European Journal of Pharmacology 697 (2012) 40–46

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Intrathecal endothelin-1 has antinociceptive effects in rat model of postoperative pain

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ARTICLE INFO

Article history:
Received 24 May 2012
Received in revised form
10 September 2012
Accepted 22 September 2012
Available online 3 October 2012

Keywords:
Dorsal root ganglion neuron
Endothelin-1
Extracellular signal-regulated protein kinase
Microglia
Postoperative pain

ABSTRACT

Endothelin-1 is known to be a potent vasoconstrictor. Administration of endothelin-1 to the central nervous system (CNS) induces antinociceptive effects. Nociceptive stimuli affect dorsal root ganglion (DRG) neurons and neurons/astrocytes/microglia in the dorsal horn of the spinal cord. Surgical incision in the plantar aspect of the rat hindpaw is a model for postoperative pain, and withdrawal thresholds reportedly decrease around the incision. We hypothesized that intrathecal endothelin-1 would have antinociceptive effects in this model, and affect DRG neurons and microglia/neurons in the dorsal horn. Intrathecal endothelin-1 partially restored the withdrawal threshold (which was decreased by plantar incision). BQ-123, and BQ-788 (specific endothelin ET_A- and ET_B-receptor antagonists, respectively) attenuated the increase in withdrawal threshold induced by endothelin-1. Phosphorylation of extracellular signal-regulated kinase (ERK) in DRG neurons and microglial activation/ERK phosphorylation in the dorsal horn were observed following the incision. Endothelin-1 decreased the incision-induced increase in the numbers of phosphorylated ERK-positive neurons in DRG and activated microglia in the dorsal horn, without affecting the numbers of phosphorylated ERK-positive neurons in the dorsal horn. BQ-123 or BQ-788 partially suppressed these endothelin-1-induced alterations. Our results show that the pain threshold, which is decreased by surgical stimuli, is partially restored by intrathecal endothelin-1 through both endothelin ET_A- and ET_B- receptors in DRG neurons and microglia in the spinal cord. Endothelin-1 administration to the CNS may be worth considering as a new candidate for the treatment of postoperative pain and to mitigate prolonged periods of pain.

1. Introduction

Endothelin-1 is a potent vasoconstrictor (Schinelli, 2006) now recognized to be implicated in many physiological or pathological functions (Schinelli, 2006; Watts, 2010). By acting within the central nervous system (CNS), endothelin-1 may play pivotal roles in the regulation of the vascular tone of cerebral arteries, the development of brain cells, the control of physiological functions outside the brain, and pain modulation (Khodorova et al., 2009; Schinelli, 2006). It is widely known that local administration of endothelin-1 to peripheral tissue or nerves causes pain (Hans et al., 2009; Khodorova et al., 2009). In contrast, its application to the CNS induces antinociception in both acute-pain and inflammatory-pain models (Khodorova et al., 2009).

Endothelins are well known to exert their effects through two subtypes of receptors (ET_A and ET_B; Khodorova et al., 2009; Schinelli, 2006; Watts, 2010). Endothelin-1 binds to both receptors equally (Khodorova et al., 2009; Schinelli, 2006). Within the CNS, the distributions of these two receptors have been investigated in the endothelial cells of brain blood vessels, in neurons, and in glia (Khodorova et al., 2009; Schinelli, 2006). The intracerebroventricular endothelin-1-induced prolongation of paw-withdrawal latencies in the hot-plate test in mice is reportedly mediated through endothelin ET_A receptors (Hasue et al., 2004). Additionally, intrathecal endothelin-1 depresses the flinching behavior elicited by plantar subcutaneous formaldehyde injection in rat through the endothelin ET_A receptors (Yamamoto et al., 1994).

The pain caused by inflammation or nerve injury is mediated via different pathophysiological mechanisms (Honore et al., 2000). Various stimuli – such as inflammation, nerve injury, or incision – are known to affect dorsal root ganglion (DRG) neurons and neurons/astrocytes/microglia in the dorsal horn of the spinal cord (Dai et al., 2004; Honore et al., 2000; Ito et al., 2009; Ji et al.,

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0014-2999/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2012.09.035

2009; Obata et al., 2006; Obata and Noguchi, 2004). It is generally recognized that the mitogen-activated protein (MAP) kinase superfamily are central elements used by mammalian cells to transduce diverse messages originating from various stimuli (Kyriakis and Avruch, 2001). Within the CNS, MAP kinases play such crucial roles as regulation of pain and inflammatory responses, neural plasticity, and cell death (Ji et al., 2009). Among the MAP kinases, activation of extracellular signal-regulated protein kinase (ERK) reportedly has an important role in persistent hyperalgesia, which induces chronic pain (Obata and Noguchi, 2004). It is known that peripheral inflammation or nerve injury activates ERK in DRG neurons and in spinal cord dorsal horn neurons (Dai et al., 2004; Wang et al., 2004). In the rat postoperative pain model with surgical incision in the hindpaw plantar area, microglia in the spinal cord dorsal horn are activated after the incision (Wen et al., 2011). However, the effects of surgical incision on DRG neurons or on neurons and microglia in the dorsal horn remain to be clarified.

In the present study, we hypothesized that endothelin-1 would normalize the withdrawal threshold in the rat postoperative pain model and modulate: (a) ERK phosphorylation within neurons in DRG and the dorsal horn and (b) microglial activation in the dorsal horn.

2. Material and methods

2.1. Rat plantar incision

The study accorded with the Guidelines Concerning Experimental Animals issued by the Japanese Association for Laboratory Animal Science. Male Sprague-Dawley rats (age, 6–8 weeks; weight, 180–230 g) were housed in groups of three or four per cage and acclimatized to the laboratory conditions for 1 week before the behavioral experiments. Efforts were made to minimize both animal suffering and the number of animals.

All animals were habituated to the testing environment for 3 days before baseline testing of the withdrawal threshold. Rats were placed in the measuring cage for 30 min acclimation, then anesthetized with sevoflurane. 100 nM endothelin-1 20 μl or 1.5 μM PD98059 (2′-amino-3′-methoxyflavone) 20 μl (Calbiochem-Novabiochem, Co., La Jolla, CA) was injected intrathecally via the L3–L4 intervertebral space in each animal. When required, 20 nM BQ-123 [cyclo(D-Trp-D-Asp-Pro-D-Val-Leu)] 20 μl or 10 nM BQ-788 (N-cis-2,6-dimethylpiperidinocarbonyl-L-gamma-methylleucyl-D-1-methoxycarbonyltrptophanyl-D-Nle) 20 μl (American Peptide, Inc., Ibaraki, Japan) was administered simultaneously with the endothelin-1. The plantar surgery was performed as previously described (Brennan et al., 1996). Briefly, a 1-cm longitudinal plantar incision of the left hindpaw was made, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally, with the muscle origin and insertion remaining intact. The skin edges were apposed. Then, the animal was allowed to recover from the anesthesia. The incision was checked daily, and upon any sign of wound infection or dehiscence the animal was excluded from the study. Endothelin-1, BQ-123, BQ-788, and PD98059 were each dissolved in saline for injection.

2.2. Measurement of withdrawal threshold

Withdrawal threshold values were determined using calibrated von Frey filaments with logarithmic incremental stiffness (0.6–26.0 g), and 50% probability withdrawal thresholds were calculated. The withdrawal thresholds were measured before induction of anesthesia and at the indicated time-points after endothelin-1 or PD98059 administration. The experiment did not start until the baseline value was roughly 20 g for the 50% probability withdrawal threshold.

2.3. Immunohistochemistry

For immunohistochemistry, rats were deeply anesthetized with sevoflurane at 30 min or 2 h after the incision, a thoracotomy was performed and they were perfused transcardially with 0.1 M phosphate-buffered saline (PBS) followed by paraformaldehyde at 4 °C. The L4 spinal segment and L4 DRGs were carefully removed and postfixed in the same fixative at 4 °C, followed by immersion in 20% sucrose in 0.1 M PBS overnight for cryoprotection. DRGs and the spinal segments were cut on a cryostat to 16 μm and 30 μm thickness, respectively, and the sections placed in 0.1 M PBS. The floating sections were pre-incubated in PBS containing 2% normal goat serum and 0.3% Triton X-100 for 1 h at room temperature. For the counting of phosphorylated ERK-positive neurons in DRGs and activated microglia in spinal segments, DRGs or spinal segments were incubated at 4 °C overnight with, respectively, a phospho-specific ERK antibody (1:500; Cell Signaling Inc., Beverly, MA), or a rabbit antibody against ionized calcium-binding adapter molecule 1 (Iba1; 1:1000; Wako Pure Chemical Industries Ltd., Tokyo, Japan). Then, the sections were incubated with the goat biotinylated anti-rabbit IgG antibody Alexa Fluor 546® (1:400; Invitrogen Corporation Inc., Carlsbad, CA) for 1 h at room temperature. For the counting of phosphorylated ERK-positive neurons in spinal segments, immunohistochemistry was performed using the ABC method (Dai et al., 2004). Briefly, the floating sections were pre-incubated for 1 h at room temperature in PBS containing 2% normal goat serum and 0.1% Tris-buffered saline, then incubated at 4 °C for 24 h in rabbit primary antibody for phospho-specific ERK (1:1500) in PBS containing 5% normal goat serum. After washing, the sections were incubated overnight at 4 °C in goat biotinylated anti-rabbit IgG antibody (1:400; Vector Laboratories Inc., Burlingame, CA). Then, the sections were incubated for 2 h at 4 °C in avidin-biotinylated horseradish peroxidase macromolecular complex (Vector Laboratories Inc., Burlingame, CA). The horseradish peroxidase reaction was developed in 3,3′-diaminobenzidine tetrahydrochloride (Dako Japan Inc., Tokyo, Japan). All DRG and spinal-segment sections were then washed, mounted on slides, air-dried, dehydrated via an alcohol gradient, cleared in xylene, and coverslipped.

The stained sections were examined under using a fluorescence microscope (Nikon, Tokyo, Japan), and images were captured using a charge-coupled-device spot camera. Counting in each section was done at 100× magnification. The numbers of immunoreactive neurons of all sizes positive for phosphorylated ERK in DRGs and in laminae I and II of the dorsal horn or of immunoreactive microglia positive for Iba1 in laminae I and II were counted.

2.4. Statistical analysis

Withdrawal thresholds (50% probability values) or immunostaining quantifications were compared by repeated measurements of one-way ANOVA followed by Fisher's least significant difference test. All data are presented as the mean ± S.E.M. P values less than 0.05 were considered significant.

3. Results

3.1. Effect of intrathecal endothelin-1 administration on withdrawal threshold (rat plantar-incision test)

It has been reported that in the rat plantar-incision test, the withdrawal threshold is decreased from 2 h until up to 3 days