

Interleukin-2-induced antinociception in morphine-insensitive rats

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ABSTRACT

AIM: To investigate interleukin-2-induced antinociception in morphine-insensitive rats. **METHODS:** Paw withdrawal latencies (PWL) to noxious radiant heat were taken as the measurement of pain threshold. **RESULTS:** Intraplantar injection of human recombinant interleukin-2 (1.5×10^4 U) significantly increased PWL in normal rats. PWL was also markedly increased by IL-2 in 45-d-post-complete Freund's adjuvant (CFA)-treated rats, which have been proven morphine-insensitive. IL-2-induced antinociception in CFA-treated rats were significantly lower than that in normal rats. IL-2-induced antinociception was partially blocked by naloxone (1 mg/kg, ip) in normal rats but remained unchanged in CFA group. **CONCLUSION:** IL-2-induced antinociception is partially mediated by μ -opioid receptors. Therapeutic applications of IL-2 may also be expanded to relieve morphine-insensitive pain.

INTRODUCTION

Interleukin-2 (IL-2), an intensely studied cytokine known as T-cell growth factor^[1], is well documented to possess diverse biological properties beyond its relation to T-cells. Rapidly accumulating evidence strongly supports the notion that IL-2 is an important modulator in the central nervous system (CNS)^[2]. Our previous studies showed that IL-2 inhibited nociceptive responses of spinal dorsal horn neurons^[3]. IL-2 also exerts notable analgesic effect in the peripheral nervous system^[4]. Previous studies have shown that IL-2 and morphine exerted similar effects in various aspects by decreasing intracellular cyclic AMP (cAMP) content, modulating neuroendocrine activity, suppressing afferent sensory transmission and serving as Ca^{2+} channel

blockers^[2,5,6]. All the evidence indicates the interplay between IL-2 and μ -opioid receptors. Previous studies have shown that 45-d-post-complete Freund's adjuvant (CFA)-treated rats are insensitive to morphine antinociception^[8,9]. In the present study, the contribution of μ -opioid receptor to IL-2-induced antinociception was evaluated in this animal model.

MATERIALS AND METHODS

Animals and groups Male Sprague-Dawley rats (260-280 g) were supplied by Shanghai Experimental Animal Center, Chinese Academy of Sciences (Grade II, Certificate No 005) and were housed in single plastic cages at a room temperature of 22 °C, available to food and water *ad libitum* with a 12-h light-dark cycle. All experiments were conducted in accordance with European Community Guidelines on Animal Care and Experimentation. Rats were randomly grouped as follows:

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Saline group: to test the combined effect of saline in normal and CFA-treated rats 15 min after injection.

Normal group: to test the effect of morphine (2 mg/kg, ip) in normal rats 15 min after injection as shown in Tab 1 and to test the effect of IL-2 (1.5×10^4 U), IL-2/Naloxone (1 mg/kg) or the vehicle of IL-2 in normal rats 10 min after injection as shown in Tab 2.

CFA group: to test the effect of morphine (2 mg/kg, ip) in CFA-treated paws 15 min after injection as shown in Tab 1 and to test the effect of IL-2 (1.5×10^4 U), IL-2/Naloxone (1 mg/kg) or the vehicle of IL-2 in CFA-treated paws 10 min after injection as shown in Tab 2.

Behavioral testing To test the antinociceptive effect of drugs, paw withdrawal reflex was induced by radiant heat as expressed in our previous studies^[4]. Briefly, from a projection bulb placed directly under the hindpaw, radiant heat beam was focused in a diameter of 1 mm and the beam center was directed at the injection point. A digital timer automatically read the duration between the start of heat stimulation to paw withdrawal. Four trials at 4-min intervals were conducted with the mean of the last three paw withdrawal latencies (PWL) taken as the measurement. This test is sensitive to detect changes in response to noxious heat^[7].

Drugs Under light methoxyflurane anesthesia, rats underwent an injection of 200 μ L CFA (Sigma, St Louis, MO) in the dorsal aspect of the left hindpaw, which was suspended in an oil/saline (1:1) emulsion. The CFA injection produced an intense inflammation, associated with thermal hyperalgesia and insensitivity to morphine^[8,9]. Human recombinant IL-2 was administered 45 d after CFA injection.

IL-2 (Shanghai Institute of Biochemistry, Chinese Academy of Sciences) was dissolved in phosphate buffer (PB) (10 mmol/L, pH 7.0). Before intraplantar injection of IL-2 or the vehicle, animals were lightly anesthetized by inspiring ethyl ether to avoid struggling and to minimize artificial responses. Site of injection was marked at the center of the plantar surface by a 4 mm-diameter circle, where radiant heat beam was focused in behavioral tests. All the intraplantar injections (sc) were given in a volume of 20 μ L and the PWL of the injected hindpaw were measured 10 min after the injection. Intensive pilot studies have been practiced by the authors to control the extent of the anesthesia. In the experiments, rats recovered soon after ethyl ether treatment and the anesthesia was light enough so as not

to alter the PWL 5 min after injection. Similar operation has been used in our published studies^[4].

Morphine was administrated at a challenge dose of (2 mg/kg, ip). Naloxone (1 mg/kg, Sigma, St Louis, MO) was given (ip) 30 min before intraplantar injection of IL-2.

Statistical analysis Changes in PWL were expressed by percentage of maximal possible effect (% MPE) according to the following formula: % MPE = (Post-drug latency - Pre-drug latency) \times 100 / (10 - Pre-drug latency). Data were then subjected to statistical evaluation using Student's *t* test followed by *post-hoc* comparison (Scheffé's *F* procedure) to confirm significant differences between groups. Criteria for significance in all analyses were defined as $P < 0.05$. Data were presented as mean \pm SD.

RESULTS

Decreased antinociceptive effect of morphine

Alterations of morphine effectiveness were investigated by measuring PWL as the pain threshold in 45-d-post-CFA ($n = 9$) rats. A single injection of morphine (2 mg/kg, ip) notably prolonged PWL in normal animals 15 min after injection ($P < 0.01$ vs saline). In consistency with previous studies^[8,9], CFA-treated paws displayed insensitivity to morphine at the same dose ($P > 0.05$ vs saline) (Tab 1).

Tab 1. Antinociceptive effect of morphine (2 mg/kg) as measured by paw withdrawal latencies (PWL). CFA, the effect of morphine (2 mg/kg) 45 d after complete Freund's adjuvant injection; Normal, the effect of morphine (2 mg/kg) in normal rats; Saline, the combined effect of saline in CFA-treated and normal rats. Changes in PWL were expressed by percentage of maximal possible effect (%MPE). Mean \pm SD. * $P < 0.01$ vs saline injection.

Group	<i>n</i>	%MPE
Saline	9	-14 \pm 4
Normal	13	55 \pm 4 ^c
CFA	9	8 \pm 24

Antinociceptive effect of IL-2 Changes in PWL 10 min after intraplantar injections at the ipsilateral paw were then measured (Tab 2). IL-2 (1.5×10^4 U) significantly increased PWL versus its vehicle in both normal ($P < 0.01$, $n = 30$) and CFA-treated ($P < 0.01$, $n = 9$) paws.

This effect of IL-2 disappeared in 25 min after injection. However, IL-2-induced antinociception in CFA group was markedly decreased than that seen in normal rats ($P < 0.05$). Naloxone (1 mg/kg) was given (ip) 30 min before intraplantar injection of IL-2. Naloxone alone produced no detectable changes in PWL but it partially blocked IL-2-induced antinociception in normal rats ($P < 0.05$ vs vehicle and $P < 0.01$ vs IL-2 without naloxone, $n = 6$). Naloxone produced no detectable effect on IL-2-induced antinociception in CFA group ($P < 0.01$ vs vehicle and $P > 0.1$ vs IL-2 injection without naloxone in CFA group, $n = 6$). Intraplantar injections of IL-2 or vehicle in the contralateral paw produced no significant changes in PWL.

Tab 2. Antinociceptive effect of human recombinant interleukin-2 (IL-2) (1.5×10^4 U) as measured by paw withdrawal latencies (PWL). Nlx, preadministration of naloxone (1 mg/kg, ip); Vehicle, the combined effect of vehicle injection in normal and 45-d-post-CFA group. ^a $P < 0.05$ vs IL-2 injection in normal rats. ^b $P < 0.01$ vs IL-2 injection without naloxone in the same group. Effect of vehicle differs significantly from all the others shown in this tablet ($P < 0.05$) so the marks are not given here for tidiness. Changes in PWL were expressed by percentage of maximal possible effect (%MPE). Mean \pm SD.

Group	IL-2 (% MPE)	IL-2/Nlx (% MPE)	Vehicle (% MPE)
Normal	79 \pm 10 ($n = 30$)	19 \pm 10 ^f ($n = 6$)	-9 \pm 2 ($n = 30$)
CFA	39 \pm 8 ^b ($n = 9$)	25 \pm 12 ($n = 6$)	-9 \pm 2 ($n = 9$)

DISCUSSION

CFA is commonly used to induce long-lasting inflammation. At the early stage such as 24 h-post CFA injection, μ -opioid receptor agonists are still effective in attenuating CFA-induced hyperalgesia^[10]. In the long term, however, the presence of chronic nociceptive stimulation produced by CFA significantly reduces the potency of morphine and facilitates the development of morphine tolerance^[8]. In consistency with previous studies^[9], intensive insensitivity to morphine analgesia was observed in our experiments on the 45th d after CFA treatment.

The present study showed that IL-2 induced similar antinociception to that of morphine in normal rats. Naloxone as a μ -opioid receptor antagonist has been

found to interfere with some functions of IL-2^[11]. In the present study, naloxone markedly decreased IL-2-induced antinociception, suggesting the involvement of μ -opioid receptor in the process.

It is noteworthy that naloxone did not completely block IL-2-induced antinociception. IL-2-induced antinociception was still remarkable when morphine/ μ -opioid receptor system had been invalidated by CFA treatment. Therefore, it is suggested that some molecules in addition to μ -opioid receptors might be responsible for IL-2-induced antinociception. The specific receptor of IL-2 (IL-2R) is a potential candidate, which is intensely localized in brain regions that are closely related to nociceptive process^[12]. To support this notion, our preliminary study showed that IL-2 exerted strong antinociceptive effect via IL-2R on peripheral afferent terminals^[3]. Particularly, we have also found that IL-2R was constitutively expressed in small and medium-sized dorsal root ganglion neurons, which are predominantly responsible for nociceptive transmission^[4]. These observations suggest that IL-2/IL-2R system may also participate in nociceptive processes.

Lines of evidence support a role of the immune system in CFA-induced inflammation^[13,14]. It is conceivable that IL-2, as a bi-directional communicator between the immune and nervous system^[15], can be used to reprogram the immune system to generate protective activities by modulating functions of other proinflammatory cytokines when the animal is confronted with injuries.

Similar aromatic residues for binding to opioid receptors have been found in the N-terminal of IL-2 and morphine^[16]. We inferred that IL-2 might directly interact with μ -opioid receptor, followed by opioid-like cellular signal transduction cascade to exert antinociceptive effect. In addition, IL-2 can also bind to other receptors such as IL-2R, triggering complex interaction between the immune and nervous system to influence nociceptive processing^[15].

Another implication of our findings is that IL-2 may be a potential drug for pain relief when morphine is ineffective as seen in the present case, which would be of interest for clinical practice.

In conclusion, the present data suggest that IL-2-induced antinociception is partially coupled with μ -opioid receptors.

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