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IL-1 receptor antagonist improves morphine and buprenorphine efficacy in a rat neuropathic pain model

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ABSTRACT

An interesting research and therapeutic problem is the reduced beneficial efficacy of opioids in the treatment of neuropathic pain. The present study sought to investigate the potential role of IL-1 family members in this phenomenon. We studied the time course of changes in *IL-1alpha*, *IL-1beta*, *IL-1 receptor type I* and *IL-1 receptor antagonist* mRNA and protein levels experienced by rats after chronic constriction injury (CCI) of the sciatic nerve using qRT-PCR and Western blot analysis. In CCI-exposed rats, spinal levels of *IL-1alpha* mRNA were slightly downregulated on the 7th day, and protein levels were not changed on the 7th and 14th days. Levels of *IL-1 receptor antagonist* and *IL-1 receptor type I* were slightly upregulated in the ipsilateral part of the spinal cord on the 7th and 14th days; however, protein levels were not changed at those time points. Interestingly, we observed that *IL-1beta* mRNA and protein levels were strongly elevated in the ipsilateral part of the dorsal spinal cord on the 7th and 14th days following CCI. Moreover, in rats exposed to a single intrathecal administration of an IL-1 receptor antagonist (100 ng *i.t.*) on the 7th and 14th day following CCI, symptoms of neuropathic pain were attenuated, and the analgesic effects of morphine (2.5 μg *i.t.*) and buprenorphine (2.5 μg *i.t.*) were enhanced. In summary, restoration of the analgesic activity of morphine and buprenorphine by blockade of IL-1 signaling suggests that increased IL-1beta responses may account for the decreased analgesic efficacy of opioids observed in the treatment of neuropathy.

1. Introduction

Neuropathic pain originates from damage or disease affecting the somatosensory system. Its treatment is imperfect; it appears refractory to most analgesics, including opioids. Surprisingly, some studies have suggested that, repeated morphine administration activates glia (Song and Zhao, 2001; Raghavendra et al., 2002; Cui et al., 2006) and increases spinal tumor necrosis factor (TNF)-alpha, interleukin (IL)-1beta and IL-6 expression in healthy animals (Raghavendra et al., 2002; Johnston et al., 2004). These pronociceptive cytokines block the analgesic effects of opioids (Gul et al., 2000; Szabo et al., 2002; Shavit et al., 2005) and have been linked to the development of paradoxical pain symptoms. It has been shown that in naïve mice, the administration of IL-1beta abolishes morphine analgesia (Shavit et al., 2005). Likewise, in a model of painful diabetic neuropathy, it has been suggested that the decreased analgesic effect of morphine is related to IL-1beta (Gul et al., 2000). There is growing recognition that neuropathic pain is a frequent manifestation of autoimmune and neurodegenerative diseases, and one key player believed to drive this pathological process is IL-1beta, which is upregulated in diseases such as Alzheimer's disease, multiple sclerosis (Schrijver et al., 1999), and epilepsy (Vezzani et al., 1999). IL-1beta signals through the type I IL-1 receptor/IL-1 accessory protein complex, leading to nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)-dependent transcription of pronociceptive cytokines such as TNF-alpha and IL-6 (Hopkins and Rothwell, 1995; Rothwell, 1997).

Remarkably, intrathecal administration of an IL-1 receptor antagonist has been shown to prevent neuronal apoptosis and consequently, to diminish the development of neuropathic pain symptoms (Relton and Rothwell, 1992; Sweitzer et al., 2001; Mika et al., 2008). This finding is in agreement with the observation that intrathecal administration of IL-1beta induces hyperalgesia in rats (Malcangio et al., 1996; Oka et al., 1993; Mika et al., 2008), in contrast to IL-1alpha, which is able to attenuate the symptoms of neuropathic pain after sciatic nerve injury (Mika et al., 2008). Although it has been suggested that nerve injury-induced activation of some members of the IL-1 family is important for the development of pain, to the best of our knowledge, the corresponding changes in mRNA and protein levels under neuropathy have not

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been studied. Identification of the precise mechanism underlying the development and maintenance of allodynia and hyperalgesia appears to be essential for the development of effective strategies for the pharmacological treatment of neuropathic pain states.

The aim of our study was to investigate the potential role of IL-1 family members in the development of neuropathic pain. In the present study, we first used qRT-PCR to analyze changes in the transcription of the *IL-1alpha, IL-1beta, IL-1 receptor I* and *IL-1 receptor antagonist* genes in the ipsilateral part of the dorsal lumbar spinal cord on the 7th and 14th days following chronic constriction injury (CCI) to the sciatic nerve in rats. Moreover, we measured their protein levels using Western blot analysis. A further goal of this study was to determine how blockade of the actions of the IL-1 family by the administration of IL-1 receptor antagonists, which we have previously shown to influence neuropathic pain symptoms in rats (Mika et al., 2008), influences the antinociceptive effects of morphine and buprenorphine in CCI-exposed rats.

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 h) with food and water *ad lib*. All experiments were performed according to the recommendations of the IASP (Zimmermann, 1983) and the NIH Guide for the Care and Use of Laboratory Animals, and all were approved by the local Bioethics Committee (Krakow, Poland).

2.2. 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 to the subarachnoid space through the atlanto-occipital membrane at the rostral level of the lumbar enlargement of the spinal cord (L4–L6). The CCI to the sciatic nerve was performed five days after catheter implantation. Intrathecal injections were carried out on the 7th and 14th days after the CCI.

2.3. Chronic constriction injury

The delivery of a 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 made around the nerve distal to the sciatic notch with 1 mm spacing until a brief twitch in the respective hind limb was observed. After CCI, the rats developed long-lasting allodynia and hyperalgesia.

2.4. Drug administration and the experimental scheme

Chemicals and their sources were as follows: recombinant rat IL-1ra antagonist (100 ng *i.t.*; R&D Systems; Biokom, Poland); morphine hydrochloride (2.5 μg *i.t.*; Polfa Kutno, Poland) and buprenorphine (2.5 μg *i.t.*; Polfa Kutno, Poland). All drugs were dissolved in sterile water. We chose the doses of *i.t.* administration for the IL-1 receptor antagonist and the time of behavioral testing based on papers from Sung et al. (2004) and Mika et al. (2008). The control groups received vehicle injections according to the same schedule. The response to the IL-1 receptor antagonist was measured 2 h after the single administration on the 7th and 14th days by the von Frey and cold plate tests. Afterwards, allodynia and hyperalgesia were assessed in the ipsilateral paw 30 min after morphine or buprenorphine injection.

2.5. Behavioral tests

2.5.1. Tactile allodynia (von Frey test)

Allodynia (pain due to a stimulus that does not normally provoke pain) was measured in the CCI-exposed rats with the use of an automatic von Frey apparatus (Dynamic Plantar Anesthesiometer Cat. no. 37400, Ugo Basile, Italy). The animals were placed in plastic cages with wire-net floors. The von Frey filaments were applied to the midplantar surface of the hind paw, and measurements were taken automatically as described previously by Mika et al. (2008). We tested the ipsilateral paws two times at 3-min intervals using von Frey filaments, and the mean value was calculated. The strength of the von Frey stimuli ranged up to 26 g.

2.5.2. Thermal hyperalgesia (cold plate test)

Thermal hyperalgesia (increased sensitivity to pain; in this case, to low temperature) was assessed using a Cold/Hot Plate Analgesia Meter (No. 05044 Columbus Instruments, USA). The temperature of the cold plate was kept at 5 °C. The animals were placed on the cold plate, and the time until the hind paw was lifted was recorded. In control rats, the reaction time of the first reacting hindpaw (left or right) was recorded, and this group of animals reacted after 29.4 ± 0.3 s. In CCI-exposed rats, the injured (right) paw always reacts first; therefore, the effect of the treatment is presented as the reaction of the ipsilateral hind paw. The cut-off latency was 30 s.

2.6. Biochemical tests

Spinal cord tissue for biochemical analysis was collected on the 7th and 14th days after the CCI. Lumbar (L5–L6) fragments of the spinal cord were removed immediately after the rats were decapitated, and the ipsilateral dorsal part was dissected. The tissue samples were processed for qRT-PCR and the Western blot procedure.

2.6.1. Analysis of gene expression by qRT-PCR

The tissue samples were placed in individual tubes containing the tissue storage reagent RNAlater (Qiagen Inc.) and were stored at −70 °C for RNA isolation. Total RNA was extracted using TRIzol reagent (Invitrogen), as previously described (Chomczynski and Sacchi, 1987). RNA concentrations were measured using a NanoDrop ND-1000 Spectrometer (NanoDrop Technologies), and RNA quality was determined by chip-based capillary electrophoresis using an RNA 6000 Nano LabChip Kit and an Agilent Bioanalyzer 2100 (Agilent) according to the manufacturer's instructions. Reverse transcription was performed on 2 μg of total RNA using Omniscript reverse transcriptase (Qiagen Inc.) at 37 °C for 60 min. Reverse transcription (RT) reactions were carried out in the presence of an RNase inhibitor (RNasin, Promega) and an oligo (dT16) primer (Qiagen Inc.). The cDNA was diluted 1:10 with H_2O, and for each reaction, ~50 ng of cDNA synthesized from the total RNA of an individual animal was used for the quantitative real-time PCR (qPCR) reaction. qPCR was performed using Assay-On-Demand TaqMan probes according to the manufacturer's protocol (Applied Biosystems), and the reactions were run on an iCycler device (BioRad, Hercules). The following TaqMan primers and probes were used: Rn00566700_m1 (IL-1alpha); Rn00580432_m1 (IL-1beta); Rn02586400_m1 (IL-1 receptor antagonist); Rn00565482_m1_m1 (IL-1 receptor type I); and Rn01527838_g1 (hypoxanthine guanine phosphoribosyl transferase). The expression of