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Role of IL-6 in Chronic Muscle Hyperalgesic Priming

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Abstract

After recovery from acute muscle pain even minor subsequent muscle use can initiate recurrence of the same mechanical hyperalgesia months or years after the initial injury. We have recently developed a model of this chronic latent hyperalgesia in the rat. In this study, we have examined the possibility that IL-6, an inflammatory mediator produced during acute muscle inflammation, can mediate the production of this chronic latent hyperalgesic state in which subsequent exposure to inflammatory mediators produce a markedly prolonged mechanical hyperalgesia. We now report that intramuscular injection of IL-6 produced mechanical hyperalgesia, lasting several hours, that was prevented by intrathecal injection of antisense to gp130, an IL-6 receptor subunit. Furthermore, following complete recovery from intramuscular IL-6-induced hyperalgesia, intramuscular PGE_2 produced a mechanical hyperalgesia that was remarkably prolonged compared to naïve controls, indicating the presence of chronic latent hyperalgesia. This ability of IL-6 to produce chronic latent hyperalgesia was prevented by intrathecal admininstration of antisense for gp130. Furthermore, gp130 antisense also prevented chronic latent hyperalgesia produced by intramuscular injection of the inflammogen, carrageenan. These results identify a role for IL-6 in acute inflammatory muscle pain and as a potential target against which therapies might be directed to treat chronic muscle pain.

Keywords

muscle pain; chronic pain; PGE2; inflammation; gastrocnemius

Introduction

Repetitive strain or trauma produces muscle inflammation that may lead to pain (Yassi, 1997, Melhorn, 1998, Latko et al., 1999, Stauber, 2004), which may re-emerge months or even years after the initial injury (Tjepkema, 2003). We have previously shown that intradermal administration of carrageenan produces cutaneous hyperalgesia, and following complete recovery of nociceptive threshold, hyperalgesia produced by subsequent administration of inflammatory mediators is significantly longer than that produced in naïve controls (Aley et al., 2000, Dina et al., 2003, Parada et al., 2003b). While we have shown that chronic latent hyperalgesia can be induced in the skin, chronic pain in muscle and other deep somatic tissue is clinically more common and refractory to existing therapies (Mense, 2003). Since chronic muscle pain is believed to be dependent, at least in part, on muscle inflammation (Barr and

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Barbe, 2002, Stauber, 2004, Sluka et al., 2007), we have recently developed a model of chronic latent muscle hyperalgesia wherein intramuscular administration of carrageenan produces hyperalgesia (lasting ~3 days), and following complete recovery of nociceptive threshold hyperalgesia produced by intramuscular injections of the inflammatory mediator prostaglandin E_2 (PGE_2) induced mechanical hyperalgesia that was markedly prolonged compared to control animals (Dina, O. et al., *submitted*). Since the pronociceptive cytokine interleukin (IL) 6 is produced in muscle exposed to carrageenan (Loram et al., 2007), we investigated whether chronic latent muscle hyperalgesia develops following transient inflammation induced by IL-6.

Experimental procedures

Animals

Adult male Sprague-Dawley rats (250–275 g at the start of the experiment) were housed in the Animal Care Facility at UCSF, under environmentally controlled conditions (7 am to 7 pm light cycles; 70–74°F) with food and water available *ad libitum*. Care and use of rats conformed to National Institutes of Health guidelines, and was approved by the UCSF Institutional Animal Care and Use Committee.

Measurement of hyperalgesia

Mechanical nociceptive thresholds were quantified using a digital force transducer (Chatillon, Model DFI2, Amtek Inc., Largo, FL). Rats were lightly restrained in a vented tubular Plexiglas holder with openings that allows easy access to the hind limb. A 6 mm diameter probe attached to the transducer was applied to the gastrocnemius muscle to deliver an increasing compression force, and the nociceptive threshold was defined as the force, in Newtons, at which the rat withdrew its hind leg. Baseline withdrawal threshold was defined as the mean of 2 readings taken at 5-min intervals. Each hind limb is treated as an independent measure and each experimental manipulation was performed on a separate group of rats. All behavioral testing was done between 10 am and 4 pm.

Intramuscular injection of agents

Rats were anesthetized with 3% isoflurane to facilitate the admininstration of IL-6 or carrageenan (in a volume of 10 μl) into the belly of the gastrocnemius muscle; injection sites were marked on the skin overlying the injection site using an indelible pen so that the same site in the muscle can be tested for mechanical nociceptive threshold. After measuring baseline mechanical nociceptive threshold, rats were briefly anesthetized with isoflurane and received a unilateral injection of IL-6 (10 ng) into the belly of the gastrocnemius muscle. Mechanical nociceptive thresholds were re-evaluated 30 min and 3 h after injection.

Chronic latent hyperalgesia (hyperalgesic priming)

Chronic latent hyperalgesia was produced in the gastrocnemius muscle using a procedure similar to one we have used to produce priming in cutaneous afferents (Aley et al., 2000). To prime muscle nociceptors, IL-6 (10 ng) or carrageenan (100 ng) was applied by deep intramuscular injection in the belly of the gastrocnemius muscle. The dose of Il-6 was determined in pilot studies as sufficient to produce robust mechanical hyperalgesia.

Antisense oligodeoxynucleotide administration

The method for intrathecal oligodeoxynucleotide (ODN) injection has been described previously (Papir-Kricheli et al., 1987, Khasar et al., 1996, Aley and Levine, 1997, Khasar et al., 1998, Alessandri-Haber et al., 2003, Parada et al., 2003a, Parada et al., 2003b, Alessandri-Haber et al., 2004, Dina et al., 2004). Administering gp130 antisense intrathecally selectively targets neurons, in particular primary afferent nociceptors. Intrathecally administered

oligodeoxynucleotides are taken up by DRG neurons {Lai, 2002, 11790477}, where they reduce expression of gp130 (Summer et al, *Pain* 2008, in press). For each ODN injection rats were briefly anesthetized with 3% isoflurane, and a 30-gauge needle inserted into the subarachnoid space on the midline, between the L4 and L5 vertebrae. ODN (80 μg in 10 μl) was slowly injected. This procedure was repeated so that ODN was administered on 3 consecutive days prior to experiments. Control animals received injections of mismatch ODN.

Antisense oligodeoxynucleotide (ODN) preparation

To delineate a contribution of IL-6, IL-6 signaling in sensory neurons was disrupted by intrathecal administration of antisense ODN to the signal transducing molecule, glycoprotein 130 (gp130), a subunit of the IL-6 receptor signaling complex necessary for its function. The antisense ODN sequence, 5′-TCC TTC CCA CCT TCT TCT G-3′, was directed against a unique sequence of rat gp130. The corresponding GenBank accession number and ODN position within the cDNA sequence are M92340 and 1834–1852, respectively (Wang et al., 1992). The mismatch ODN sequence, 5′-T**A**C T**A**C **T**CA C**A**T TC**A** TC**A** G-3′, corresponds to the gp130 subunit antisense sequence with 6 bases mismatched (denoted by bold letters). The 19-mer antisense- and mismatch ODN for gp130 were purchased from Invitrogen (San Francisco, CA). The dose of ODN, 80 μg, was based on prior dose–response curve studies (Summer et al., 2006).

Statistical analyses

Group data are expressed as mean ± SEM of n distinct observations. Statistical comparisons were made by a two-tailed Student's t test (for one or two independent populations) and by one-way ANOVA for comparing multiple treatments, using StatView statistical software. $P<0.05$ was considered statistically significant.

Results

Intramuscular IL-6 induces hyperalgesia

IL-6 administration produced robust mechanical hyperalgesia at these time points (n=4, filled symbols, Fig. 1), while threshold was unchanged in rats in which saline was injected into the gastrocnemius muscle (n=4, open symbols, Fig. 1); nociceptive thresholds had returned to baseline levels when evaluated 120 h (5 days) after IL-6 injection.

Intrathecal injection of antisense ODNs for gp130, an IL-6 receptor subunit (n=6), but not mismatch ODN (n=6) (80 μg/20 μl, once daily for 3 days), significantly reduced the magnitude of acute IL-6–induced hyperalgesia (Fig. 2). We have previously shown that this protocol for ODN administration decreases expression of gp130 by 64% on Western analysis (compared to β-actin control) (Summer et al., 2007).

IL-6 induces chronic latent hyperalgesia

Complete recovery from acute IL-6 hyperalgesia occurred by day 5 (see Figure 1). To determine the ability of a single exposure to IL-6 to produce chronic latent muscle hyperalgesia, we assessed the response to a new inflammatory challenge 10 days after injection of IL-6. After verifying the return to baseline withdrawal threshold (time 0) the inflammatory mediator PGE_2 (1 μg) was injected into the muscle previously exposed to IL-6. In saline pretreated control animals (open squares or open circles, both n=4), intramuscular PGE_2 induced a short-lived hyperalgesia that completely resolved within 4 h. In contrast, in the IL-6–pretreated muscle (filled circle, n=4) the duration of PGE_2-induced hyperalgesia was greatly enhanced at both 4 and 24 h post PGE_2 administration (2-way ANOVA, $P<0.001$, Figure 3).

IL-6 receptor antisense inhibits carrageenan and IL-6-induced chronic latent hyperalgesia

We have previously observed that administration of carrageenan into the rat gastrocnemius muscle produces chronic latent muscle hyperalgesia observed when a subsequent injection of PGE_2 produces a long-lasting hyperalgesic response (Dina et al., *submitted*). Since muscle inflammation is associated with increase in IL-6 in the muscle (Kim et al., 2007, Loram et al., 2007, Ono et al., 2007), to evaluate the contribution of IL-6 to carrageenan-induced chronic latent muscle hyperalgesia, we injected antisense (n=6) or mismatch (n=6) ODNs against gp130 (Figure 4). Antisense or mismatch ODNs (80 μg/20 μl) were administered intrathecally once daily for 3 days prior to intramuscular carrageenan (100 μg) and then daily for 5 days. PGE_2 hyperalgesia was evaluated 6 days after the last gp130 ODN administration (i.e. 11 days after carrageenan) when nociceptive threshold had returned to baseline. While neither antisense nor mismatch ODN treatments affected the magnitude of acute PGE_2 hyperalgesia (mismatch vs. antisense $P>0.05$, t-test 30 min post-PGE_2 time point), antisense ODN to gp130 (filled circles, n=6) treatment completely prevented the development of chronic latent hyperalgesia (mismatch vs. antisense $P<0.0001$, repeated measures ANOVA, Fig. 4).

Discussion

We have previously shown that intradermal administration of carrageenan produces cutaneous hyperalgesia, and following complete recovery of nociceptive threshold, a state of chronic latent hyperalgesia wherein subsequent administration of an inflammatory mediator produces a hyperalgesic response that is markedly longer than that produced in naïve controls (Aley et al., 2000, Dina et al., 2003, Parada et al., 2003b). Since the potent hyperalgesic inflammatory mediator, IL-6 is produced in muscle following injection of carrageenan (Loram et al., 2007) and chronic pain in muscle and other deep somatic tissue is clinically more common, we evaluated the role of IL-6 in inflammatory mediator-induced hyperalgesic priming in muscle. The current study has demonstrated that intramuscular injection of IL-6 also produces an acute mechanical hyperalgesia in the gastrocnemius muscle that resolves by 120 h (5 days). This IL-6-induced muscle hyperalgesia is blocked by antisense ODN treatment to ‘knock down’ the IL-6 receptor (gp130) in primary afferent nociceptors. In addition, prior administration of IL-6 induces chronic latent mechanical hyperalgesia that is revealed after return to baseline nociceptive thresholds as a markedly prolonged PGE_2 hyperalgesia that was still unattenuated after 14 days, compared with <4 h in the cytokine-naïve rat. This IL-6-induced chronic latent mechanical appears to be mediated via its receptor on the primary afferent nociceptor.

Of note, while we measured hyperalgesia by mechanically stimulating the muscle through skin, it is very unlikely that nociceptive changes involved significant cutaneous component, since we have previously shown that while intradermal injection of prostaglandin E_2 or bradykinin sensitizes cutaneous nociceptors, subcutaneous injection of these inflammatory hyperalgesic agents were ineffective {Khasar, 1993, 8100992}. Furthermore, it has previously been reported that intraplantar carrageenan increased COX2 expression in skeletal muscle when given in 150 μl of 3% solution, but not when given in 50 μl of a 1% solution {Nantel, 1999, 10556918}, i.e. significant diffusion of carrageenan only occurs at a concentration and volume far greater than what we have used in our current study (10 μl of a 1 % solution), suggesting that it is extremely unlikely that cutaneous nociceptors would be affected following deep injections in the gastrocnemius muscle.

There are several lines of evidence implicating a role for IL-6 in chronic muscle pain. For example, in rats repetitive motion induces an increase in muscle IL-6 (as well as other cytokines) in a muscle pain model (Al-Shatti et al., 2005); while these authors did not explicitly evaluate nociception, there was a suggestion of repetitive motion-induced muscle pain in this model (Barr and Barbe, 2002, Barbe et al., 2003). Of note, administration of IL-6 sensitizes group IV muscle afferents to mechanical stimulation (Hoheisel et al., 2005). In humans, IL-6

is increased following whiplash injury in trapezius muscle in patients with muscle hyperalgesia (Gerdle et al., 2007), and in muscles following eccentric exercise (Rosendal et al., 2005) (Steensberg et al., 2000, Tomiya et al., 2004). Also, it has been recently shown that in rats administration of carrageenan into the masseter muscle increased IL-6 1 and 3 h post-carrageenan (Ono et al., 2007), and carrageenan administered into the gastrocnemius muscle produced a several-fold increase in IL-6 (and other cytokines) 6 and 24 h post-administration (Loram et al., 2007).

Most studies that have attempted to elucidate mechanisms of chronic muscle pain have focused on evaluating nociceptive mechanisms over short time periods (minutes or hours). However, nociceptor activation triggers a sequence of neuronal events that, over time, may eventually lead to chronic neuroplastic changes {Zieglgänsberger, 2005 #653}. The transition from acute to chronic pain is likely to involve several systems including changes in second messenger signaling in the periphery as well as centrally {Vadivelu, 2005, 16534290; Mense, 2004, 15138681}, but, to date, these mechanisms are poorly understood. In our current study, we show that IL-6 not only produced acute hyperalgesia, but also produced a long-lasting 'priming' effect (chronic latent hyperalgesia (Aley et al., 2000, Dina et al., 2003, Parada et al., 2003b)), a state in which the duration of PGE_2 hyperalgesia was markedly prolonged, being unattenuated even 14 days after injection of PGE_2. This chronic latent pain condition is dependent on IL-6 receptors on the primary afferent nociceptor. Clinically, one of the most important aspects of inflammatory pain is the development of chronic pain following recovery from acute inflammation, seen in a number of painful disorders, in particular those associated with musculoskeletal pain, e.g. repetitive strain injuries (Melhorn, 1998). We have hypothesized that this process involves cellular mechanisms different from those of acute inflammatory pain. Of note, the chronic latent hyperalgesia induced in muscle is markedly more prolonged than what we observed for cutaneous inflammatory hyperalgesia {Joseph, 2003, 14499430}, suggesting that this mechanism for the transition from acute to chronic mechanical hyperalgesia plays an even more important role in muscle than in skin.

In conclusion, we describe a novel experimental model for chronic muscle mechanical hyperalgesia induced by intramuscular IL-6. This model may have clinical significance since it tracks the transition from acute to chronic peripheral muscle hyperalgesia, and has the potential to reveal cellular processes by which acute inflammation can create a state of enhanced susceptibility to inflammatory mediators or subsequent mechanical stimulation. These findings have begun to clarify mechanisms underlying chronic muscle pain and to provide information for future strategies for the prevention and treatment of chronic musculoskeletal pain.

Abbreviations

IL-6
: interleukin-6

PGE_2
: prostaglandin E_2

gp130
: glycoprotein 130

ODN
: oligodeoxynucleotide

ANOVA
: analysis of variance

N.S.

not significant

SEM

standard error of the mean

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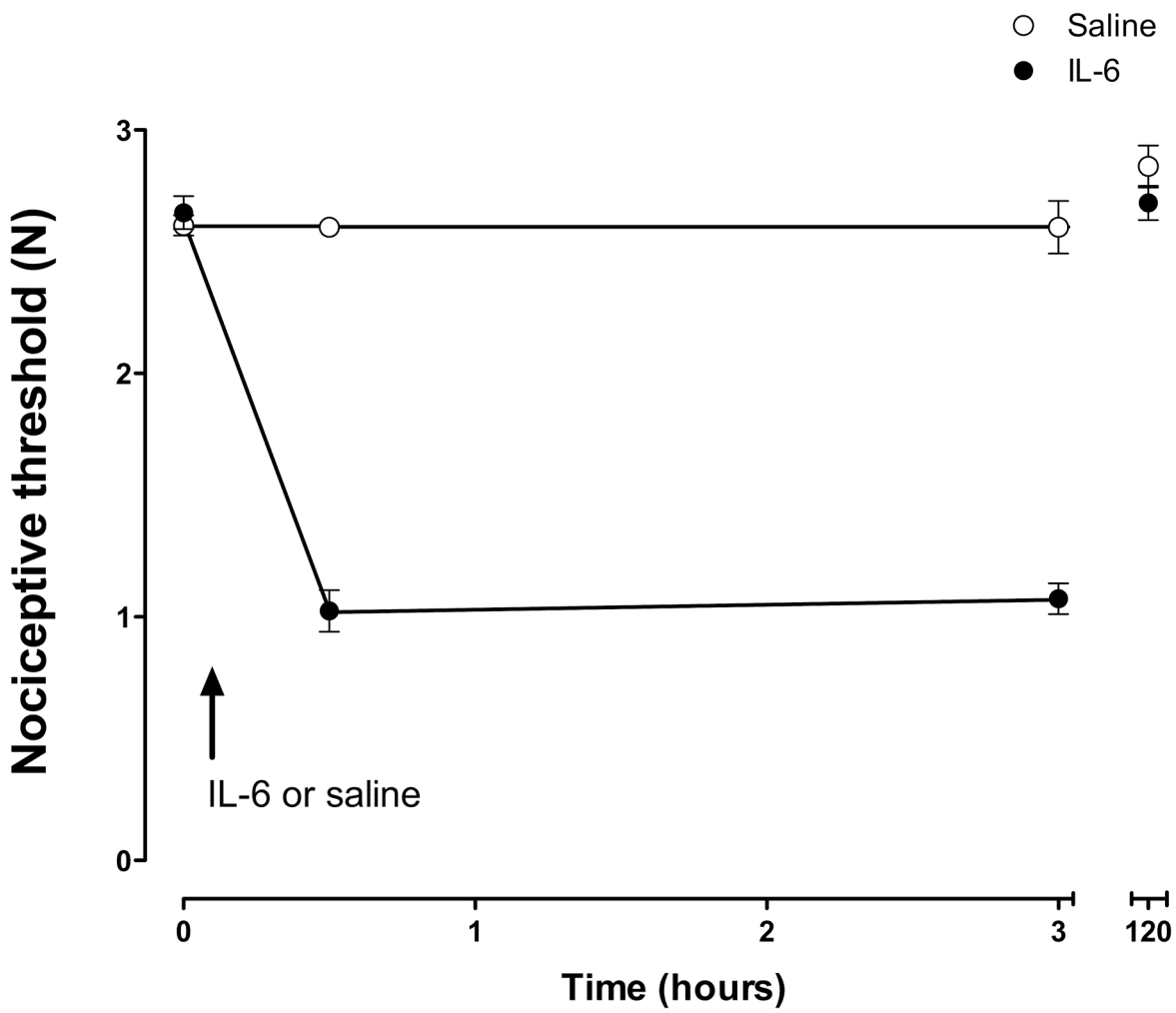

Figure 1. IL-6 induces mechanical hyperalgesia in gastrocnemius muscle

IL-6 (10 ng in 10 μl, filled circles), injected into the belly of the gastrocnemius muscle, decreased nociceptive threshold by ~60% within 30 min, an effect that was maintained at approximately the same level for at least 3 h. Nociceptive thresholds had returned to baseline values by 120 h (5 days). Control animals injected with saline (0.9% NaCl, 10 μl, open circles) exhibited no significant change in threshold.

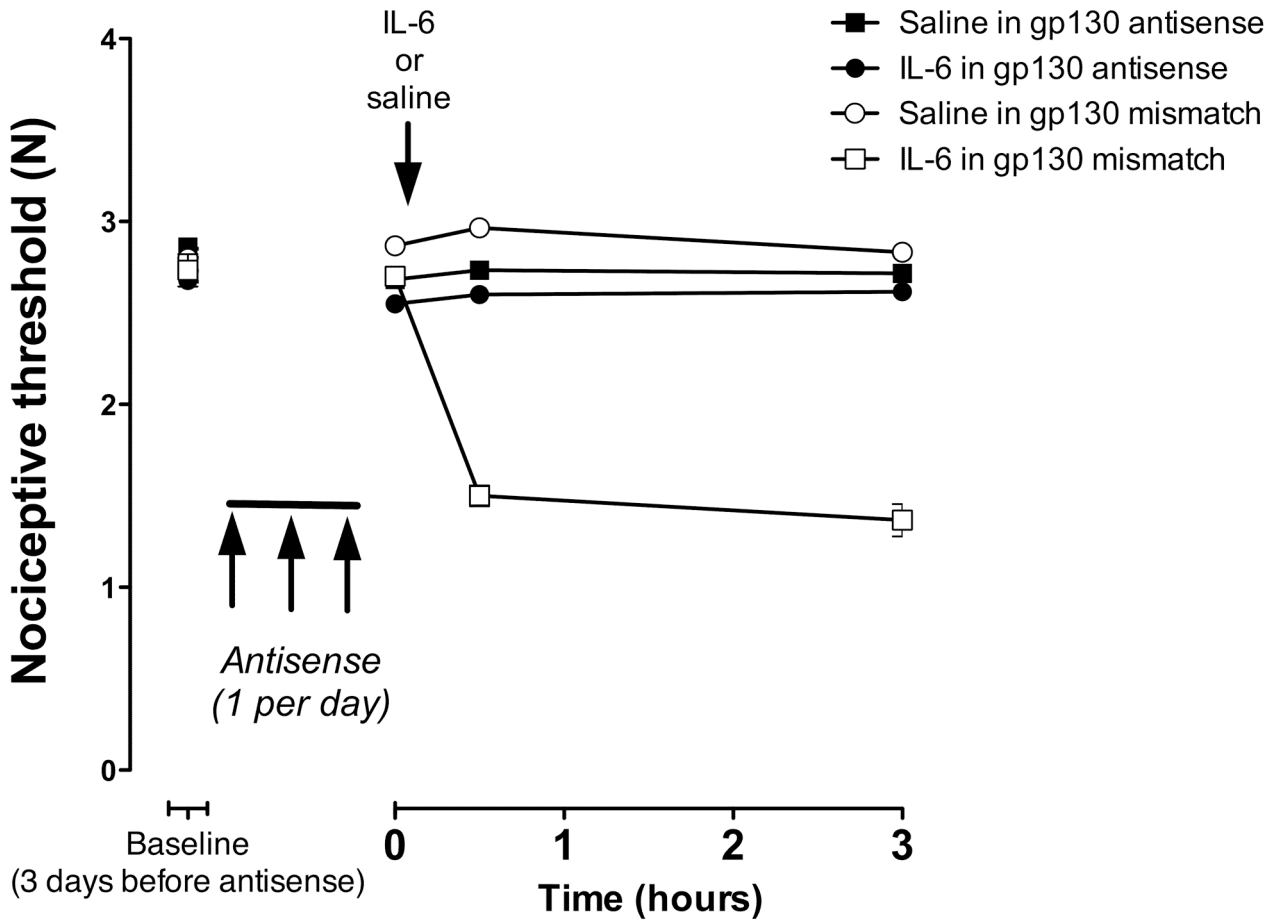

Figure 2. Admininstration of IL-6 receptor antisense prevents IL-6 hyperalgesia

Intrathecal administration of antisense ODN directed against the IL-6 receptor subunit gp130 daily for 3 days completely prevented hyperalgesia induced by subsequent intramuscular IL-6 (filled circle). Administration of IL-6 in rats receiving mismatch ODN did not affect the magnitude of IL-6-induced muscle hyperalgesia (open squares) compared to IL-6 in naïve rats (*cf.* Figure 1, filled circles). Administration of IL-6 antisense (closed squares) or mismatch (open squares) did not affect baseline nociceptive thresholds. Of note, some of the error bars are very small and so are hidden within the symbol.

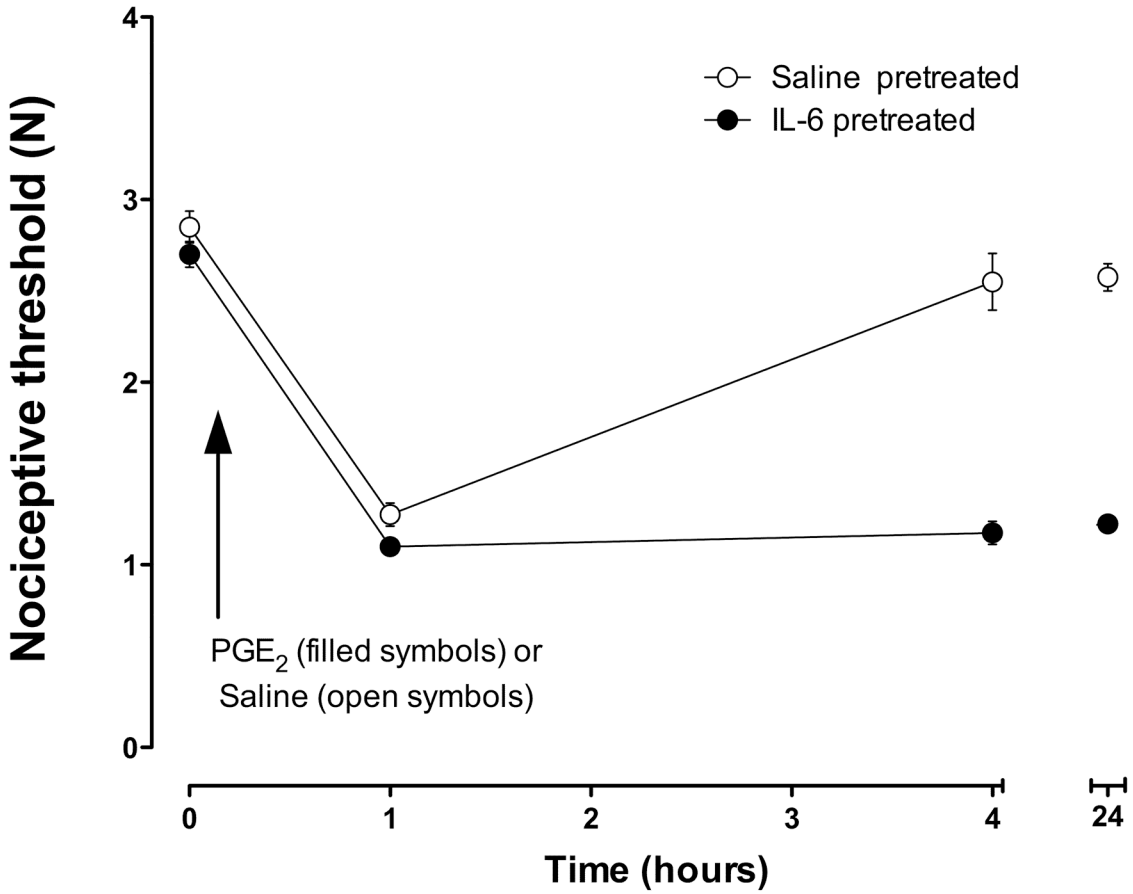

Figure 3. IL-6 induces a chronic latent hyperalgesic priming in muscle

Ten days after IL-6 administration, following complete recovery from the hyperalgesia (leg withdrawal threshold returned to baseline after 5 days), PGE_2 (1 μg) was injected into the muscle. In saline pretreated rats (open symbols), PGE_2-induced hyperalgesia had completely resolved within 4 h, but in the cytokine-pretreated muscles (filled symbols), hyperalgesia was greatly prolonged, being undiminished 24 h after PGE_2 admininstration.

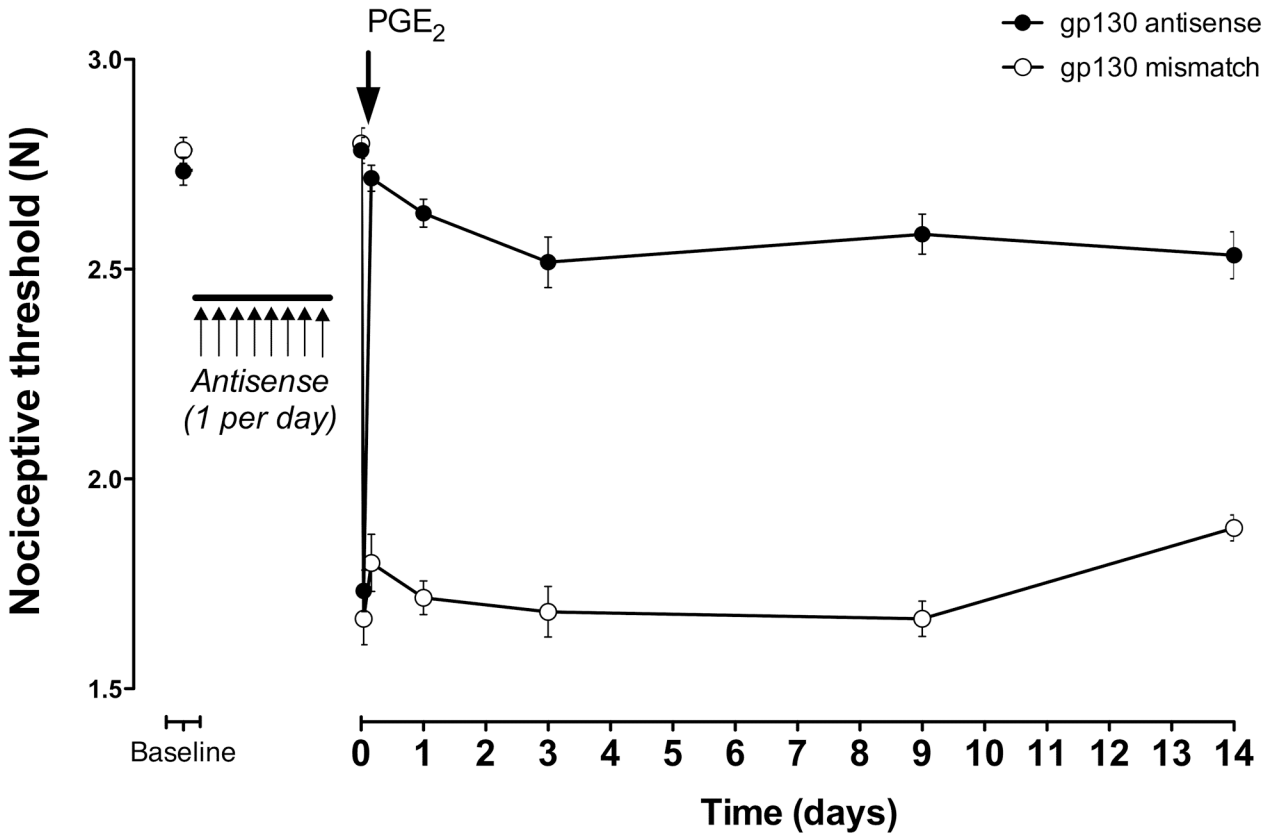

Figure 4. IL-6 receptor antisense inhibits chronic latent hyperalgesia induced by carrageenan

Intrathecal injections of ODN antisense or mismatch for the IL-6 receptor subunit gp130 were given once daily for 3 days prior to carrageenan and daily thereafter for an additional 5 days. Intramuscular PGE_2 injected 6 days after the final intrathecal ODN administration, produced hyperalgesia that persisted for more than 14 days in the mismatch ODN treated rats (open symbols) compared to < 4 h in the antisense ODN treated rats (closed symbols).