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Antiallodynic effect through spinal endothelin-B receptor antagonism in rat models of complex regional pain syndrome

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HIGHLIGHTS

- Each CRPS-I and -II rat model was made by O-ring application or by spinal nerve ligation.
- The level of ET-1 in the spinal cord was increased in both CRPS models.
- Intrathecal ET-B receptor antagonist increased the withdrawal threshold in both CRPS types.
- Intrathecal ET-A receptor antagonist did not affect the withdrawal threshold in either CRPS type.
- Intrathecal ET-B receptor antagonist decreased the spinal ET-1 level in both CRPS rats.

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ABSTRACT

Complex regional pain syndrome (CRPS) is a very complicated chronic pain disorder that has been classified into two types (I and II). Endothelin (ET) receptors are involved in pain conditions at the spinal level. We investigated the role of spinal ET receptors in CRPS. Chronic post-ischemia pain (CPIP) was induced in male Sprague–Dawley rats as a model for CRPS-I by placing a tourniquet (O-ring) at the ankle joint for 3 h, and removing it to allow reperfusion. Ligation of L5 and L6 spinal nerves to induce neuropathic pain was performed as a model for CRPS-II. After O-ring application and spinal nerve ligation, the paw withdrawal threshold was significantly decreased at injured sites. Intrathecal administration of the selective ET-B receptor antagonist BQ 788 dose-dependently increased the withdrawal threshold in both CRPS-I and CRPS-II. In contrast, ET-A receptor antagonist BQ 123 did not affect the withdrawal threshold in either CRPS type. The ET-1 levels of plasma and spinal cord increased in both CRPS types. Intrathecal BQ 788 decreased the spinal ET-1 level. These results suggest that ET-1 is involved in the development of mechanical allodynia in CRPS. Furthermore, the ET-B receptor appears to be involved in spinal cord-related CRPS.

1. Introduction

Complex regional pain syndrome (CRPS) is a debilitating chronic pain disorder that is difficult to treat satisfactorily [12]. Therefore, clinicians often experience emotional stress while treating such patients, while the patients often feel frustration and suffer a loss of quality of life. CRPS is classified into type I (previously called reflex sympathetic dystrophy) and type II (previously termed causalgia) [13]. CRPS-I occurs after fracture, soft tissue injury, or crush injury [5]. CRPS-II is similar, but also exhibits clinically verified nerve injury [25]. Typical symptoms of CRPS are allodynia and hyperalgesia [14].

Although the underlying mechanisms of CRPS have been studied increasingly over the past decade, knowledge of the detailed mechanisms is lacking because of the complex pathophysiology of this syndrome. Therefore, CRPS treatment remains a problematic issue and effective studies that shed more light on this disorder are clearly needed.

Recently, the chronic post-ischemia pain (CPIP) model was proposed as a CRPS-I animal model [4]. A classical neuropathic pain model induced by spinal nerve ligation (SNL) may be considered an animal model of CRPS-II.

Endothelins (ET) are one of the many signaling systems involved in various chronic pain conditions. Behaviorally, ET-1 induces pain,

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whereas local or systemic ET-receptor antagonists attenuate a variety of nociceptive states [1,3,7,11,15,18–20,26]. However, little is known about the role of ET receptors found in the spinal cord in nociception [23].

Therefore, we investigated the modulatory role of spinal ET receptors using rat models of CRPS-I and CRPS-II, as well as the possible modification of the ET-1 levels in plasma and spinal cord.

2. Materials and methods

2.1. Animal preparation

This study was approved by The Institutional Animal Care and Use Committee of Chonnam National University. Male Sprague–Dawley rats weighing 100–120 and 320–340 g were used in all experiments. While in the home–cage environment, the animals had free access to a standard rat diet and tap water. Room temperature was maintained at 20–23 °C with a 12:12-hour light/dark cycle.

2.2. Animal model of CRPS-I (CPIP) and CRPS-II (SNL)

Both CRPS-I model and CRPS-II model were produced by O-ring application and by spinal nerve ligation as described previously [4,17]. In CRPS-I, a Nitrile 70 Durometer O-ring (Orings West, Seattle, WA, USA) with 7/32 in. internal diameter was placed around the rat's left hind limb just proximal to the ankle joint for 3 h under sevoflurane anesthesia, and then removed. In CRPS-II, the left L5 and L6 spinal nerves of rats were isolated adjacent to the vertebral column during sevoflurane anesthesia and tightly ligated with a 6-0 silk suture. Sham rats received the same procedure, except that the O-ring was cut so that it fit loosely around the ankle without occluding the blood flow to the hind paw or without ligation of the spinal nerves. Animals were considered to have CRPS-I and CRPS-II when a paw-flinching response occurred upon applying a bending force of <5 and <4 g, respectively. Following procedure, the mechanical sensitivity of the injured paw was daily evaluated for 21 days.

2.3. Implantation of intrathecal catheter

At 2 and 5 days after hind paw ischemia and reperfusion or spinal nerve ligation, a polyethylene-10 tube was inserted into the subarachnoid space through a slit made in the atlantooccipital membrane under sevoflurane anesthesia [27]. Rats with neurological deficit after catheterization were excluded and euthanized immediately with an overdose of volatile anesthetics. There was a recovery period of 5 days after catheterization before commencing the behavioral study.

2.4. Drugs

Cyclo[D-Trp-D-Asp-Pro-D-Val-Leu] (BQ 123, Tocris Cookson Ltd., Bristol, UK) was used as an ET-A receptor antagonist while N-[(cis-2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl-1-(methoxycarbonyl)-D-tryptophyl-D-norleucine sodium salt (BQ 788, Tocris) was administered as an ET-B receptor antagonist. BQ 123 and BQ 788 were dissolved in 0.9% saline or dimethylsulfoxide (DMSO), respectively. Intrathecal administration of these agents was performed using a hand-driven, gear-operated syringe pump. The drugs were delivered as a 10 μL solution, followed by an additional 10 μL normal saline to flush the catheter.

2.5. Assessment of mechanical allodynia

To determine withdrawal threshold, rats were placed individually in plastic cages with a plastic mesh floor. The animals were tested after acclimation to the environment, typically 20–30 min after placement in the cage. The paw withdrawal threshold in response to mechanical stimulation was measured using the up and down method [2] by applying calibrated von Frey filaments (Stoelting, Wood Dale, IL, USA) to the hind paw from underneath the cage through openings in the mesh floor. A series of eight von Frey filaments (0.4, 0.7, 1.2, 2.0, 3.6, 5.5, 8.5, and 15 g) were applied vertically to the plantar surface of the hind paw for 5 s while the hair was bent. Brisk withdrawal or paw flinching was considered a positive response. The absence of a response in the animals at a pressure of 15 g was considered the cutoff value.

2.6. Experimental paradigm

The rats were allocated to receive BQ 123 or BQ 788 on the day of the experiment, while the control animals received solvent alone (saline or DMSO, respectively). Animals were tested only once. All experiments were carried out by an observer blinded to the drug treatments.

2.7. Effects of intrathecal BQ 123 and BQ 788

The effects of the ET-A receptor antagonist BQ 123 (20 and 50 μg) and the ET-B receptor antagonist BQ 788 (10, 20, and 40 μg) were investigated in CPIP and neuropathic pain-state rats. Measurement of the mechanical threshold prior to ischemia–reperfusion induction or spinal nerve ligation was taken as the baseline threshold. The withdrawal threshold was determined at 15, 30, 60, 90, 120, 150, and 180 min after intrathecal administration of the drugs. The withdrawal threshold measured immediately before intrathecal delivery of drugs was taken as the control. The highest drug doses were selected based on their lack of neurologic impairment with maximal solubility from pilot experiments. Hence, the highest drug doses administered were considered the maximum doses.

2.8. Measurement of ET-1 levels

The levels of ET-1 in plasma and spinal cord were measured in sham, CRPS and ET-B receptor antagonist BQ 788-delivered rats. In CRPS models, ET-1 levels were determined 7 and 10 days after ischemia–reperfusion or spinal nerve ligation, and 60–90 min after BQ 788 administration. The ET-1 level was quantified using ELISA kits obtained from Assay Designs (Ann Arbor, Michigan, USA).

2.9. General behavior

Behavioral changes in response to BQ 123 and BQ 788 treatment were evaluated in additional rats 5, 10, 20, 30, 40, 50, and 60 min after intrathecal administration of the highest drug doses. Motor functions were determined by examining the righting and placing-stepping reflexes. Righting was evaluated by placing the rat horizontally with its back on a table, which normally gives rise to an immediate coordinated twisting of the body to an upright position. Placing-stepping reflexes were evoked by drawing the dorsum of either hind paw across the edge of the table. Rats normally attempt to place their paws forward into a walking position. Pinna and corneal reflexes was also evaluated and considered present or absent. Abnormal behavior, including serpentine movement or tremors, was also monitored.