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Hemopressin, an inverse agonist of cannabinoid receptors, inhibits neuropathic pain in rats

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ABSTRACT

Direct-acting cannabinoid receptor ligands are well known to reduce hyperalgesic responses after nerve injury, although their psychoactive side effects have damped enthusiasm for their therapeutic development. Hemopressin (Hp) is a nonapeptide that selectively binds CB1 cannabinoid receptors (CB_1 receptors) and exerts antinociceptive action in inflammatory pain models. We investigated the effect of Hp on neuropathic pain in rats subjected to chronic constriction injury (CCI) of the sciatic nerve, and explored the mechanisms involved. Oral administration of Hp inhibits mechanical hyperalgesia of CCI-rats up to 6 h. Hp treatment also decreases Egr-1 immunoreactivity (Egr-1Ir) in the superficial layer of the dorsal horn of the spinal cord of CCI rats. The antinociceptive effect of Hp seems to be independent of inhibitory descending pain pathway since methysergide ($5HT_{1A}$ receptor antagonist) and yohimbine (α-2 adrenergic receptor antagonist) were unable to prevent Hp antinociceptive effect. Hp decreased calcium flux on DRG neurons from CCI rats, similarly to that observed for AM251, a CB_1 receptor antagonist. We also investigated the effect of Hp on potassium channels of CCI rats using UCL 1684 (a blocker of Ca^{2+}-activated K^+ channels) which reversed Hp-induced antinociception. Furthermore, concomitant administration of URB-584 (FAAH inhibitor) but not JZL-184 (MAGL inhibitor) potentiates antinociceptive effect of Hp in CCI rats indicating an involvement of anadamide on HP-induced antinociception. Together, these data demonstrate that Hp displays antinociception in pain from neuropathic etiology through local effects. The release of anandamide and the opening of peripheral K^+ channels are involved in the antinociceptive effect.

1. Introduction

Neuropathic pain is defined as a chronic or persistent pain state that results from an injury or dysfunction of the nervous system, and includes clinical symptoms of hyperalgesia, allodynia and spontaneous ongoing pain [13]. Due to its persistent condition, neuropathic pain represents a major public health problem. A variety of therapeutic approaches, including opioid analgesics, tricyclic antidepressants, anticonvulsants, and local anesthetics have been used to treat neuropathic pain. But due to the complexity of the mechanisms involved, the treatment is often ineffective [13]. The use of Δ9-THC (derived from *Cannabis sativa*) for the treatment of various neurological disorders, including chronic pain, is supported by experimental and clinical data [6,10,23]. Although they are seen as promising target for the development of medications, clinical and preclinical studies have shown that Δ9-THC and other CB1 ligands generally produce undesirable effect in the central nervous system. CB1 agonists are generally at risk for psychoactive effects and dependence, limiting the optimization of doses in clinical trials and preclinical studies [28]. Thus, development of drugs capable of binding to the cannabinoid receptors without

Abbreviations: CB1 receptors, type 1 cannabinoid receptors; Hp, hemopressin; CCI, chronic constriction injury; PB, phosphate buffer; DRG, dorsal root ganglia; Egr1-IR, EGR-1 immunoreactivity; Met, methysergide; Yoh, yohimbine.

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psychoactive effects provide therapeutic potential without the risk of adverse effects, making it a valuable tool for the treatment of several disorders related to the cannabinoid system [28]. Hemopressin (Hp), a nonapeptide (PVNFKFLSH) derived from the hemoglobin α1 chain was previously shown to target CB_1 receptor, and to modulate its signaling [19]. Hp exhibits antinociceptive effects in inflammatory pain models [18,19]. In this sense, it was demonstrated that Hp inhibits carrageenan-induced hyperalgesia only at the injured paw; without antinociceptive effect observed in the contralateral, uninflamed paw, indicating that the effect of Hp is limited to tissue injury-induced pain [19]. Also, intrathecal administration of Hp induces significant antinociception in the first and second phases of the formalin test [18]. The effects of Hp on carrageenan-induced hyperalgesia are independent of route of administration (oral, local, or intrathecal) [19]. More interesting is the fact that neurological side effects that are typically associated with antinociceptive doses of CB_1 receptor ligands, including hypothermia, catalepsy and hypoactivity, were not reported with antinociceptive doses of Hp [19]. This, taken with the fact that the effects of Hp on carrageenan-induced hyperalgesia were found to be independent of route of administration, raises the possibility that Hp could be developed as a novel class of drug that modulates CB_1 receptor for the treatment of pain.

Since the majority of the previous studies focused on inflammatory pain and relatively little information is available regarding the role of Hp in alleviating chronic pain, in this study the effects of Hp on neuropathic pain using chronic constriction injury model (CCI) were examined.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 160–180 g, age-matched, were used throughout this study. Animals were maintained under controlled light cycle (12/12 h) and temperature (22 ± 2 °C) with free access to food and water. Throughout the experiments, animals were managed using the principles and guidelines for the care of laboratory animals in studies involving pain and were approved by the Ethics Committee on the Use of Animals of Hospital Sírio-Libanês (CEUA, protocol number 2008/07).

2.2. Induction of neuropathic pain

Rats were anesthetized with halothane (2.5%) (Cristália) and subjected to chronic constriction injury (CCI) of the sciatic nerve according to the method of Bennett and Xie [3]. In the procedure, the sciatic nerve of the right paw was exposed at the middle of the thigh by blunt dissection through the biceps femoris. Proximal to the sciatic nerve's trifurcation (about 7 mm), the nerve was freed of adhering tissue and four ligatures (4.0 chromic gut) were tied loosely around it with about 1 mm spacing. Great care was taken to tie the ligatures, so that the diameter of the nerve was seen to be just barely constricted. The incisions were sutured in layers using silk suture wire (5-0) (Ethicon). As a control, rats were sham-operated by exposing the sciatic nerve without nerve compression ligation or constriction. Experiments were conducted on the 14th day after CCI induction.

2.3. Behavioral analysis

Pain threshold was measured using a paw pressure apparatus (Ugo Basile®, Italy), essentially as described [31]. Briefly, a force with increasing magnitude (16 g/s) was applied to the right hind paw of rats. When animals reacted by withdrawing the paw, the force (in grams) needed to induce this response represented the pain threshold. Antinociceptive activity was expressed as the increase in the force needed to induce the withdrawal response in treated versus control animals.

2.4. Pharmacological treatments

2.4.1. Hemopressin

Hemopressin (Proteimax Biotechnology) was administered orally at the doses of 0.5 or 0.25 mg/kg as described [19].

2.4.2. Methysergide and yohimbine

Methysergide (Met) was administered intraplantar at the dose of 5 mg/kg; 100 μl, and yohimbine (Yoh) was administered intrathecally (30 μg/animal; 50 μl). Both were administered 30 min before Hp (0.25 mg/kg, orally). Mechanical hyperalgesia was evaluated in sham-operated and CCI rats by the paw pressure 1 h after Hp administration. Both groups (Sham and CCI) were treated with only Hp, Met or Yoh, or the combinations Hp + Met and Hp + Yoh. Met and Yoh were purchased from Sigma–Aldrich®.

2.4.3. UCL1684

Intraplantar injection of UCL1684 (10 μg/paw; 100 μl) was administered concomitantly with oral Hp (0.25 mg/kg). Mechanical hyperalgesia was evaluated in sham-operated and CCI rats by the paw pressure test. Both groups (Hp and Hp + UCL1684) were tested 1 h after Hp administration. UCL1684 was purchased from Sigma–Aldrich®.

2.4.4. URB597 and JZL184

Intraplantar injection of URB597 (100 μg/paw) and JZL184 (100 μg/paw) was administered concomitantly with oral Hp (0.25 mg/kg). Mechanical hyperalgesia was evaluated in sham-operated and CCI rats by the paw pressure test. The animals were treated with Hp, JZL184, URB597, Hp + JZL184 or Hp + URB597 and were evaluated 1 h after treatments. JZL184 and URB597 were purchased from Sigma®.

2.5. Immunohistochemistry

One hour after the hemopressin administration (0.25 mg/kg) rats were transcardially perfused with phosphate-buffered saline and 4% paraformaldehyde (Sigma–Aldrich®) in 0.1 M phosphate buffer, pH 7.4 (PB). The spinal cords (L4 and L5) were removed, left in the same fixative for 5–8 h and then cryoprotected overnight in 30% sucrose. Thirty μm frozen sections were immunostained for Egr-1 expression. The spinal cord sections were incubated free floating with a rabbit polyclonal antibody against the nuclear protein which is the product of the early response genes *egr-1* (also known as Zif268) (Santa Cruz Biotechnology, Santa Cruz, CA), diluted 1:500 in PB containing 0.3% Triton X-100 plus 5% of normal goat serum. Incubation with the primary antibody was conducted overnight at 24 °C. After three washes (10 min each) in PB, the sections were incubated with biotinylated goat anti-rabbit sera (Vector Labs, Burlingame, CA) diluted 1:200 in PB for 2 h at 24 °C. The sections were washed again in PB and incubated with the avidin–biotin–peroxidase complex (ABC Elite; Vector Labs). After the reaction with 0.05% 3,3′-diaminobenzidine and a 0.01% solution of hydrogen peroxide in PB and intensification with 0.05% osmium tetroxide in water, the sections were mounted on gelatin- and chromoalumen-coated slides, dehydrated, cleared, and coversliped. The material was then analyzed on a light microscope, and digital images were collected. A quantitative analysis was performed on the density of nuclei representative of the immunoreactivity for Egr-1 (Egr1-IR) in the dorsal horn of the spinal cord (DHSC; laminae I–VI of the L4–L5 dorsal horn). Measurements were taken from 10 different sections for each animal analyzed,