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Blockade of IL-18 signaling diminished neuropathic pain and enhanced the efficacy of morphine and buprenorphine

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ABSTRACT

Currently, the low efficacy of antinociceptive drugs for the treatment of neuropathic pain is a major therapeutic problem. Here, we show the potential role of interleukin (IL)-18 signaling in this phenomenon. IL-18 is an important molecule that performs various crucial functions, including the alteration of nociceptive transmission in response to neuropathic pain. We have studied the changes in the mRNA and protein levels (qRT-PCR and Western blot analysis, respectively) of *IL-18*, *IL-18-binding protein* (*IL-18BP*) and the *IL-18 receptor* (*IL-18R*) over time in rats following chronic constriction injury (CCI) of the sciatic nerve. Our study demonstrated that the spinal levels of *IL-18BP* were slightly downregulated at days 7 and 14 in the rats subjected to CCI. In contrast, the *IL-18* and *IL-18R* mRNA *expression* and protein levels were elevated in the ipsilateral spinal cord on days 2, 7 and 14. Moreover, in rats exposed to a single intrathecal administration of IL-18BP (50 and 100 ng) 7 or 14 days following CCI, symptoms of neuropathic pain were attenuated, and the analgesia pursuant to morphine and buprenorphine (0.5 and 2.5 μg) was enhanced. In summary, the restoration of the analgesic activity of morphine and buprenorphine via the blockade of IL-18 signaling suggests that increased IL-18 pathway may account for the decreased analgesic efficacy of opioids for neuropathic pain.

1. Introduction

Interleukin-18 (IL-18), a member of IL-1 family, is an important regulator of innate and acquired immune responses. IL-18 is known to be expressed in various cell types and is upregulated under pathological conditions, including tissue injury and inflammation (Mika et al., 2013; Xie et al., 2007). IL-18 is a pro-inflammatory cytokine similar in structure and mechanism of action to IL-1 beta. Formation of active IL-18 by macrophages requires cleavage of an inactive precursor by caspase-1 protease, also termed the IL-1 converting enzyme (ICE). The issue of whether IL-18 is a true member of the IL-1 family remains to be resolved, but similarities in its structures, cleavage, receptors and signaling mechanism suggest a close relationship with IL-1 (Dinarello et al., 1998). IL-18, like IL1beta, with which it shares structural homology, is produced as a 24 kDa inactive precursor lacking a signal peptide (Pro-IL-18). Like that of IL-1, the IL-18R complex is a heterodimer containing an α (IL-1Rrp) chain responsible for extracellular binding of IL-18 and a nonbinding, signal-transducing beta (AcPL) chain (Parnet et al., 1996; Novick et al., 1999).

In 2008, Miyoshi et al. showed that the spinal levels of both IL-18 and the IL-18 receptor (IL-18R) are enhanced after L5 spinal nerve ligation. Inhibiting the IL-18 signaling pathways using anti-IL-18 antibodies suppressed nerve injury-induced tactile allodynia (Miyoshi et al., 2008). Moreover, the intrathecal injection of IL-18 induced behavioral, morphological, and biochemical changes similar to those observed after peripheral nerve injury (Miyoshi et al., 2008). Therefore, IL-18 appears to be an important pronociceptive factor. Based on immunohistological studies, Miyoshi et al. (2008) and Daigo et al. (2012) suggested that IL-18 mediates spinal microglia-astrocyte interactions. IL-18 is known to be upregulated in several human autoimmune and inflammatory diseases; therefore, this factor might represent a novel therapeutic target (Plater-Zyberk et al., 2001; Ten Hove et al., 2001; Carrascal et al., 2003; Boraschi and Dinarello, 2006; Novick et al., 1999). IL-18 is known to be regulated by its endogenous inhibitor, IL-18 binding protein (IL-18BP), which is constitutively expressed and secreted (Novick et al., 1999; Kim et al., 2000). IL-18BP binds to IL-18 with high affinity and blocks its biological activity at a 1:1 M ratio (Kim et al., 2000). In our opinion, this naturally occurring molecule represents an interesting inhibitor that warrants investigation in neuropathic pain models. It has previously been shown that neutralizing IL-18 using IL-18BP reduces systemic or local inflammation (Plater-Zyberk et al., 2001; Ten Hove et al., 2001; Carrascal et al., 2003; Boraschi and Dinarello, 2006) and that certain pronociceptive cytokines, such as IL-1beta, block the

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analgesic effects of opioids (Gul et al., 2000; Szabo et al., 2002; Shavit et al., 2005). We have previously shown that the analgesic activity of morphine and buprenorphine can be restored via the blockade of IL-1beta signaling during neuropathic pain (Pilat et al., 2015). In 2012, Chen et al. found that chronic morphine treatment induced an increase in the mRNA expression of IL-18 in microglia and IL-18R in astrocytes; these authors hypothesized that both factors may be responsible for the development of opioid tolerance. It is known that opioid tolerance and neuropathic pain share the characteristic of diminished morphine analgesia, resulting in the suggestion of a common underlying mechanism (Mao et al., 1995; Mayer et al., 1999; Raghavendra et al., 2002; Watkins et al., 2005).

In the present study, we used qRT-PCR to analyze changes in the transcription of the *IL-18*, *IL-18R* and *IL-18BP* genes in the ipsilateral dorsal lumbar spinal cord of rats 2, 7 and 14 days following chronic constriction injury (CCI) to the sciatic nerve. Moreover, we measured the protein levels of these factors using Western blot analysis. A further goal of this study was to determine how the blockade of IL-18 signaling via the intrathecal administration of IL-18BP, which influences the neuropathic pain symptoms of rats, influences the antinociceptive activity of morphine and buprenorphine in rats subjected to CCI.

2. Materials and methods

2.1. Animals

Male Wistar rats (200–350 g) were housed in cages lined with sawdust under a standard 12/12 h light/dark cycle (lights on at 08:00) with food and water ad libitum. All experiments were performed in accordance with the recommendations of the International Association for the Study of Pain (Zimmermann, 1983) and the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local Bioethics Committee (Krakow, Poland).

2.1.1. Intrathecal catheter implantation

The rats were chronically implanted with intrathecal (*i.t.*) catheters according to the methods of Yaksh and Rudy (1976) under pentobarbital anesthesia (60 mg/kg; *i.p.*). The catheter (PE 10, INTRAMEDIC, Clay Adams, Becton Dickinson and Company, Rutherford, NJ, USA.) was flushed with 70% ethanol and then with sterile water prior to insertion. It was carefully introduced through the atlanto-occipital membrane to the subarachnoid space at the rostral level of the spinal cord lumbar enlargement (L4-L6). CCI of the sciatic nerve was performed 5 days after catheter implantation. The intrathecal injections were performed 7 and 14 days after CCI.

2.1.2. Chronic constriction injury

CCI was performed according to Bennett and Xie (1988). The right sciatic nerve was exposed under sodium pentobarbital anesthesia (60 mg/kg; *i.p.*). Four ligatures (4/0 silk) were generated around the nerve distal to the sciatic notch at 1-mm intervals until a brief twitch in the corresponding hind limb was observed. After CCI, the rats developed allodynia and hyperalgesia.

2.1.3. Drug administration and the experimental scheme

The chemicals used and their sources were as follows: recombinant IL-18BP (50, 100 ng *i.t.*; R&D Systems; Biokom, Poland), morphine hydrochloride (0.5, 2.5 μg *i.t.*; Polfa Kutno, Poland); buprenorphine (0.5, 2.5 μg *i.t*; Polfa Kutno, Poland) and naloxone (5 μg i.t.; Tocris, Poland). All drugs were dissolved in sterile water. The control groups received vehicle injection according to the same schedule. The response to IL-18BP was measured 2 h after a single administration on days 7 and 14 using the von Frey and cold plate tests. Afterwards, behavioral tests (allodynia, 30 min. And hyperalgesia, 35 min.) were assessed in the ipsilateral paw after morphine or buprenorphine injection. The naloxone was administered 15 min before an IL-18BP (100 ng) and the antiallodynic and antihyperalgesic properties were measured by von Frey and cold plate tests 7 days after chronic constriction injury of the rat sciatic nerve.

2.2. Behavioral tests

2.2.1. Tactile allodynia (von Frey test)

Allodynia was measured in the rats subjected to CCI using an automated von Frey apparatus (Dynamic Plantar Anesthesiometer, Cat. No. 37400, Ugo Basile, Italy) just as it was previously published (Makuch et al., 2013; Rojewska et al., 2014). The rats were placed in plastic cages with a wire net floor 5 min before the experiment. The rats were moving freely on the surface of the metal mesh in an enclosed area. Was moved the machine's touch stimulator until under the animal and using an angled adjustable mirror the filament was placed below the surface of the paw. The von Frey filament was applied to the midplantar surface of the hind foot, and measurements were taken automatically. The animal's response, which was paw withdrawal reflex, was recorded automatically using the following metric: the force at which the paw was withdrawn, in grams. The strength of the von Frey stimuli was up to 26 g.

2.2.2. Cold hyperalgesia (cold plate test)

Hyperalgesia was assessed using a Cold/Hot Plate Analgesia Meter (No. 05,044, Columbus Instruments, USA). The temperature of the cold plate was maintained at 5 °C. The animals were placed on the cold plate, and the time until lifting the hind paw was recorded; because the ipsilateral paw of the rats subjected to CCI always reacts first to a cold stimulus, this response reflects the reactivity of the ipsilateral hind paw. The cut-off latency was 30s.

2.2.3. Nociceptive threshold (tail flick test)

The tail-flick test is performed to evaluate the pain threshold to a thermal stimulus by a Tail-Flick Analgesic Meter (Analgesia Meter; Ugo Basile, Comerio, Italy), which is a standard method used in our study (Mika et al., 2009b). During the procedure, an animal is placed on the apparatus surface and gently held by an experimenter. In this test, a beam of light is focused on a dorsal tail surface, at 2 cm from the tail tip. When the animal flicks its tail, the timer stops automatically and the recorded time (latency) is measured. The animals were tested only once in this test, the cut-off time was 9 s and the control group responded to 3.8 ± 0.15.

2.3. Microglial and astroglial cell cultures

Primary cultures of microglia and astroglia were prepared from 1-day-old Wistar rat pups as previously described by Zawadzka and Kaminska (2005). Briefly, the cells were isolated from the rat cerebral cortices and were plated at a density of 3×10^5 cells/cm^2 in culture medium consisting of high glucose Glutamax DMEM (Gibco, USA) supplemented with heat-inactivated 10% fetal bovine serum (Gibco, USA), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (Gibco, USA) on poly-L-lysine (1 mg/ml; Po282 Sigma)-coated 75-cm^2 culture flasks and were maintained at 37 °C in 5% CO2. The culture medium was replaced after 3 days. The loosely adherent microglia were recovered after 9 days via mild shaking and centrifugation. After replacing the media to remove the no adherent cells, primary glia in 75-cm^2 culture flasks were placed on a rotary shaker and shaken at 37 °C for 24 h (200 rpm). The culture medium, which contained oligodendrocytes, was removed, and the astrocytes were plated on 10-cm^2 culture dishes and were maintained for 3 days at 37 °C in 5% CO2. Then, the astrocytes were trypsinized (0.005% Trypsin EDTA solution T4049; Sigma-Aldrich, USA). The microglia and astrocytes were suspended in culture medium and plated at a final density of 2×10^5 cells on 24-well plates for mRNA analysis. The adherent cells were incubated for 48 h in culture medium before analysis. The primary microglial and astrocyte cultures were treated with lipopolysaccharide (LPS, 100 ng/ml, Sigma-Aldrich, USA)