Nociceptive Effect of Subcutaneously Injected Interleukin-12 Is Mediated by Endothelin (ET) Acting on ET$_B$ Receptors in Rats


Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, São Paulo, Brazil (W.A.V., R.O.M., I.R.S.S., T.M.C., C.A.P., S.H.F., F.Q.C.); and National Institute of Biological Standards and Control, South Mimms, Hertfordshire, United Kingdom (S.P.)

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ABSTRACT

Interleukin-12 (IL-12) is an inflammatory Th1-driving cytokine that has been clinically used as immune therapy and vaccine adjuvant. Recently, it was reported that patients receiving IL-12 presented hyperalgesia. In the present study, we investigated the mechanical hyperalgesic effect of IL-12 in rats using two tests: 1) paw constant pressure and 2) electronic pressure meter. In both tests, intraplantar administration of IL-12 (3–30 ng paw$^{-1}$) caused a dose- and time-dependent mechanical hyperalgesia, which peaked between 3 to 5 h, remaining significantly different from control levels until 7 h and resolved 24 h postinjection. However, the same doses of IL-12 did not induce thermal hyperalgesia, determined using the Hargreaves test. Pretreatments with effective doses of indomethacin (2.5 mg kg$^{-1}$), atenolol (1 mg kg$^{-1}$), 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-t-butylthiodiolindol-2-yl]-2,2-dimethylopropanoic acid, sodium (MK886) (5-lipoxygenase activating protein inhibitor; 1 mg kg$^{-1}$), or cyclo[9-Trp-$\gamma$-Asp-Pro-$\gamma$Val-Leu] (BQ123) (endothelin (ET)$_B$ receptor antagonist; 30 nmol paw$^{-1}$) did not inhibit IL-12-evoked mechanical hyperalgesia (10 ng paw$^{-1}$). However, dexamethasone (2 mg kg$^{-1}$), morphine (3–12 μg paw$^{-1}$), and N-cys-2,6 dimethylpiperidinocarboynd-$\gamma$-methylleucylo-$\alpha$-1-methoxyarylcarboyo-$\gamma$-norleucine (BQ788) (ET$_B$ receptor antagonist; 3–30 nmol paw$^{-1}$) did inhibit IL-12 hyperalgesia. Furthermore, neither pretreatment with effective doses of antiserum against rat-TNF-$\alpha$ (50 μl paw$^{-1}$) nor against IL-18 (10 μg paw$^{-1}$) inhibited the IL-12-induced hyperalgesia. Likewise, antiserum against IL-12 (10 ng paw$^{-1}$) did not alter IL-18-induced hyperalgesia. In conclusion, we demonstrated for the first time that IL-12 is a prohyperalgesic cytokine that induces mechanical hyperalgesia mediated by endothelin action on the ET$_B$ receptor. Therefore, endothelin receptor antagonism could be beneficial in controlling IL-12 therapy-induced pain or hyperalgesia.

Interleukin-12 (IL-12) is the prototypic member of a heterodimeric family of cytokines that includes IL-23 and IL-27 (for review, see Brombacher et al., 2003). It is produced by a variety of cells including monocytes, neutrophils, B lymphocytes, macrophages, and dendritic cells, stimulated by pathogenic organisms such as bacteria, parasites, viruses, and fungi. IL-12 regulates both innate and adaptive immunity, being a key cytokine that regulates Th1 differentiation (for review, see Brombacher et al., 2003; Watford et al., 2004). Besides its importance in host-protective responses to most intracellular infectious microorganisms, the Th1 response is required for the development of autoimmune diseases including arthritis, myocarditis, encephalomyelitis, diabetes, and lupus (Joosten et al., 1997; for review, see Brombacher et al., 2003; Watford et al., 2004).

Recent findings consistently demonstrated that IL-12 induces pain in humans. For instance, 1) patients that received intravenous rhIL-12 therapy for metastatic renal cancer or malignant melanoma presented arthralgias involving primarily the shoulders and fingers (Gollob et al., 2000); 2) patients that received intraperitoneally injected rhIL-12 for Mullerian carcinoma, gastrointestinal primary malignant...
cies, and mesothelioma treatment had headache and abdominal pain (Lenzi et al., 2002); 3) pain and bladder spasms were adverse effects related to the intravesical treatment with rhIL-12 for cell carcinoma of the bladder (Weiss et al., 2003); and 4) mild to moderate pain at the site of injection has been reported in patients that received peritumoral injection of IL-12-transduced autologous fibroblasts (Kang et al., 2001). However, the mechanisms underlying IL-12-induced pain have not yet been investigated.

At the present time, it is accepted in the literature that a cascade of cytokines constitutes a link between inflammatory stimuli and release of the final mediators that directly sensitizes the nociceptors, such as prostanoids and sympathetic amines (Cunha et al., 1992). In rats, inflammatory stimuli induce resident cells to release TNF-α, which in turn activates two pathways: 1) TNF-α → IL-6 → IL-1β → prostaglandins (Cunha et al., 1992), and 2) TNF-α → cytokine-induced neutrophil chemoattractant 1 (rat IL-8 related chemokine; Lorenzetti et al., 2002) → sympathetic amines (Nakamura and Ferreira, 1987; Cunha et al., 1991). The sequential role of cytokines leading to the release of final mediators was further substantiated in rats by Safiie-Garabedian et al. (1997) and, more recently, in mice by Cunha et al. (2005). Furthermore, cytokines may also stimulate the release of other directly acting mediators, such as endothelin (ET) (Verri et al., 2004).

Thus, in the present study we investigated whether the local injection of IL-12 induced mechanical hyperalgesia, as well as the involvement of other cytokines, prostanoid, sympathetic amines, leukotrienes, and endothelin in this process. We found that IL-12 induces mechanical hyperalgesia in a dose- and time-dependent manner, mediated by endothelin acting via the ETB receptor.

Materials and Methods

Animals. Male Wistar rats (180–220 g) were housed in temperature-controlled rooms (22–25°C) with access to water and food ad libitum. All experiments were conducted in accordance with National Institutes of Health guidelines for the welfare of experimental animals and with the approval of the Ethics Committee of the Faculty of Medicine of Ribeirão Preto (University of São Paulo, São Paulo, Brazil). The animals were used only in a single experimental group.

Mechanical Hyperalgesic Tests. Hyperalgesia was assessed using two different methods: the constant pressure rat paw and the electronic pressure-meter tests. A different investigator performed each test, prepared the solution, and injected subcutaneously in the hindpaw of rats. Multiple paw treatments with saline did not alter basal reaction time, which was similar to that observed in noninjected paws.

The Constant Pressure Rat Paw Test. Mechanical hyperalgesia was tested in rats as previously described (Ferreira et al., 1978). In this method, a constant pressure of 20 mm Hg (measured using a sphygmomanometer) is applied (via a syringe piston moved by compressed air) to a 15-mm² area on the dorsal surface of the hindpaw and discontinued when the rat presents a typical “freezing reaction”. This reaction is comprised of brief apnea, concomitant with retraction of the head and forepaws, and reduction in the escape movements that animals normally make to free themselves from the position imposed by the experimental situation. Usually, the apnea is associated with successive waves of muscular tremor. For each animal, the latency to the onset of the freezing reaction is measured after administration (zero time) and at different times after administration of the hyperalgesic agents. The intensity of mechanical hyperalgesia is quantified as the reduction in the reaction time, calculated by subtracting the value of the second measurement from the first (Ferreira et al., 1978). Reaction time was 31.9 ± 0.2 s (mean ± S.E.M.; n = 36) before injection of the hyperalgesic agents.

A shortened reaction time is prevented by steroidal and nonsteroidal anti-inflammatory drug treatment before an inflammatory stimuli injection (Cunha et al., 1992; Lorenzetti et al., 2002). This method has been used to demonstrate the peripheral effect of morphine (Fadnoura et al., 1978; Smith et al., 1982), the contribution of eicosanoids, sympathetic amines, adenosine 3’5’ cyclic monophosphate (cAMP), and of cytokines to the development of peripheral inflammatory hyperalgesia (Ferreira and Nakamura, 1979a; Cunha et al., 1992, 2000; Ferreira et al., 1993). These concepts and findings have been extensively confirmed with other methodologies such as formalin-induced flinching and others (Vegar et al., 1976; Vivancos et al., 2004).

The Electronic Pressure-Meter Test. The paw hyperalgesia was also measured with an electronic pressure-meter. The rats were placed in acrylic cages (12 × 20 × 17 cm high) with a wire grid floor 15 to 30 min before beginning the tests. During this adaptation period, the paws were poked two to three times. Before paw stimulation, the animals should be quiet, without exploratory or toilet movements, and not resting over the paws. In these experiments a pressure-meter, which consisted of a hand-held force transducer adapted with a 0.7-mm² polypropylene tip (electronic von Frey anesthesiometer; IITC Inc. Life Science Instruments, Woodland Hills, CA), was used. The investigator was trained to apply the polypropylene tip perpendicularly in between the five distal footpads with a gradual increase in pressure. A tilted mirror below the grid provided a clear view of the animal’s hindpaw. The test consisted of poking the hindpaw to provoke a flexion reflex followed by a clear flinch response after the paw withdrawal. The electronic pressure-meter automatically recorded the intensity of stimulus when the paw was withdrawn. The stimulation of the paw was repeated until the animal presented three similar measurements (with the difference between the highest and the lowest measurement being no more than 10 g). If the results were inconsistent, the experimenter used another animal (~1:25 animals). The animals were tested before and after treatments, and the results are expressed by the delta reaction force (grams) that was calculated by subtracting the value of the measurements after treatment from that of the first measurement before treatment (Vivancos et al., 2004). The reaction force was 43.6 ± 0.3 g (mean ± S.E.M.; n = 36) before injection of the hyperalgesic agents.

Thermal Test: Hargreaves’ Plantar Test. The Hargreaves’ test was performed as previously described (Hargreaves et al., 1988), using a standard apparatus (Ugo Basile, Comerio, Italy). The test consists of placing the rat in a transparent acrylic box and applying a thermal radiant stimulus with a mobile infrared heat lamp positioned underneath the targeted hindpaw. The latency of the paw withdrawal response was measured automatically with the help of a photoelectric-sensitive device. The latency of the withdrawal response of each hindpaw was determined before and at 1, 3, and 5 h after IL-12 (3, 10, and 30 ng in 50 μl) or saline (50 μl) i.pl. injection. The intensity of thermal hyperalgesia was expressed as the reduction in the reaction time, calculated by subtracting the value of the post-treatment measurement from the pretreatment.

Protocols. The IL-12-induced mechanical or thermal hyperalgesia was assessed using the following protocols.

Dose- and Time-Dependent Mechanical or Thermal Hyperalgesia Induced by IL-12. To determine whether IL-12 induces mechanical and thermal hyperalgesia, the cytokine (3–30 ng in 50 μl) was injected i.pl., and the nociceptive responses were measured 1, 3, 5, 7, and 24 h later.

Role of Eicosanoids (Prostanoids and Leukotrienes), Sympathetic Mediators, and Morphine Treatment in IL-12-Induced Mechanical Hyperalgesia. The participation of nociceptive mediators in IL-12 (10 ng in 50 μl)-induced mechanical hyperalgesia

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was determined 1, 3, and 5 h after i.pl. injection of IL-12. The rats were treated with dexamethasone (1 h before, 2.0 mg kg\(^{-1}\) s.c.; Verri et al., 2004), indomethacin [30 min before, 2.5 mg kg\(^{-1}\) s.c., diluted in Tris (2-amino-2-hydroxymethyl-propan-1-3-diol)/HCl, pH 5.0; Cunha et al., 1992], atenolol (30 min before, 1.0 mg kg\(^{-1}\) s.c.; Nakamura and Ferreira, 1987) or 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-butythioindol-2-yl]-2,2-dimethylpropanoic acid, sodium (MK886; 24 h reinforcement dose 1 h before, 1.0 mg kg\(^{-1}\) s.c., per oral, diluted in 0.1% methylcellulose in water; Tonussi and Ferreira, 1999). Additionally, it was determined that opioid modulation of IL-12 induced hyperalgesia was not affected by morphine. Because the peripheral effect of morphine lasts approximately 1 h (Ferreira and Nakamura, 1979b), morphine (3–12 μg in 50 μl i.pl.; Ferreira and Nakamura, 1979b) was injected 4 h after IL-12 administration (10 ng in 50 μl) and evaluated 1 h after its injection. Naloxone (1.0 mg kg\(^{-1}\) i.p.; Ferreira and Nakamura, 1979b) was administered 30 min before and evaluated 1 h after morphine (6 μg paw\(^{-1}\)). The selected doses of dexamethasone, indomethacin, atenolol, MK886, and morphine inhibit carrageenan- or LPS-induced mechanical hyperalgesia (Ferreira and Nakamura, 1979b; Nakamura and Ferreira, 1987, 1988; Cunha et al., 1992; Tonussi and Ferreira, 1999; Lorenzetti et al., 2002; Verri et al., 2004), and did not affect the mechanical thresholds of normal animals (data not shown).

**Role of TNF-α and IL-18 on IL-12-Induced Hyperalgesia and of IL-12 on IL-18-Induced Hyperalgesia.** Antiserum to rat TNF-α (15 min, 50 μl i.pl.; Ferreira et al., 1993), anti-IL-18 antibody (15 min, 10 μg, 50 μl i.pl.), or control serum (50 μl i.pl.) was injected before IL-12 (10 ng, 50 μl) injection. Furthermore, anti-IL-12 antibody (15 min, 10 ng, 50 μl i.pl.) or control serum (50 μl i.pl.) was administered before IL-12 (40 ng, 50 μl) injection. The effects of the antiserum to rat TNF-α, anti-IL-18 antibody and anti-IL-12 antibody (doses described above) upon the TNF-α (2.5 pg in 50 μl), IL-12 (40 ng in 50 μl), or IL-12 (10 ng in 50 μl) induced-mechanical hyperalgesia were also determined, respectively. The hyperalgesic responses were measured 1, 3, and 5 h after stimulus i.pl. injection.

**Role of Endothelin and Its Receptors in IL-12-Induced Mechanical Hyperalgesia.** BQ123 (30 min, 30 nmol in 50 μl i.pl., an ET\(_A\) receptor antagonist) or BQ788 (30 min, 30–30 nmol in 50 μl i.pl., an ET\(_B\) receptor antagonist) was injected before IL-12 (10 ng in 50 μl i.pl.) or endothelin-1 (ET-1; 10 pmol in 50 μl i.pl.; Da Cunha et al., 2004) administration. Animals may also be pretreated with indomethacin or atenolol (doses described above) before ET-1 (10 pmol in 50 μl) injection. The hyperalgesic responses were measured 1, 3, and 5 h after IL-12 i.pl. injection.

**Drugs, Cytokines, Antibodies, and Antisera.** The following materials were obtained from the sources indicated: atenolol (Sigma-Aldrich, St. Louis, MO); human IL-18 (referred to as IL-18; Peprotech Inc., Rocky Hill, NJ); anti-human-IL-18 antibody (referred to as anti-IL-18 antibody; Peprotech Inc.); BQ123 sodium salt (lot A21510; Novabiochem, La Jolla, CA); BQ788 sodium salt (lot B32622; Calbiochem, La Jolla, CA); dexamethasone (Sigma-Aldrich); human endothelin-1 (referred to as ET-1, American Peptide Company, Sunnyvale, CA); indomethacin (Prodome, Campinas, São Paulo, Brazil); methylcellulose (Sigma-Aldrich); MK886 (lot B39328; Calbiochem, Darmstadt, Germany); morphine sulfate (Cristalia, Itapira, São Paulo, Brazil); naloxone, hydrochloride (Sigma-Aldrich), human IL-12 (referred to as IL-12; lot 95/544), rat recombinant TNF-α, sheep antisera to rat TNF-α, and sheep preimmune serum (National Institute of Biological Standards and Control, South Mimms, Hertfordshire, UK), and Tris (Merck, Darmstadt, Germany). The preimmune serum was obtained from the sheep before the immunization procedure. The LPS content of the above materials, as measured in a Limulus Amoebocyte Lysate test, was of the order of 0.25 IU mg\(^{-1}\), which is equivalent to a little over 10\(^{-15}\) g of LPS in a hypernociceptive dose of TNF-α (2.5 pg). The threshold hypernociceptive dose of LPS in the above model is 100 ng, i.e., 10\(^{-7}\) g (Ferreira et al., 1993). Therefore, the doses of the hypernociceptive agents used contained amounts of LPS up to eight log\(_{10}\) values less than the threshold hypernociceptive dose of LPS.

**Statistical Analysis.** Results are presented as means ± S.E.M. of measurements made on four to five animals in each group. Two-way analysis of variance (ANOVA) was used to compare the groups and doses at all times (curves) when the hyperalgesic responses were measured at different times after the stimulus injection. The analyzed factors were treatments, time, and time versus treatment interaction. When there was a significant time versus treatment interaction, one-way ANOVA followed by Bonferroni’s \(t\) test was performed for each time. On the other hand, when the hyperalgesic responses were measured once after the stimulus injection, the differences between responses were evaluated by one-way ANOVA followed by Bonferroni’s \(t\) test. Statistical differences were considered to be significant at \(P < 0.05\).

**Results**

**IL-12-Induced Dose- and Time-Dependent Mechanical Hyperalgesia.** Injection of IL-12 into the hindpaw of rats induced significant dose- (3, 10, and 30 ng in 50 μl) and time (1, 3, 5, 7, and 24 h)-dependent mechanical hyperalgesia determined by either the constant pressure rat paw test (Fig. 1A) or the electronic pressure-meter test (Fig. 1B). The mechanical hyperalgesic time course of IL-12 determined by both methods peaked 3 h after the administration of the higher dose (30 ng) and 5 h after the injection of other doses (3 and 10 ng) and was maintained at a similar level until 7 h
after injection, decreasing thereafter and returning to control levels 24 h later (Fig. 1). All doses of IL-12 induced significant hyperalgesia after 1, 3, 5, and 7 h in both methods, except for the dose of 3 ng in the 1st h. Therefore, for the other experiments the dose of 10 ng of IL-12 was used, and the mechanical hyperalgesia was determined 1, 3, and 5 h after the cytokine injection, except in the morphine group, in which it was measured 5 h after IL-12 injection. To determine whether IL-12 induces thermal hyperalgesia, the cytokine (same doses) was injected i.pl., and the thermal hyperalgesic response was measured 1, 3, and 5 h later. However, injection of IL-12 into the hindpaw of rats did not induce significant thermal hyperalgesia (data not shown).

**Effects of Dexamethasone, Indomethacin, Atenolol, MK886, and Morphine on IL-12-Induced Mechanical Hyperalgesia.** The pretreatment of the rats with a glucocorticosteroid (dexamethasone; 2.0 mg kg⁻¹) significantly inhibited IL-12 (10 ng)-induced mechanical hyperalgesia determined by either the constant pressure rat paw test (Fig. 2A) or the electronic pressure-meter test (Fig. 2B). However, the treatment of the animals with a standard cyclooxygenase inhibitor (indomethacin; 2.5 mg kg⁻¹), β-adrenergic antagonist (atenolol; 1.0 mg kg⁻¹), or 5-lipoxygenase activating protein inhibitor (MK886; 1.0 mg kg⁻¹) was ineffective in inhibiting IL-12 (10 ng)-induced mechanical hyperalgesia in both tests (Fig. 2, A and B). These results suggest that prostanoids, sympathetic amines, or leukotrienes are not involved in IL-12-induced mechanical hyperalgesia. The fact that dexamethasone inhibited the IL-12-induced hyperalgesia suggests that this cytokine is not directly sensitizing the nociceptor but, rather, that it is acting via the release of glucocorticosteroid-sensitive secondary mediators. Moreover, the treatment with an opioid agonist (morphine, 3, 6, and 12 μg i.pl.) also inhibited in a dose-dependent manner the IL-12 (10 ng)-induced mechanical hyperalgesia, and an opioid antagonist (naloxone, 1.0 mg kg⁻¹; Fig. 2, C and D) prevented the analgesic effect of morphine (6 μg i.pl.).

**Effects of Antiserum against Rat TNF-α or IL-18 Antibody on IL-12-Induced Hyperalgesia and of IL-12 Antibody on IL-18-Induced Hyperalgesia.** The pretreatment of rats with antiserum against rat TNF-α (50 μl) or anti-IL-18 antibody (10 μg) did not alter IL-12 (10 ng)-induced mechanical hyperalgesia determined by both methods. Furthermore, anti-IL-12 antibody (10 ng) did not alter IL-18 (40 ng)-induced mechanical hyperalgesia (Fig. 3). As expected, the antiserum against rat TNF-α, IL-18 antibody, and IL-12 antibody inhibited TNF-α (2.5 pg in 50 μl), IL-18 (40 ng in 50 μl), and IL-12 (10 ng in 50 μl)-induced mechan-

![Fig. 2. Effects of dexamethasone, indomethacin, atenolol, MK886, and morphine on IL-12-induced mechanical hyperalgesia. A and B, the animals were pretreated with dexamethasone (Dexa; 1 h, 2.0 mg kg⁻¹ s.c., glucocorticosteroid), indomethacin (Indo; 30 min, 2.5 mg kg⁻¹ s.c., cyclooxygenase inhibitor), atenolol (Atn; 30 min, 1.0 mg kg⁻¹ s.c., β-adrenergic antagonist), or MK886 (24 h plus 1 h, 1.0 mg kg⁻¹, per oral, 5-lipoxygenase-activating protein inhibitor) before IL-12 (10 ng in 50 μl i.pl.) administration. The intensity of hyperalgesia was measured 1, 3, and 5 h after IL-12 injection by the constant pressure paw test (panel A) or the electronic pressure-meter test (panel B). C and D, after the administration of IL-12 (10 ng) in 50 μl i.pl.), the animals were treated with saline (Sal, 200 μl) or morphine (4 h after IL-12 injection, 3–12 μg in 50 μl i.pl.). The animals also received an injection of naloxone (1.0 mg kg⁻¹ in 500 μl i.pl.) 30 min before morphine (6 μg paw⁻¹) injection. The intensity of hyperalgesia was measured 5 h after IL-12 injection by the constant pressure paw test (panel C) or the electronic pressure-meter test (panel D). Before the injection of the hyperalgesic agents, the reaction time and reaction force were 32.0 ± 0.2 s and 43.2 ± 0.7 g (means ± S.E.M.; n = 12 groups), respectively. Bars represent means ± S.E.M. of four to five rats per group, except for the vehicle bar in A and B that represents means ± S.E.M. of four groups (one for each drug treatment). *, P < 0.05 compared with the respective control (one-way ANOVA followed by Bonferroni’s t test).
IL-12 is a proinflammatory cytokine (for review, see Watford et al., 2004), and recently, patients receiving IL-12 as an immune therapy for cancer treatment reported hyperalgesia (Gollob et al., 2000; Kang et al., 2001; Lenzi et al., 2002; Weiss et al., 2003). Therefore, in the present study, we investigated the possible hyperalgesic effect of IL-12 and its pharmacological susceptibility. We report that IL-12 induced significant dose- and time-dependent mechanical hyperalgesia in rats, determined by either constant pressure paw or electronic pressure-meter tests. The IL-12 effects were restricted to the ipsilateral paw (data not shown), and the hyperalgesic dose of IL-12 (10 ng/paw) was at least 50% lower than the local dose of IL-12 used in humans as a vaccine adjuvant (Portielje et al., 2005). The IL-12-induced mechanical hyperalgesia was dose-dependently inhibited by the local administration of morphine, and the nonspecific opioid receptor antagonist blocked this analgesic effect. These results are in line with the observation that opiates directly block hyperalgesia induced by various mediators such as endothelin (Menéndez et al., 2003) and prostaglandins E2 and I2 (Ferreira and Nakamura, 1979b). There is evidence that morphine, besides acting on the central nervous system, has a

**TABLE 1**

Effects of antisera against TNF-α, IL-12, and IL-18 and their respective control sera on TNF-α-, IL-12-, and IL-18-induced mechanical hyperalgesia, respectively

<table>
<thead>
<tr>
<th>Group/Time</th>
<th>Constant Pressure Test</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Δ reaction time, s</td>
<td>1 h</td>
<td>3 h</td>
<td>5 h</td>
</tr>
<tr>
<td>Saline + saline</td>
<td>0.9 ± 0.3</td>
<td>1.5 ± 0.6</td>
<td>1.3 ± 0.5</td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>a-Control + saline</td>
<td>1.1 ± 0.5</td>
<td>1.6 ± 0.8</td>
<td>2.0 ± 0.7</td>
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<tr>
<td>a-Control + TNF-α</td>
<td>9.4 ± 0.6*</td>
<td>16.2 ± 0.4*</td>
<td>12.6 ± 1.1*</td>
<td>8.1 ± 1.9*</td>
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<tr>
<td>α-TNF-α + TNF-α</td>
<td>2.0 ± 0.4*</td>
<td>3.9 ± 1.1*</td>
<td>3.5 ± 0.5*</td>
<td>8.3 ± 1.4*</td>
</tr>
<tr>
<td>a-Control + IL-12</td>
<td>15.5 ± 0.6*</td>
<td>17.2 ± 0.8*</td>
<td>18.6 ± 0.5*</td>
<td>12.4 ± 1.7*</td>
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<tr>
<td>α-IL-12 + IL-12</td>
<td>1.2 ± 0.6</td>
<td>1.5 ± 0.7*</td>
<td>1.4 ± 0.6*</td>
<td>6.8 ± 0.7*</td>
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<td>α-Control + IL-18</td>
<td>8.3 ± 1.4*</td>
<td>16.3 ± 0.8*</td>
<td>14.7 ± 0.5*</td>
<td>15.4 ± 6.2*</td>
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<tr>
<td>α-IL-12 + IL-18</td>
<td>1.2 ± 0.4*</td>
<td>4.6 ± 1.1*</td>
<td>1.9 ± 0.6*</td>
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<td>1 h</td>
<td>3 h</td>
<td>5 h</td>
</tr>
<tr>
<td>Saline + saline</td>
<td>0.7 ± 0.3</td>
<td>0.5 ± 0.3</td>
<td>0.7 ± 0.5</td>
<td>1.6 ± 0.5</td>
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<tr>
<td>a-Control + saline</td>
<td>1.6 ± 0.5</td>
<td>1.8 ± 0.9</td>
<td>1.9 ± 0.6</td>
<td>8.3 ± 1.4*</td>
</tr>
<tr>
<td>a-Control + TNF-α</td>
<td>12.9 ± 8.6*</td>
<td>17.9 ± 1.4*</td>
<td>14.5 ± 1.6*</td>
<td>8.1 ± 0.5*</td>
</tr>
<tr>
<td>α-TNF-α + TNF-α</td>
<td>8.1 ± 1.9*</td>
<td>8.3 ± 1.4*</td>
<td>8.1 ± 0.5*</td>
<td>12.4 ± 1.7*</td>
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<tr>
<td>a-Control + IL-12</td>
<td>6.8 ± 0.7*</td>
<td>7.7 ± 0.8*</td>
<td>8.7 ± 1.4*</td>
<td>15.4 ± 6.2*</td>
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<td>α-IL-12 + IL-12</td>
<td>15.4 ± 6.2*</td>
<td>27.6 ± 3.8*</td>
<td>22.8 ± 2.6*</td>
<td>1.0 ± 0.8*</td>
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<td>α-Control + IL-18</td>
<td>1.0 ± 0.8*</td>
<td>7.3 ± 0.5*</td>
<td>10.0 ± 0.9*</td>
<td>1.0 ± 0.8*</td>
</tr>
<tr>
<td>α-IL-12 + IL-18</td>
<td>1.0 ± 0.8*</td>
<td>7.3 ± 0.5*</td>
<td>10.0 ± 0.9*</td>
<td>1.0 ± 0.8*</td>
</tr>
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</table>

* P < 0.05 compared with the a-control + saline group. † P < 0.05 compared with the a-control + respective cytokine group (one-way ANOVA followed by Bonferroni’s t test).
peripheral effect (Ferreira and Nakamura, 1979b; Smith et al., 1982). It is noteworthy that, differently of mechanical hyperalgesia, IL-12 i.pl. injection did not induce thermal hyperalgesia determined using the Hargreaves test. Similarly, doses of TNF-α and IL-18 that induced mechanical hyperalgesia did not induce thermal hyperalgesia (Cunha et al., 1992; Ferreira et al., 1993; Woolf et al., 1997).

The IL-12-induced hyperalgesia was not affected by treatment of the rats with indomethacin, atenolol, and MK886, suggesting that prostanoids, sympathetic amines, and leukotrienes are not involved in the onset of the hyperalgesia induced by this cytokine. It has been demonstrated that hyperalgesia induced by IL-6/IL-1β and the chemokines cytokine-induced neutrophil chemoattractant 1 or IL-8 is dependent on prostaglandin synthesis and on the release of sympathetic amines, respectively (Cunha et al., 1992, 2000; Ferreira et al., 1993; Lorenzetti et al., 2002). Furthermore, the release of these cytokines is stimulated by TNF-α, which is produced in response to inflammatory stimuli, such as carrageenan and LPS (Cunha et al., 1992, 2000; Ferreira et al., 1993; Lorenzetti et al., 2002). It appears that TNF-α does not participate in the IL-12-induced hyperalgesia, since it was observed that antiserum against TNF-α failed to alter the response. This is consistent with the negative results obtained with indomethacin and atenolol, because these compounds inhibit the hyperalgesia induced by TNF-α (Cunha et al., 1992; Ferreira et al., 1993).

Recently, we have shown that the mechanism by which IL-18 induces mechanical hyperalgesia in rats depends on endothelin acting on ETβ receptors (Verri et al., 2004). IL-12 and IL-18 have synergic actions in several biological processes including interferon-γ production, T cell proliferation, and enhancement of cell-mediated cytotoxicity (for review, see Biet et al., 2002). However, the IL-12 hyperalgesia is not dependent on IL-18 since an effective dose of anti-IL-18 antibody did not affect the IL-12-induced process. Further investigating the relation between IL-12 and IL-18, the IL-18-induced mechanical hyperalgesia was not affected by anti-IL-12 antibody, suggesting that the hyperalgesia induced by these cytokines is independent one of each other. The IL-12-induced hyperalgesia was inhibited by dexamethasone. It is important to mention that the patients under IL-12 treatment that reported pain, described in the above clinical study (Gollob et al., 2000; Lenzi et al., 2002; Weiss et al., 2003), were not receiving glucocorticosteroid therapy. Glucocorticosteroids are known inhibitors of the synthesis of eicosanoids (prostaglandins and leukotrienes), proinflammatory cytokines such as TNF-α, IL-2, IL-18, IL-6, and IL-18 (for review, see Goulding, 1998; Kodama et al., 2002) and endothelin (Dschietzig et al., 2001). Thus, taking into account the above results indicating that prostanoids, leukotrienes, TNF-α, and IL-18 were not involved in the IL-12-induced hyperalgesia, the possible involvement of endothelin and its receptor subtypes was addressed.

It appears that endothelin is involved in the IL-12-induced hyperalgesia, and this effect is mediated via the ETβ receptor, since BQ788 (ETβ receptor antagonist) but not BQ123 (ETα receptor antagonist) inhibited the IL-12 hyperalgesia. In this context, ET-1 induces time and dose-dependent hyperalgesia, which was inhibited by BQ788, but not by BQ123 (Da Cunha et al., 2004). Furthermore, neither indomethacin nor atenolol affects ET-1-induced hyperalgesia (Ferreira et al., 1989; Da Cunha et al., 2004; Verri et al., 2004). In agreement with our results, ETβ receptors mediate phenylbenzoquinone-induced abdominal writhing (Griswold et al., 1999), carrageenan-primed knee joint articular incapacitation (De-Melo et al., 1998), and cytokine- (IL-18) or ET-1-induced mechanical hyperalgesia (Da Cunha et al., 2004; Verri et al., 2004). However, there is also evidence that ETα mediates nociceptive behavior (flinching) induced by direct application of ET-1 on the sciatic nerve (Davar et al., 1998), as well as ET-1-induced thermal hyperalgesia (Menéndez et al., 2003) and ET-1 potentiation of capsaicin-induced nociception (Piovezan et al., 1998). In fact, it has also been reported that the antinociception mediated by endothelin via the ETβ receptor is opioid-sensitive (Khodorova et al., 2003). However, other investigators have reported that both ETα and ETβ mediate nociception in abdominal writhing (Raffa et al., 1996) and carrageenan-induced mechanical hyperalgesia (Baamonde et al., 2004). These apparent discrepancies could be due to differences in experimental nociceptive models, the dose of endothelin, and time intervals of the nociceptive responses, which might detect the hyperalgesia of different sets of primary sensory neurons.

In conclusion, we demonstrated here for the first time that IL-12 induces mechanical hyperalgesia mediated by endothelin action on ETβ receptors in an opioid-sensitive manner in rats. This finding not only highlights a possible adverse effect of IL-12 therapy but also suggests that selective antagonism of the endothelin ETβ receptor could be of value to prevent IL-12 therapy-induced clinical pain and hyperalgesia in humans.
IL-12 Hyperalgesia: Endothelin Action on ETB Receptors

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Address correspondence to: Prof. Dr. Fernando de Queiroz Cunha, Department of Pharmacology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, Avenida Bandeirantes, 3900, 14049-900-Ribeirão Preto, São Paulo, Brazil. E-mail address: fdcunha@fmrp.usp.br