Interleukin-18 Induces Mechanical Hypernociception in Rats via Endothelin Acting on \( \text{ET}_B \) Receptors in a Morphine-Sensitive Manner

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ABSTRACT

Interleukin (IL)-18 has an important role in the pathogenesis of arthritis, which is accompanied by movement limitation secondary to inflammatory articular nociception. Therefore, we investigated the possible mechanical hypernociceptive effect of IL-18 in rats using the paw constant pressure and the electronic pressure-meter tests. In both tests, intraplantar administration of IL-18 (20–60 ng paw\(^{-1}\)) caused a dose- and time-dependent mechanical hypernociception, which peaked 3 h and reached control levels 24 h after injection. Pretreatments with indomethacin (2.5 mg kg\(^{-1}\)), atenolol (1 mg kg\(^{-1}\)), or 3-[1-(\(\rho\)-chlorobenzyl)-5-(isopropyl)-3-butylthioindol-2-yl]-2,2-dimethylpropanoic acid; Na (MK886) (5-lipoxygenase-activating protein inhibitor; 1 mg kg\(^{-1}\)) did not inhibit IL-18-evoked hypernociception (40 ng paw\(^{-1}\)), whereas dexamethasone (2 mg kg\(^{-1}\)) inhibited the process. IL-18-evoked hypernociception was not inhibited by pretreatment with antisera to rat tumor necrosis factor-\(\alpha\) (50 \(\mu\)l paw\(^{-1}\)) or IL-1 receptor antagonist (300 pg paw\(^{-1}\)). Pretreatment with \(N\)-cys-2,6-dimethylpiperidinocarbonyl-L-\(\gamma\)-methylleucyl-D-1-methoxyxycarbonyl-D-norleucine (BQ788) (\(\text{ET}_B\) receptor antagonist; 3–30 nmol paw\(^{-1}\)), but not with cyclo[\(\omega\)-Trp-\(\omega\)-Asp-Pro-\(\omega\)-Val-Leu] (BQ123) (\(\text{ET}_A\) receptor antagonist; 30 nmol paw\(^{-1}\)), dose dependently inhibited the IL-18-induced hypernociception. Pretreatment with morphine (3–12 \(\mu\)g paw\(^{-1}\)) also dose-dependently inhibited the IL-18-induced hypernociception. Moreover, endothelin-1-induced hypernociception also was inhibited by BQ788, but not by BQ123, indomethacin, or atenolol. In conclusion, we demonstrated for the first time that IL-18 is a prohypernociceptive cytokine that induces mechanical hypernociception mediated by endothelin, via \(\text{ET}_B\) receptor. Therefore, inhibition of the endothelin \(\text{ET}_B\) receptor could be beneficial on controlling inflammatory hypernociception of diseases in which IL-18 plays a role in their pathogenesis.

Interleukin (IL)-18, identified as an interferon (IFN)-\(\gamma\)-inducing factor, is a member of the IL-1 family due to its structural homology and because it shares IL-1\(\beta\)-converting enzyme (caspase 1) to cleave its precursor pro-IL-18, yielding an active 18-kDa glycoprotein (for review, see Nakanishi et al., 2001). IL-18 mRNA is expressed by various cell types, including macrophages, dendritic, osteoblasts, and intestinal epithelial cells (McInnes et al., 2000). IL-18 has a variety of biological functions, including the stimulation of the proliferation of activated T cells, enhancement of activation of natural killer cells lytic activity, and IFN-\(\gamma\) production by T helper 1 (Th1), CD8\(^+\), and natural killer cells in mice and in humans (Hoshino et al., 1999; for review, Nakanishi et al., 2001). Although IL-18 itself cannot induce strong IFN-\(\gamma\) expression, IL-18 fully induces IFN-\(\gamma\) production in synergy with IL-12. IL-18 itself cannot induce Th1 differentiation, but it potentiates IL-12-driven Th1 development (Hoshino et al., 1999).

Recent reports indicate a role for IL-18 in the pathogenesis of several inflammatory diseases. In humans, IL-18 expression has been reported in sepsis, hepatitis C virus infection, Crohn’s disease, and type I diabetes (McInnes et al., 2000; for

ABBREVIATIONS: IL, interleukin; IFN, interferon; Th1, T helper 1; TNF, tumor necrosis factor; CIA, collagen type II-induced arthritis; LTB\(_4\), leukotriene B\(_4\); i.pl., intraplantar; IL-1ra, interleukin-1 receptor antagonist; ET, endothelin receptor; LPS, lipopolysaccharide; ANOVA, analysis of variance.
review, see Nakanishi et al., 2001). IL-18 messenger and protein are present in significant levels in the rheumatoid arthritis synovium in humans and in experimental model (Plater-Zyberk et al., 2001), where it induces and sustains articular Th1 cell responses and independently promotes tumor necrosis factor (TNF-α) production. IL-18-deficient mice developed significantly reduced incidence and severity of collagen-induced arthritis (CIA) compared with wild-type mice, associated with suppressed TNF-α production and Th1 immune responses ex vivo. This reduction in disease and immune response was completely reversed by the administration of recombinant IL-18 (Wei et al., 2001). These data clearly demonstrate that IL-18 is of importance during developing and sustained inflammatory diseases. We have recently reported that IL-18 administration promoted neutrophil accumulation in vivo, an important event involved in the pathogenesis of tissue lesions in arthritis (Leung et al., 2001). IL-18 activates and attracts neutrophils by inducing the production of TNF-α, which in turn induces the synthesis of leukotriene B4 (LTB4), a well known chemoattractant of neutrophils (Canetti et al., 2001; Leung et al., 2001). This finding is consistent with the previous observation that inhibition of LTB4 synthesis attenuated the severity of CIA (Nickerson-Nutter and Medvedeff, 1996).

Limitation of movement secondary to inflammatory hyperalgesia is a serious problem to patients and animals presenting inflammatory arthropathies. There are two groups of directly acting inflammatory hyperalgesic mediators that satisfy the experimental and clinical criteria for agents that directly sensitize nociceptors: eicosanoids and sympathetic amines. The capacity of prostaglandins and sympathetic amines to sensitize nociceptors has been shown in human and in animals using both behavioral and electrophysiological techniques (Hannington-Kiff, 1974; Nakamura and Ferreira, 1987; Lorenzetti et al., 2002). It is well accepted in the literature that the release of eicosanoids and sympathetic amines is secondary to the generation of a cascade of cytokines, in which TNF-α has a pivotal role (Cunha et al., 1992). TNF-α initiates two pathways, each of which involve the release of cytokines and the final nociceptive mediators that sensitize the nociceptor. The two pathways are 1) inflammatory stimuli → TNF-α → IL-6 → IL-1β → prostaglandins; and 2) TNF-α → cytokine-induced neutrophil chemoattractant-1 (rat IL-8 related chemokine; Lorenzetti et al., 2002) → sympathetic amines (Nakamura and Ferreira, 1987). Besides the mediators described above, there is consistent evidence that endothelins and LTB4 also participate in the genesis of inflammatory hyperalgesia (Levine et al., 1984; Ferreira et al., 1989). Endothelins potentiate the prostaglandin E2-induced locomotion incapacitation in dogs (Ferreira et al., 1989), beyond directly inducing nociceptive behavior (Raffa et al., 1996; Davar et al., 1998) by a morphine-sensitive manner (Menéndez et al., 2003). LTB4 induces a leukocyte migration-dependent nociception in animals (Levine et al., 1984) and humans (Bisgaard and Kristensen, 1985).

In view of the evidence that IL-18 has an essential role in CIA, in the present study we investigated the possible mechanical hypernociceptive effect of IL-18 and the nociceptive pathways involved in its effect. It was observed that IL-18 induces a dose- and time-dependent mechanical hypernociceptive response in rats, which is mediated by endothelin via its ETB receptor activation, in an opioid-sensitive manner.

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). The animals were used only in a single experimental group.

Mechanical Hypernociceptive Tests

In this article, we have used the term hypernociception (increased nociception) to describe the behavioral response induced by mechanical pressure in rats. Although the terms allodynia (pain from stimuli that are not normally painful) and hyperalgesia (an increased sensation to painful stimuli that may follow damage to soft tissue containing nociceptors or injury to a peripheral nerve) describe distinct nociceptive symptoms in human (Bisgaard and Kristensen, 1985; Vrinten et al., 2000), in animal models they are used indistinguishably to describe the increase in mechanical nociceptor sensitivity. In fact, there is up to now, no evidence that different second messenger events mediate allodynia and hyperalgesia. The use of the terms hypersensitivity or hyperexcitability also were avoided because they have specific meaning in immunology and electrophysiology, respectively.

Hypernociception was measured at different times after intraplantar (i.pl.) injection into the hindpaws of rats using two different methods: the constant pressure rat paw and the electronic pressure-meter tests. Different individuals performed each test, prepared solutions to be injected and performed the injections. Multiple paw treatments with saline did not alter basal reaction time, which was similar to that observed in noninjected paws.

Constant Pressure Rat Paw Test

Mechanical hypernociception was tested in rats as described previously (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 presented 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 before administration (zero time) and at different times after administration of the hypernociceptive agents. The intensity of mechanical hypersensitivity 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 31.5 ± 0.1 s (mean ± S.E.M.; n = 36) before injection of the hypernociceptive agents. A shortened reaction time is prevented by steroidal and nonsteroidal anti-inflammatory drug treatment before an inflammatory stimulus injection (Cunha et al., 1992; Lorenzetti et al., 2002). This method has been used to demonstrate the contribution of eicosanoids, sympathetic amines, and cytokines in the development of peripheral inflammatory hypernociception (Ferreira and Nakamura, 1979a; Cunha et al., 1992, 1999, 2000; Ferreira et al., 1993). These concepts and findings have been extensively confirmed with other methodologies such as formalin-induced flinches and others (Vinegar et al., 1976; Bisgaard and Kristensen, 1985). Furthermore, this method is able to discriminate peripheral and central analgesic effects of drugs (Ferreira et al., 1978).
Electronic Pressure-Meter Test

The paw hypernociception also was measured with an electronic pressure-meter. The rats were placed in acrylic cages (12 × 20 × 17 cm in height) with wire grid floor, 15 to 30 min before beginning tests. During this adaptation period, the paws were poked 2 to 3 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 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 maximal value of calibration range in which the pressure was linearly detectable by the equipment was 80 g. The stimulation of the paw was repeated until the animal presented three similar measurements (the difference between the highest and the lowest measurement should be no more than 10 g). If the results were inconsistent (~1:25 animals), the experimenter used another animal. 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 first measurement before treatment (Vivancos et al., 2004). The reaction force was 43.8 ± 0.3 g (mean ± S.E.M.; n = 36) before injection of the hypernociceptive agents.

Protocols

The IL-18-induced mechanical hypernociception was evaluated by the following protocols.

Dose- and Time-Dependent Mechanical Hypernociception Induced by IL-18. To evaluate whether IL-18 induces mechanical hypernociception, the cytokine (20–60 ng in 50 μl) was injected i.pl., and the nociceptive response was measured 3 h later. The time course of IL-18 injected at the dose of 40 ng in 50 μl i.pl. was measured 1, 3, 5, and 24 h after injection.

Role of Eicosanoids (Prostanoids and Leukotrienes), Sympathetic Mediators, and Morphine Treatment on IL-18-Induced Mechanical Hypernociception. The participation of nociceptive mediators in IL-18 (40 ng in 50 μl)-induced mechanical hypernociception was evaluated 3 h after i.pl. injection of IL-18. The animals were treated with dexamethasone (1 h before, 2 mg kg⁻¹ s.c.; Cunha and Ferreira, 1986), indomethacin (30 min before, 2.5 mg kg⁻¹ s.c., diluted in Tris/HCl, pH 8.0; Cunha et al., 1992), atenolol (30 min before, 1 mg kg⁻¹ s.c.; Nakamura and Ferreira, 1987), 3-[1-(p-chlorobenzyl)-5-(isopropyl)-3-butythioindol-2-yl]-2,2-dimethylpropanoic acid, sodium (MK886; 24-h reinforcement dose 1 h before, 1 mg kg⁻¹ per oral, diluted in 0.1% methylcellulose in water; Tonussi and Ferreira, 1999), or morphine (2 h after IL-18 injection, 3–12 μg in 50 μl i.pl.; Ferreira and Nakamura, 1979b). The effect of naloxone (30 min before morphine, 1 mg kg⁻¹ i.p.; Ferreira and Nakamura, 1979b) on the analgesic effect of morphine (6 μg paw⁻¹) also was tested. The doses of dexamethasone, indomethacin, atenolol, MK886, and morphine inhibit carrageenan- or LPS-induced mechanical hypernociception (Ferreira and Nakamura, 1979b; Cunha and Ferreira, 1986; Nakamura and Ferreira, 1987; Cunha et al., 1992; Tonussi and Ferreira, 1999; Lorenzetti et al., 2002) and did not affect the mechanical thresholds of normal animals (data not shown).

Role of TNF-α and IL-1β on IL-18-Induced Mechanical Hypernociception. Antiserum to rat TNF-α (15 min, 50 μl i.pl.; Ferreira et al., 1993), control serum (50 μl i.pl.), or IL-1 receptor antagonist (IL-1ra) (30 min, 300 pg in 50 μl i.pl.; Cunha et al., 2000) was administered before IL-18 (40 ng; 50 μl) injection. The effects of the antisera to rat TNF-α and IL-1ra (doses described above) upon the TNF-α (2.5 pg; 50 μl) or IL-1β (0.5 pg; 50 μl)-induced mechanical hypernociception also was evaluated, respectively. The hypernociceptive response was measured 3 h later.

Role of Endothelin and Its Receptors on IL-18-Induced Mechanical Hypernociception. BQ123 (30 min, 30 nmol in 50 μl i.pl.; an ET₃ receptor antagonist) or BQT88 (30 min, 3–30 nmol in 50 μl i.pl.; an ETA receptor antagonist) was injected before IL-18 (40 ng in 50 μl i.pl.) or endothelin-1 (ET-1; 10 pmol in 50 μl i.pl.; J. M. Cunha, G. A. Rae, S. H. Ferreira, and F. Q. Cunha, manuscript submitted for publication) administration. Animals also may be pretreated with indomethacin or atenolol (doses described above) before ET-1 (10 pmol in 50 μl) injection. The hypernociceptive response was measured 3 h later.

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-1β; Peprotech Inc., Rocky Hill, NJ); BQ123, sodium salt (Uio), (Tetra-Asp-Pro-Val-Leu); lot A21510, Novabiochem, La Jolla, CA; BQT88, sodium salt N-cys-2,6 dimethylpiperidinocarbonyl-L-γ-methylleucyl-d-1-methoxycarbonyl-D-norleucine; 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); rat recombinant IL-1β, rat recombinant IL-1ra, rat recombinant TNF-α, sheep antiserum to rat TNF-α and sheep preimmune serum (National Institute of Biological Standards and Control, South Mimms, Hertfordshire, UK); and Tris (Merek, Darmstadt, Germany). The LPS content of the above materials, as measured in a Limulus Amoeocyte Lysate test, was of the order of 0.25 μg ml⁻¹, which is equivalent to a little over 10⁻¹⁵ g of LPS in a hypernociceptive dose of IL-18 (0.5 pg). The threshold hypernociceptive dose of LPS in the above-mentioned 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 8 log₁₀ values less than the threshold hypernociceptive dose of LPS.

Statistical Analysis

Results are presented as mean ± S.E.M. of measurements made on four to five animals in each group. Differences between responses were evaluated by one-way analysis of variance (ANOVA) followed by Bonferroni’s t test. Statistical differences were considered to be significant at P < 0.05.

Results

IL-18-Induced Dose- and Time-Dependent Mechanical Hypernociception. Injection of IL-18 (50 μl) into the hindpaw of rats induced a dose- (20, 40, and 60 ng; Fig. 1, a and b, left) and time (1, 3, 5, and 24 h; Fig. 1, a and b, right)-dependent mechanical hypernociception determined by either the constant pressure rat paw test (Fig. 1a) or the electronic pressure-meter test (Fig. 1b), respectively. The hypernociceptive response was statistically significant for the three (20, 40, and 60 ng) doses, and the higher dose (60 ng) was significantly different from the lowest dose (20 ng) when evaluated by the electronic pressure-meter test. With the constant pressure paw test, the two doses, 40 and 60 ng, were significantly different from control group (Fig. 1a, left). The mechanical hypernociceptive time course of IL-18 (40 ng) determined by both methods peaked 3 h after the cytokine
injection, decreasing thereafter and reached the control levels 24 h later (Fig. 1, right). Therefore, for the rest of the experiments, a dose of 40 ng of IL-18 was used, and the mechanical hypernociception was determined 3 h after the cytokine injection.

**Effects of Dexamethasone, Indomethacin, Atenolol, MK886, and Morphine on IL-18-Induced Mechanical Hypernociception.** The pretreatment of the rats with a glucocorticosteroid (dexamethasone; 2 mg kg$^{-1}$), but not with a standard cyclooxygenase inhibitor (indomethacin; 2.5 mg kg$^{-1}$), β-adrenergic antagonist (atenolol; 1 mg kg$^{-1}$), or 5-lipoxygenase-activating protein inhibitor (MK886; 1.0 mg kg$^{-1}$), significantly inhibited IL-18 (40 ng)-induced mechanical hypernociception determined by either the constant pressure paw test (Fig. 2a, left) or the electronic pressure-meter test (Fig. 2b, left). These results suggest that prostanoids, sympathetic amines or leukotrienes are not involved in IL-18-induced mechanical hypernociception. The fact that dexamethasone inhibited the IL-18-induced hypernociception suggests that this cytokine is not directly sensitizing the nociceptor, but it is acting via the release of secondary mediators. Moreover, the treatment with an opioid agonist (morphine; 3–12 μg i.pl.) also inhibited in a dose-dependent manner the IL-18 (40 ng)-induced mechanical hypernociception. The analgesic effect of morphine (6 μg i.pl.) was prevented by an opioid antagonist (naloxone, 1 mg kg$^{-1}$; Fig. 2, a and b, right).

**Effects of Antiserum against Rat TNF-α and IL-1 Receptor Antagonist on IL-18-Induced Mechanical Hypernociception.** The pretreatment of rats with antiserum against rat TNF-α (50 μl) or IL-1ra (300 pg) did not alter IL-18 (40 ng)-induced mechanical hypernociception determined by both methods (Fig. 3, a and b). As expected, the antiserum against rat TNF-α and IL-1ra inhibited TNF-α (2.5 pg in 50 μl) and IL-1β (0.5 pg in 50 μl)-induced hypernociception, respectively. These results suggest that TNF-α and IL-1β are not mediating the IL-18-induced mechanical hypernociception.

**Effects of Endothelin ETA and ETB Receptor Antagonists on IL-18-Induced Mechanical Hypernociception.** The ETB receptor antagonist BQ788 inhibited the IL-18 (40 ng) and the ET-1 (10 pmol)-induced mechanical hypernociception.

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**Fig. 1.** IL-18 induces a dose- and time-dependent mechanical hypernociception. The intensity of hypernociception was measured 3 h after IL-18 (20, 40, and 60 ng in 50 μl i.pl.) or 1, 3, 5, and 24 h after IL-18 (40 ng in 50 μl i.pl.) administration by the constant pressure paw test (a) or the electronic pressure-meter test (b). Before the injection of the hypernociceptive agents, the reaction time and reaction force were 31.2 ± 0.2 s and 43.7 ± 0.8 g (mean ± S.E.M.; n = 6 groups), respectively. Bars represent mean ± S.E.M. of five rats per group. *, P < 0.05 compared with the respective control (one-way ANOVA followed by Bonferroni’s t test).
hypernociception in both methods (Fig. 4). The inhibition of IL-18-induced hypernociception was dose-dependent for BQ788 (3–30 nmol). However, the ETA receptor antagonist (BQ123) at the dose of 10 nmol (Fig. 4, a and b, right) and 30 nmol (data not shown) did not inhibit the ET-1 effect. As expected, the IL-18-induced hypernociception was not inhibited by the dose of 30 nmol of BQ123 (Fig. 4, a and b, left). These results suggest that ET-1 acted on ETB receptors mediating the IL-18-induced mechanical hypernociception. Moreover, neither indomethacin nor atenolol attenuated ET-1-induced mechanical hypernociception (Fig. 4, a and b, right).

Discussion

IL-18 is a cytokine with a pleiotropic role in the regulation of the Th1 immune response that costimulates IFN-γ production in the presence of IL-12 (for review, see Nakanishi et al., 2001). It has an important role in the pathophysiology of arthritis, an autoimmune disease accompanied by articular nociception (Plater-Zyberk et al., 2001; Wei et al., 2001). Therefore, in the present study we have investigated the possible hypernociceptive effect of IL-18. It was observed that IL-18 induces a dose- and time-dependent mechanical hypernociceptive response in rats determined by either constant pressure paw or electronic pressure-meter tests. The IL-18-induced hypernociception was not affected by treatment of the animals with indomethacin, atenolol, and MK886, suggesting that prostanoids, sympathetic amines, and leukotrienes are not involved in the onset of IL-18-induced hypernociception, respectively. The results also suggest that IL-18 may trigger a novel mechanical nociceptive pathway, different from those activated by other described hypernociceptive cytokines. It had been demonstrated that hypernociception induced by IL-6/IL-1β and by the chemokines cytokine-in-
duced neutrophil chemoattractant-1 or IL-8 are dependent on prostaglandin synthesis and on sympathetic amine release, respectively (Cunha et al., 1992; Ferreira et al., 1993; Cunha et al., 2000; Lorenzetti et al., 2002). Furthermore, the release of these cytokines is stimulated by TNF-α, which in turn is produced in response to unspecific stimuli, such as carrageenan and LPS (Cunha et al., 1992, 2000; Ferreira et al., 1993; Lorenzetti et al., 2002). Moreover, confirming that TNF-α and IL-1β do not participate in IL-18-induced hypernociception, it was observed that antiserum against TNF-α and IL-1ra were ineffective in the process. This is consistent with the negative results obtained with indomethacin and atenolol, because these compounds inhibit the hypernociception induced by TNF-α and IL-1β (Cunha et al., 1992, 2000; Ferreira et al., 1993).

Glycocorticosteroids are inhibitors of the synthesis of eicosanoids, proinflammatory cytokines (for review, see Goulding, 1998), and also endothelin (Dschiethzig et al., 2001). Moreover, dexamethasone also inhibits the expression of the endothelin ET_A and ET_B receptors (Nambi et al., 1992). The fact that IL-18-induced hypernociception was inhibited by dexamethasone but eicosanoids, TNF-α and IL-1β do not participate in the process, led us to evaluate the possible involvement of endothelin and its receptor subtypes in IL-18-induced hypernociception. There is evidence that endothelin induces mechanical hypernociception by a mechanism independent of prostanoids and sympathetic amines (Ferreira et al., 1989; J. M. Cunha, G. A. Rae, S. H. Ferreira, and F. Q. Cunha, manuscript submitted for publication). Moreover, depending on the nociceptive method, the reaction time and reaction force were 31.6 ± 0.2 s and 44.4 ± 0.6 g (mean ± S.E.M.; n = 8 groups), respectively. Bars represent mean ± S.E.M. of four to five rats per group. *, P < 0.05 compared with the respective control (one-way ANOVA followed by Bonferroni’s t test).

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on activation of ET_\text{A} receptors, or they can be mediated via both receptor subtypes. Thus, selective ET_\text{A} receptor antagonists prevent overt nociception, i.e., flinches or linking induced by the application of ET-1 subcutaneously into the rat or mouse plantar hindpaws (Piovezan et al., 2000; Gokin et al., 2001) and endothelin-induced potentiation of capsaicin-induced linking in mouse (Piovezan et al., 2000). Likewise, ETA receptor antagonist also prevents the flinches induced by topical application of endothelin in the rat sciatic nerve (Davar et al., 1998; Fareed et al., 2000). However, there is also evidence in the literature demonstrating that activation of both endothelin receptors (ETA and ETB) is responsible for nociception (Raffa et al., 1996). On the other hand, there are also studies demonstrating that ETB receptor, instead of mediating nociceptive response, mediates antinociception. Khodorova et al. (2002, 2003) reported that ET-1-induced flinches are inhibited by a selective ETB agonist (IRL 1620) and are enhanced by ETB receptor antagonist. These apparent discrepancies could be due to differences in experimental nociceptive models and also in the time intervals of the nociceptive responses, which might detect the hypernociception of different sets of primary sensory neurons. The ETA and ETB receptors might play different roles in these sets of primary sensory neurons. In fact, using Khodorova et al. (2002, 2003) experimental design and doses, we confirmed that ET-1-induced flinch depends on ETA activation, whereas ETB mediates antinociception. However, ET-1 in the dose used in the present study did not induce flinches in the rats (data not shown). It seems that in the immediate phase of the overt nociception, ETA mediates nociception, whereas ETB mediates antinociception. Nevertheless, in the later phase of the overt nociception (inflammatory phase) or in mechanical hypernociceptive models, ETB mediates hypernociception. Reinforcing this hypothesis, there is evidence that ET-1, but
not ET-3 and sarafotoxin S6c (ET B agonists), potentiate the first phase of formalin-induced flinches (immediate noninflammatory overt nociception), whereas ET-1, ET-3, and sarafotoxin S6c potentiate the second phase (inflammatory phase) of this test (Piovezan et al., 1997) and also induce long-lasting articular incapacitation in rats when injected in carrageenan-primed knee joints (De-Melo et al., 1998).

The ability of endothelin to induce hypernociception by a mechanism independent of endogenous release of prostaglandin and sympathetic amines points to a direct effect of endothelin on the nociceptor. This view is further substantiated by evidence that endothelin can induce firing in peripheral nociceptors (Gokin et al., 2001), a finding attributable to direct action of endothelin on these cells, activating or potentiating an inward current by suppressing an outward current that contributes to the resting potential (Zhou et al., 2001).

Furthermore, the IL-18-induced mechanical hypernociception was dose dependently inhibited by the local administration of morphine, and this analgesic effect was blocked by naloxone. There is evidence that morphine, besides acting on the action of endothelin on these cells, activating or potentiating an inward current by suppressing an outward current that contributes to the resting potential (Zhou et al., 2001).

In conclusion, we demonstrated here for the first time that IL-18, a key proinflammatory cytokine of pleiotropic functions, induces hypernociceptor mediated by endothelin, acting on ET B receptors, in an opioid-sensitive manner. This finding not only reveals a novel pathway of cytokine-induced hypernociception but also the highly specific nature of the inductive cascade suggests that selective inhibition of the endothelin ET B receptor could be of considerable beneficial potential to the control of hypernociception of inflammatory diseases in which IL-18 plays a dominant role.

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References


