact sciatic nerve and is upregulated following nerve injury (Grothe et al., 2000; Ito et al., 1998). Intact nerves display low levels of gp130 mRNA, yet following nerve injury there is also a marked increase in gp130 mRNA (Ito et al., 1998). However, it has been recently reported that dorsal root ganglia protein levels of gp130 are unaffected by axotomy (Gardiner et al., 2002).

Despite a wealth of literature describing the marked upregulation of interleukin-6 following nerve injury, data on the functional effects of interleukin-6 in sensory processing is limited. Oka et al. (1995) found that intracerebroventricular injections of interleukin-6 induced thermal hyperalgesia in naive rats, whereas intracisternal interleukin-6 had anti-nociceptive effects in an acute orofacial pain model, yet hyperalgesic effects in an inflammatory orofacial pain model (Choi et al. 2003). Intrathecal interleukin-6 had no effect on thermal withdrawal latencies in uninjured rats (DeLeo et al., 1996). We have recently reported that spinal administration of interleukin-6 elicits anti-nociceptive effects following nerve injury, which are not observed under normal conditions (Flatters et al. 2003b). Intraplantar interleukin-6 increased mechanical thresholds of inflamed rat hindpaws, but had little effect in normal conditions (Czlonkowski et al., 1993). In vitro, interleukin-6 only increased the heat-evoked CGRP release from nociceptors in rat skin in the presence of soluble interleukin-6 receptors, (Opree and Kress, 2000), results which have reproduced in vivo (Obreja et al., 2002). Studies using interleukin-6-deficient mice have not been conclusive in establishing the role of endogenous interleukin-6 in nociception. Two studies found no difference in the thermal and mechanical withdrawal latencies of interleukin-6 knockout mice and wild-type mice (Bianchi et al., 1999; Murphy et al., 1999b). Whilst others report increased thermal thresholds in interleukin-6 knockout animals (Zhong et al., 1999) or decreased mechanical and thermal thresholds (Xu et al., 1997). The reasons for the different outcomes of these studies are unclear, although the 'knock-out' of interleukin-6 appears to result in compensatory effects such as a threefold increase in TNFα levels (Fattori et al., 1994).

The aim of this study was to investigate the functional effect of interleukin-6 administration on peripheral nociceptive transmission in naive animals and following nerve injury. Due to the lack of interleukin-6 receptor antagonists and previous inconclusive reports following the genetic manipulation of endogenous interleukin-6 levels, we have administered interleukin-6 exogenously in different experimental settings in an attempt to ascertain the possible role of this cytokine in peripheral sensory processing. Specifically, we have employed a combination of in vitro electrophysiology recording from peripheral nociceptive fibres, in vivo electrophysiology recording from convergent dorsal horn neurones and behavioural studies examining withdrawal latencies. Preliminary reports of our findings have been published in abstract form (Flatters et al., 2002a).

2. Materials and methods

Guidelines for animal research by UK Home Office and the International Association for the Study of Pain (Zimmermann, 1983) were adhered to in all of the experiments undertaken for this study which were also approved by UCL Ethics Committee.

2.1. In vitro skin-nerve preparation

Male Sprague–Dawley rats (160–215 g, Charles River, UK) were sacrificed by CO_2 overdose and the hindpaw skin of one hindpaw with attached saphenous nerve was dissected as previously described by Reeh (1986). The skin was pinned 'hairy side down' to expose the corium side and the end of the nerve threaded through a hole (2 mm diameter) into a separate recording chamber to lay on a mirror. The skin chamber was perfused (15 ml/min) with a physiological salt solution (PSS) of the following composition (in mM): NaCl, 138.6; KCl, 3.5; $CaCl_2$, 1.5; $MgCl_2$, 1.2; $NaHCO_3$, 21.0; NaH_2PO_4, 0.58; glucose, 10.0, which was perfused with 95% O_2 and 5% CO_2 and maintained at 33 °C. The aqueous solution in the recording chamber was overlaid by paraffin oil, the epineurium was then pulled back to dissect out fine filaments which were placed on a single platinum wire in the paraffin layer to record single-unit activity. Single units were identified following electrical stimulation of the nerve trunk via a bipolar platinum electrode, which enabled the measurement of the conduction velocity of the nociceptive fibres (distance between stimulating electrode and recording electrode divided by time taken for action potential propagation). Receptive fields were then located by gently prodding the skin with a blunt glass rod and the mechanical thresholds determined with calibrated von Frey hairs.

A hollow metal cylinder (8 mm diameter) was placed on the receptive field and perfused with PSS at a rate of 5 ml/min with the temperature regulated by an electrical continuous flow heater (Peltier device) which was under the influence of a Marlow temperature controller. A thermocouple placed in the receptive field cylinder recorded the temperature of the solution at the skin. Using the Marlow device, the temperature of solution at the skin was increased to 49 °C and maintained for 30 s then cooled back to 35 °C. Following a control thermal stimulation, interleukin-6 (20 or 50 ng/ml, human recombinant interleukin-6, Sigma-Aldrich)

was perfused onto the receptive field for 10 min, and thermal stimulation was performed in the continued presence of interleukin-6. The thermal response was then repeated following a 10 min washout period with PSS. In addition, the effect of the soluble interleukin-6 receptor (human recombinant interleukin-6 receptor, shIL-6R, R&D Systems) was investigated by perfusing soluble interleukin-6 receptor solution (25 ng/ml) for 10 min at 35 °C, prior to exposure to 20 ng/ml interleukin-6. Interleukin-6 was only applied once to each skin preparation. In control fibres, thermal stimulation was repeated three times in the absence of interleukin-6. The resultant heat ramp and the action potentials elicited were recorded using CED 1401 interface and Spike 2 software, which enabled different fibres to be distinguished and their responses to be quantified.

2.2. Spinal nerve ligation surgery

Male Sprague–Dawley rats were housed in groups of five in plastic cages with artificial lighting which had a fixed 12 h light–dark cycle. Food and water were available ad libitum. The spinal nerve ligation (SNL) model of neuropathic pain first described by Kim and Chung (1992) was used. Briefly, rats (130–150 g) were anaesthetised with halothane (Fluothane, Zeneca, UK) in a 50% O_2, 50% N_2O gaseous mixture. A small piece of paravertebral muscle and part of the left transverse process of the L5 lumbar vertebra were removed to expose the three branches of the sciatic nerve, L4, L5 and L6. The L5 and L6 spinal nerves were isolated and tightly ligated using 6-0 silk leaving L4 spinal nerve uninjured. Muscle and skin were closed with sutures (silk 3.0) and wound clips, respectively. The surgery for the sham-operated rats was identical to the spinal nerve ligated rats with the exception of the ligation of L5 and L6 spinal nerves.

2.3. In vivo electrophysiology

Experiments were performed 14–18 days after the surgery and on uninjured (naive) animals of a similar weight as previously described (Flatters et al. 2002b, 2003a,b). Rats were initially anaesthetised with 3.5% halothane in a 33% O_2, 66% N_2O gaseous mixture until areflexia was produced. Cannulation of the trachea was performed and this route supplied anaesthesia thereafter. Rats were secured in a stereotaxic frame, and a laminectomy was performed to expose segments L4–L5 of the spinal cord. The dura of the exposed spinal cord was removed, and the spine was held rigid by clamps caudal and rostral to the laminectomy. The halothane concentration was lowered (2.5–2.8%) whilst the surgery was performed and then held at 1.9–2.2% for the duration of the experiment. This level of anaesthesia maintained areflexia with respiratory and cardiovascular parameters uncompromised. Throughout the experiment, the core body temperature of the rat was monitored and maintained (36.5–37 °C) by means of a heating blanket connected to a rectal thermal probe via an automatic feedback control unit. At the end of the experiment, rats were killed with an overdose of halothane.

A parylene-coated tungsten electrode was lowered into the cord using a SCAT microdrive (Digitmer UK), which enabled the depth of the neurone from the surface of the dorsal horn of the spinal cord to be measured. In spinal nerve ligated and sham-operated animals neurones ipsilateral to the surgery were recorded. Extracellular recordings were made from single convergent dorsal horn neurones responding to both innocuous (brush) and noxious (pinch) stimuli applied to the receptive fields in the plantar region of the hindpaw. All neurones in this study had A-fibre and C-fibre responses established by electrical stimulation that resulted in the construction of a post-stimulus time histogram as previously described (Flatters et al., 2002b).

Responses of convergent dorsal horn neurones were recorded following defined innocuous and noxious mechanical stimulation of the receptive field in the hindpaw using von Frey hairs with bending forces of 8.51 g and 28.84 g, respectively. Each hair (8.51 g hair followed by the 28.84 g hair) was applied for 15 s, and the number of action potentials this stimulation generated was recorded. The spontaneous activity of the neurone was taken into account by recording the number of action potentials over a 15 s period prior to the stimulation by the 8.51 g von Frey hair. This background measurement was then subtracted from the response to each of the von Frey hairs. Following mechanical stimulation, a jet of 32 °C water was applied to the receptive field for 15 s via a syringe; this was repeated with a jet of 45 °C water for 15 s, and the difference between the two was taken as the neuronal response to noxious heat.

Responses to mechanical and thermal stimulation were determined at 10-min intervals until the number of action potentials evoked to each stimulus did not differ by more than 15%. The last four tests were then averaged for each stimulus to comprise the pre-drug control response. Human recombinant interleukin-6 (40 and 100 ng dissolved in 20 μl saline, Sigma-Aldrich, UK) was then injected, in a cumulative manner, into the toe containing the receptive field of the spinal neurone. Tests were then performed every 10 min over a 120-min time course following each dose of interleukin-6. Effects of interleukin-6 were again expressed as percentages of this pre-drug control value for every evoked response, allowing each neurone to act as its own control. A similar protocol was followed in separate experiments to assess the effect of saline injections on the evoked neuronal responses.

2.4. Thermal behavioural testing

Male Sprague–Dawley rats (200–250 g) were randomly assigned to groups of six. Nociceptive responses to a noxious thermal stimulus were examined by measuring the latency to withdrawal of the hindpaws from a focused beam of radiant heat to the plantar surface using a Ugo Basile Plantar Test

apparatus. Animals (one group at a time) were placed in six perspex transparent chambers (dimensions: 18 × 29 × 13 cm) with a thin glass floor and allowed to acclimatise for 5–10 min before withdrawal latencies were measured on both hindpaws. With a cut-off of 31.8 s, withdrawal latencies were measured prior to (predose) and then 1 and 3 h following a 10 μl intraplantar injection of either human recombinant interleukin-6 (0.01, 0.1 and 1 μg) or saline. At each time point, both hindpaws of each rat were tested twice, and then an average of these two readings was taken. To avoid sensitisation of the paws, several minutes elapsed before a paw was tested for the second time.

2.5. Statistical methods

In in vitro electrophysiological studies analysis of variance (ANOVA) was used to test for differences in conduction velocity, mechanical threshold and first heat response between the four fibre groups (heat alone, 20 ng interleukin-6, 20 ng interleukin-6 + soluble interleukin-6 receptors and 50 ng interleukin-6). Paired *t*-tests were used to test the effect of interleukin-6 and soluble interleukin-6 receptors on nociceptive fibres compared to the first (control) heat response. In in vivo electrophysiological studies, ANOVA was used to test for differences in control neuronal responses prior to peripheral interleukin-6 administration for naive, sham and neuropathic groups. Then, paired *t*-tests were used to test the differences between the control responses and maximal effects following peripheral interleukin-6 application. In the behavioural study, ANOVA (for repeated measures) followed by Tukey's HSD test was used to compare behavioural withdrawal latencies after intraplantar interleukin-6 administration to predose values. All tests used a 5% level of significance, $P < 0.05$.

3. Results

3.1. In vitro effects of peripheral interleukin-6

Responses were obtained from a total of 47 nociceptive fibres which were classified as 45 C-fibres with a mean conduction velocity of 0.79 ± 0.03 m/s and 2 Aδ-fibres with a mean conduction velocity of 2.8 ± 0.13 m/s. The 47 fibres had a mean mechanical threshold of 1.95 ± 0.15 g. There was no significant difference between the mean conduction velocity or mechanical threshold for the four fibre groups, heat alone, 20 ng interleukin-6 (20 ng IL-6), 50 ng interleukin-6 (50 ng IL-6) and 20 ng interleukin-6 + soluble interleukin-6 receptors (20 ng IL-6 + sIL-6R) (Table 1, ANOVA). However, the number of action potentials elicited in the control heat response (prior to drug administration) for the 50 ng interleukin-6 fibre group (50 ng IL-6) and the 20 ng interleukin-6 + soluble interleukin-6 receptors group (20 ng IL-6 + sIL-6R) was significantly higher than in the control heat response of the heat alone group ($P < 0.05$, unpaired *t*-test, Table 1). Therefore, the effects of the drug treatments were compared to their own pre-drug control response.

Figure 1 shows sample traces of fibre responses illustrating the effect of interleukin-6 on responses to noxious heat. Fig. 1A illustrates 20 ng interleukin-6 had little effect on firing elicited in response to noxious heat (spikes which occurred within the heat ramp). In comparison, 50 ng interleukin-6 (Fig. 1B) induced a marked inhibition of firing to noxious heat. Repeated application of noxious heat produced some desensitisation in the response, with a reduction of 37% in the third response compared to the first ($n = 16$, Fig. 2). The 50 ng sample of interleukin-6 produced a significant 60% inhibition of responses to noxious heat which was reversible upon washout ($n = 11$, $P < 0.01$, Fig. 2). The 20 ng sample of interleukin-6 was ineffective alone ($n = 9$), but produced a significant 46% inhibition of the heat response when applied in the presence of the soluble interleukin-6 receptors ($n = 9$, $P < 0.01$, Fig. 2). This inhibition was not reversed by washout, escalating to a 79% inhibition of this response ($P < 0.01$, Fig. 2). There was no significant difference between the number of action potentials elicited in the second heat response compared to those in the first heat response for the heat alone fibre group. This shows that the inhibition shown in Fig. 2 by different drug treatments is a result of a pharmacological action and not due to desensitisation.

3.2. In vivo effects of peripheral interleukin-6

As we have previously reported (Flatters et al. 2002b, 2003a,b), spinal nerve-ligated rats displayed marked behavioural sensitivity to innocuous mechanical and cold stimuli,

Table 1
Control responses and characteristics of nociceptive fibres

Experimental fibre group	Heat alone $n = 16$	20 ng IL-6 $n = 11$	20 ng IL-6 + sIL-6R $n = 9$	50 ng IL-6 $n = 11$
Control heat response (number of action potentials)	78 ± 12	72 ± 14	145 ± 22	134 ± 35
Conduction velocity (m/s)	0.73 ± 0.04	1.16 ± 0.28	0.72 ± 0.04	0.95 ± 0.1
Mechanical threshold (g)	2.18 ± 0.28	1.96 ± 0.21	1.69 ± 0.27	1.83 ± 0.37

Control responses (prior to drug administration) expressed as the mean ± S.E.M. of the total number of action potentials evoked by the 30 s noxious heat stimulation of the nociceptive fibres in the four experimental groups, ((heat alone, 20 ng interleukin-6 (20ng IL-6), 20 ng interleukin-6 + soluble interleukin-6 receptors (20 ng IL-6 + sIL-6R) and 50 ng interleukin-6 (50 ng IL-6)). In addition, the mean ± S.E.M. of the conduction velocity and mechanical threshold of the four experimental groups of nociceptive fibres are shown, expressed in m/s and g, respectively. All fibres were recorded from skin-saphenous nerve preparations of naive rats, $n = 9–16$.

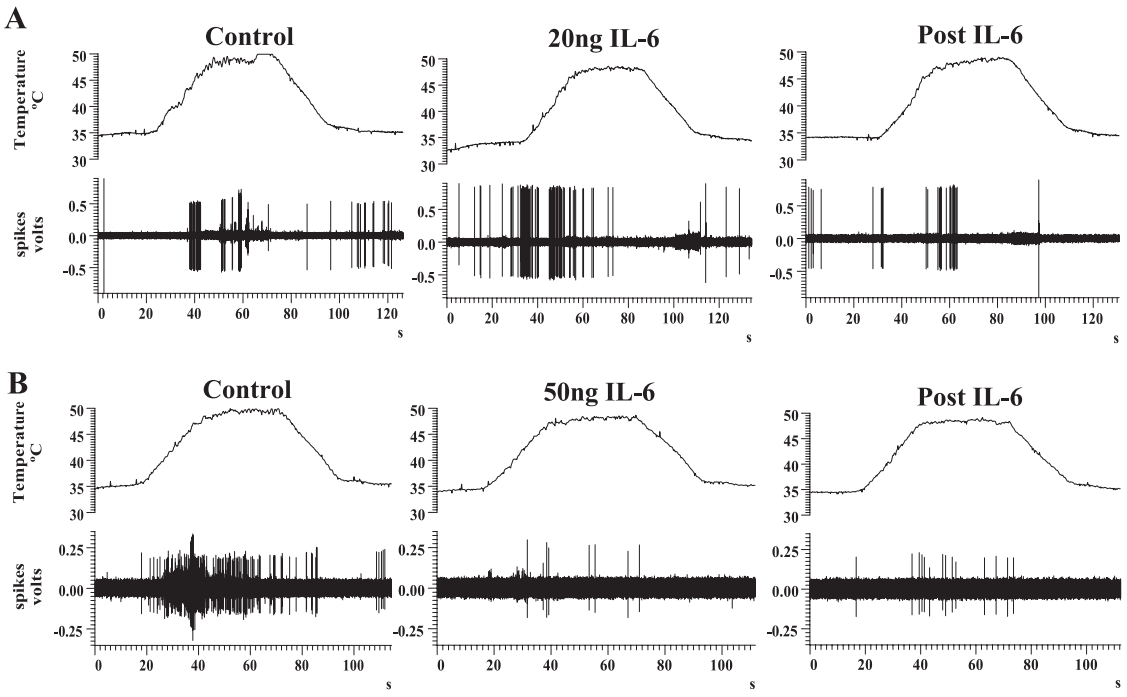

Fig. 1. Single nociceptive fibre firing in response to noxious heat before interleukin-6 (control), in the presence of interleukin-6 (20 ng/50 ng IL-6) and after 10 min wash-out (post-IL-6). (A) An example of the mild facilitatory effect of 20 ng interleukin-6 on the firing induced by noxious heat. (B) An example of the marked inhibitory effect of 50 ng interleukin-6 on the firing induced by noxious heat.

whereas the sham-operated rats did not show any sensitivity to these stimuli. Of the neurones exposed to peripheral interleukin-6, there was no significant difference (ANOVA) between the three animal groups in the depth of these neurones or in their pre-drug control neuronal responses obtained prior to interleukin-6 administration (Table 2).

Administration of interleukin-6 (40 and 100 ng) into peripheral receptive fields produced a significant inhibition of spinal neuronal responses to innocuous (von Frey 8.51 g) and noxious (von Frey 28.84 g) mechanical stimulation in naive rats ($P<0.05$). A 100 ng sample of interleukin-6 induced the greatest inhibition of mechanically evoked responses in naive rats with 70% and 57% inhibition for von Frey hairs 8.51 and 28.84 g, respectively (Fig. 3). In sham-operated rats, peripheral interleukin-6 administration also induced inhibitions of up to 38% on mechanical responses, although these effects were only significant on

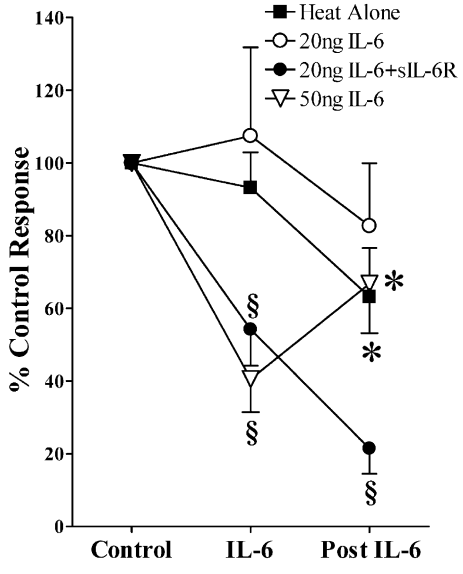

Fig. 2. Effect of interleukin-6 (IL-6) and interleukin-6 soluble receptors (sIL-6R) on single nociceptive fibres. Number of action potentials elicited during the heat ramp for each fibre were expressed as a percentage of the control response, and the plots show the mean ± S.E.M. for each fibre population, $n=9–16$. *$P<0.05$, §$P<0.01$ (paired t-test) mean response is significantly different from control (pre-drug) response.

Table 2
Mean depth and pre-drug control responses of spinal neurones

Neuronal characteristic	Naive $n=10$	Sham $n=9$	Neuropathic $n=9$
Depth of neurone (μm)	748 ± 48	708 ± 43	636 ± 16
Innocuous mechanical (von Frey 8.51 g, APs)	169 ± 53	153 ± 32	178 ± 48
Noxious mechanical (von Frey 28.84 g, APs)	498 ± 99	582 ± 93	538 ± 98
Noxious heat (APs)	418 ± 72	357 ± 40	421 ± 75

Mean depth and pre-drug control responses of neurones from naive, sham neuropathic (spinal nerve ligated) rats that were exposed to peripheral interleukin-6. Values are the mean ± S.E.M. of the neuronal population. Neuronal responses to natural stimulation are expressed as the total number of action potentials (APs) evoked by the 15 s stimulus application. No significant differences were found in any of the pre-drug control responses or depth of the neurones between the three animal groups (ANOVA).

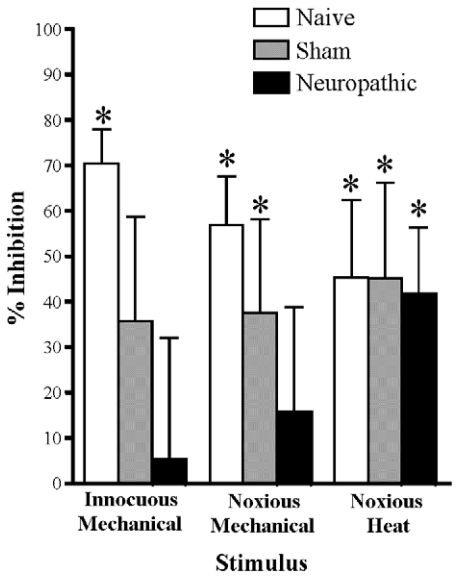

Fig. 3. Effect of peripheral interleukin-6 (100 ng) on neuronal responses to innocuous mechanical stimulation (8.51 g von Frey), noxious mechanical stimulation (28.84 g von Frey) and noxious heat (45 °C water jet normalised by response to 32 °C water jet) in naive, sham and neuropathic rats. Graph shows the mean ± S.E.M. of the maximum effect, expressed in terms of % inhibition, which occurred for the neurone population over the 120-min time course, $n = 7–13$. *$P < 0.05$ (paired t-test) degree of inhibition is significantly different from the control response prior to peripheral interleukin-6 administration for the animal group in question. N.B. Peripheral saline markedly facilitated the neuronal responses to innocuous and noxious mechanical stimuli and slightly facilitated the response to noxious heat compared to responses prior to injection. The inhibitory effects induced by peripheral interleukin-6 in naive rats are significantly different from the effects of peripheral saline for the mechanical responses, but not the response to noxious heat ($P < 0.05$, unpaired t-test).

those responses to noxious mechanical stimulation ($P < 0.05$, Fig. 3). Contrastingly, in neuropathic rats, peripheral interleukin-6 (40 and 100 ng) did not significantly affect responses to either innocuous or noxious mechanical stimulation (Fig. 3). In contrast to the differential effects on mechanical neuronal responses, peripheral interleukin-6 (40 and 100 ng) significantly inhibited neuronal responses to noxious heat to a similar degree (41–46% inhibition) in naive, sham and neuropathic rats ($P < 0.05$, Fig. 3).

Separate experiments examined the effect of peripheral saline injections on spinal neuronal responses to mechanical and thermal stimulation in naive rats (data not shown). Saline never caused an inhibition and, indeed, led to clear facilitations (65–130%) of the neuronal responses to both innocuous (von Frey 8.51 g) and noxious (von Frey 28.84 g) mechanical stimulation compared to responses prior to injection. These effects are significantly different from the inhibitory effects induced by peripheral interleukin-6 on the mechanical responses in naive rats ($P < 0.05$, unpaired t-test). The effect of saline on the neuronal response in naive rats to noxious heat was slight, 15% facilitation of the pre-injection control response and not significantly different from this response following peripheral interleukin-6 in naive rats.

3.3. Behavioural effects of peripheral interleukin-6

To investigate a possible behavioural correlate of our findings, we examined the effect of peripheral interleukin-6 administration on thermal nociceptive measures in naive rats, since we had shown an inhibitory effect on these responses both in vivo and in vitro using electrophysiological approaches. Intraplantar administration of interleukin-6 produced a significant increase in the withdrawal latencies of the ipsilateral paw (Fig. 4A). The 0.01 μg interleukin-6 sample caused a significant increase at 1 h post administration and at 3 h post administration ($P < 0.01$, ANOVA for repeated measures). The 0.1 μg sample of interleukin-6 and

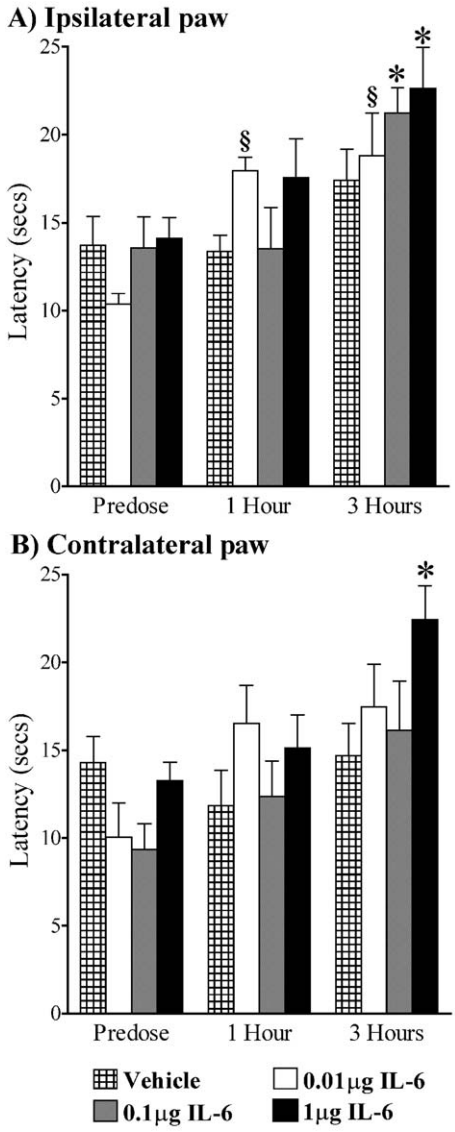

Fig. 4. (A and B) Effect of intraplantar interleukin-6 (IL-6) on paw withdrawal latencies to a focused heat stimulus for the ipsilateral paw and contralateral paw, respectively. Each column shows the mean withdrawal latency ± S.E.M. for each group of six rats. *$P < 0.05$, §$P < 0.01$ (ANOVA followed by Tukey's HSD test) significant increase in latency compared to group's predose reading.

1 μg interleukin-6 also caused a significant increase in the ipsilateral withdrawal latency at 3 h post administration ($P<0.05$). Contralateral withdrawal latencies (Fig. 4B) were unaffected by interleukin-6, with the exception of the highest dose at 3 h following administration ($P<0.01$). The vehicle (saline) group showed no significant difference in the withdrawal latencies of either the ipsilateral or the contralateral paw over the time course of the experiment in keeping with our electrophsyiological findings with this stimulus.

4. Discussion

In this study, we have presented for the first time a profile of the functional effects of peripheral interleukin-6 on nociceptive transmission, in naive and neuropathic states using different electrophysiological techniques. In addition, we have shown that the inhibitory effects of interleukin-6 seen electrophysiologically are completely in accordance with the behavioural effects of interleukin-6.

In vitro, higher concentrations of interleukin-6 profoundly inhibited the nociceptive fibre responses to noxious heat. Lower concentrations of interleukin-6 were ineffective, but markedly inhibited fibre heat responses when given with the soluble interleukin-6 receptors, supporting a specific action of the cytokine. Similar effects of interleukin-6 and its receptor have been described on heat evoked CGRP release from the rat skin; 20 ng interleukin-6 and noxious heat did not evoke significant CGRP release but noxious heat plus 20 ng interleukin-6 and soluble interleukin-6 receptors significantly increased CGRP release from peripheral fibres (Opree and Kress, 2000). These authors concluded that normal rat skin does not express interleukin-6 receptors but possesses gp130, so that a significant effect is only seen following the addition of soluble interleukin-6 receptors. However, we found a significant effect was induced by a higher interleukin-6 concentration. Thus, we suggest it is possible that interleukin-6 receptors are present in normal rat skin, but their expression is too low for 20 ng interleukin-6 to produce a significant effect. In addition, we show that gp130 must be expressed in normal skin as we observe a significant inhibition of the heat response following 20 ng interleukin-6 and soluble interleukin-6 receptors. Our results clearly show an inhibitory effect of interleukin-6 on C-fibre evoked responses yet the cytokine (at the same doses and its soluble receptor) increases heat-evoked release of CGRP from fine afferents (Opree and Kress, 2000). This suggests the intriguing possibility that interleukin-6 is a pro-inflammatory yet anti-nociceptive cytokine.

In vivo, peripheral interleukin-6 administration into the receptive fields of convergent dorsal horn neurones again induced inhibitory effects that depended on the animal group and the modality of stimulation. The in vivo effects of peripheral interleukin-6 in naive animals mirrored the observations from the in vitro experiments, showing the inhibitory effects of this cytokine on C-fibre activity as well as spinal neuronal activity. Peripheral interleukin-6 markedly inhibited neuronal responses to mechanical stimulation in naive rats but had little effect on the same responses in neuropathic rats. Saline produced a significant facilitation of the responses to mechanical stimulation which has been seen in previous electrophysiological studies (Carpenter et al., 2000). Although this facilitatory effect of saline could potentially offset the inhibitory activity of interleukin-6, the results clearly show that the ability of peripheral interleukin-6 to reduce innocuous responses is attenuated by nerve injury. Interestingly, peripheral interleukin-6 significantly inhibited the neuronal responses to noxious heat in naive, sham and neuropathic rats to near identical extents. It is unclear why neuronal responses to heat remain unchanged whilst the inhibition of mechanical responses is reduced in neuropathic rats. This modality-specific inhibition could perhaps reflect differential changes in phenotypes of different populations of afferent fibres, reorganisations and differential modifications in the expression of interleukin-6 or other receptors such as the vanilloid receptors and mechanosensors after nerve injury. Whatever the case, the effects of interleukin-6 are highly specific.

The inhibitory effect of peripheral interleukin-6 on neuronal responses had a clear behavioural counterpart as demonstrated by the effect of intraplantar interleukin-6 administration on thermal withdrawal latencies in naive rats. Each dose of interleukin-6 administered elicited a significant increase in the withdrawal latencies of the ipsilateral paw. A contralateral effect was only observed after 3 h with the top dose of interleukin-6 (1 μg). This contralateral effect could suggest that perhaps interleukin-6 is producing at this late stage systemic actions. Two previous studies examined intraplantar injections of interleukin-6 on behavioural nociceptive measures (Cunha et al., 1992; Czlonkowski et al., 1993). Mechanical thresholds were unaltered 5, 10 and 20 min following intraplantar injections of interleukin-6 (1–50 ng) in uninjured rats (Czlonkowski et al., 1993). In contrast, Cunha et al. (1992) reported that intraplantar interleukin-6 (0.001–1 ng) induced a long-lasting (6 h) but bilateral mechanical hyperalgesia in naive rats, which was present in both paws from 30 min postinjection. No vehicle control group was included in this study, and so the effects seen could simply be due to sensitisation of both paws. In contrast, here using thermal behavioural testing, we report a prolonged anti-nociceptive effect of intraplantar interleukin-6 at a higher dose range (10–1000 ng) with contralateral effects only seen at 3 h post-injection at the highest dose. These differences may have arisen as a result of differing dose ranges or the modality of sensory testing.

We provide a detailed study of the actions of interleukin-6 on nociception in the periphery under normal and pathological conditions. In summary, we find in naive rats peripheral administration of interleukin-6 is anti-nociceptive in three different in vitro and in vivo experimental settings; producing a significant inhibition of heat responses of

nociceptive C-and Aδ-fibres in vitro, spinal neuronal responses in vivo, as well as a behavioural anti-nociceptive effect in naive rats. However, peripheral interleukin-6 administration in neuropathic rats induced differential effects, with the inhibition of spinal neuronal responses to noxious heat, but not mechanical neuronal responses.

In conclusion, these studies suggest that under normal conditions, any physiological or pathological change that caused increased levels of interleukin-6 in the periphery could result in an attenuation of nociception and that these effects are subject to plasticity since nerve injury partially reverses such effects in a modality selective manner. Interleukin-6 may thus potentially be proinflammatory (Van Snick, 1990), yet attenuate nociception. This latter action would be in line with the reported neuroprotective actions of this mediator (Toulmond et al., 1992), and overall, these findings suggest that the repertoire of actions of cytokines may be subtle and state dependent.

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