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Centrally mediated antinociceptive effects of cannabinoid receptor ligands in rat models of nociception

Aldric Hama *, Jacqueline Sagen

The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, Miami, FL 33136, USA

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ABSTRACT

The endogenous nonapeptide hemopressin (HE) demonstrates potent block of the cannabinoid subtype-1 (CB1) receptor in vitro and robust antinociception in vivo. The current study evaluated the effects of centrally administered HE in mechanistically distinct pre-clinical rat models of pain—the hot plate test and the hind paw formalin test. The non-subtype selective CB receptor agonist WIN 55,212-2 was tested concurrently as a positive control. In the hot plate test, neither intrathecal (i.t.) HE nor WIN 55,212-2 significantly altered the latency to respond to noxious heat. By contrast, i.t. HE and WIN 55,212-2 significantly reduced pain-related behaviors in the formalin test. Possible HE functionality as a CB1 receptor antagonist at the spinal level was evaluated in the formalin test. Intrathecal pretreatment with HE did not attenuate the antinociceptive effect of i.t. WIN 55,212-2. However, pretreatment with the CB1 receptor antagonist rimonabant did; i.t. rimonabant pretreatment was not antinociceptive. Potential supraspinal antinociceptive activity of HE was also evaluated. Whereas intracerebroventricular (i.c.v.) injection of WIN 55,212-2 reduced pain-related behaviors in the formalin test, interestingly, i.c.v. HE increased behaviors. In the current study, an antinociceptive effect with the CB receptor ligand HE was obtained under the specific condition of tissue injury and not in the uninjured state. Thus, HE could be a useful analgesic peptide with a novel spinal mechanism of action.

1. Introduction

Although the use of *Cannabis sativa* for the treatment of various neurological disorders, including chronic pain, is supported by clinical data, adverse effects, such as cognitive impairment and hallucination, limit its widespread clinical use (Iskedjian et al., 2007; Russo et al., 2007). Given socio-political controversy surrounding the medical use of *C. sativa* and its bioactive CB components, as well as the side-effects, alternate CB receptor ligands are needed for clinical use.

The nonapeptide hemopressin (HE), derived from the α-chain of hemoglobin, was isolated from rat brain homogenates and demonstrated hypotensive effects in rats in vivo (Rioli et al., 2003). Hemopressin binds to rat brain cannabinoid subtype-1 (CB1) receptors with sub-nanomolar potency, which is slightly less than that of the CB1 receptor antagonist rimonabant (Heimann et al., 2007). Hemopressin blocks the effect of the CB receptor agonist HU-210 in in vitro functional assays. Furthermore, in the absence of an agonist in these assays, HE behaves as an inverse agonist, similar to rimonabant, which also demonstrates inverse agonist activity. Given these in vitro effects, it is surprising that such a peptide would demonstrate robust antinociceptive effects, which are consistently observed with CB receptor agonists. Nonetheless, carrageenan-induced hind paw hypersensitivity to noxious stimulation was markedly attenuated with intrathecal (i.t.) injection of HE (Heimann et al., 2007). No antinociceptive effect was noted in the contralateral, uninflamed paw, indicating that the effect of HE was limited to tissue injury-induced pain. Neurological responses typically associated with antinociceptive doses of CB1 receptor agonists, including hypothermia, catalepsy and hypoactivity, were not reported with antinociceptive doses of HE (Heimann et al., 2007; Martin et al., 1991). Thus, it is possible that HE induces an antinociceptive effect via a novel CB1 receptor-mediated mechanism.

An additional attractive feature of HE over a small molecule compound is that it may be possible to insert its gene into cells. Cells engineered to express analgesic substances, such as HE, implanted into the subarachnoid space could be a long-term solution to managing chronic pain (Eaton, 2006; Jeon, 2011). Long-term antinociception has been demonstrated in various animal pain models with cells that produce and release endogenous analgesic substances into the intrathecal space (Hentall and Sagen, 2000). Presently, there is limited information concerning the efficacy of i.t. HE across a range of pain models.

The primary objective of the current study was to determine the efficacy of i.t. injected HE in response to noxious thermal stimulation and in a rat pain model of peripheral, acute inflammation. Given that

Abbreviations: CB, cannabinoid; A_{50}, 50% antinociceptive dose; HE, hemopressin; i.c.v., intracerbroventricular; i.t., intrathecal; MPE, maximum possible effect.

* Corresponding author at: University of Miami Miller School of Medicine, The Miami Project to Cure Paralysis, 1095 NW 14th Terrace (R-48), Miami, FL 33136, USA. Tel.: +1 305 243 5618; fax: +1 305 243 3923.
E-mail address: ahama@miami.edu (A. Hama).

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brain CB1 receptors also mediate the neurological effects of systemically administered CB receptor agonists, a possible antinociceptive effect of HE was also evaluated following injection into the lateral ventricle (i.c.v). The nonselective CB receptor agonist WIN 55,212-2 was used as a positive control in these tests.

A secondary objective of the current study was to evaluate a possible in vivo CB1 receptor antagonist effect of HE, since HE has been shown to potently bind to the CB1 receptor and block the effects of a CB receptor agonist in vitro. Rats were i.t. pretreated with HE and then i.t. injected with an antinociceptive dose of WIN 55,212-2 in the formalin test. As a positive control, rats were pretreated with rimonabant prior to i.t. injection of WIN 55,212-2.

2. Materials and methods

Procedures were reviewed and approved by the University of Miami Animal Care and Use Committee and followed recommendations of the National Research Council's *Guide for the Care and Use of Laboratory Animals*. Male Sprague–Dawley rats (250–275 g; Harlan, IN) were used in these experiments. Upon arrival, rats were allowed to acclimate to the animal facility for 5–7 days prior to surgery. Rats were housed two per cage and allowed free access to food and water. Following cannulation surgeries, however, rats were singly housed. At the end of the studies, rats were euthanized with CO_2.

2.1. Surgical procedures

For all surgical procedures, aseptic surgical techniques were used, including the use of sterile instruments, gloves and personal protective equipment. Following shaving of the rat skin, the surgical area was swabbed with chlorhexidine. Rats were anesthetized and maintained on isoflurane in O_2 for the duration of the surgical procedures. Rats were allowed at least 3 days to recover from surgery prior to use in experiments.

2.1.1. Intrathecal catheters

The method of implanting an i.t. catheter in rats has been described elsewhere (Yaksh and Rudy, 1976). Briefly, rats were anesthetized and the head secured in a stereotaxic unit. The atlanto-occipital membrane was exposed and cut and an i.t. catheter (ReCathCo, Allison Park, PA) was threaded down the i.t. space. The catheter was secured to the musculature with sutures and the skin incision was closed with cyanoacrylate. After flushing the catheter with 10 μl saline, the externalized catheter was melted shut. At the end of testing, prior to euthanasia with CO_2 overdose, 5 μl of 5% lidocaine was i.t. injected to assess the location of the catheter tip. An acute bilateral flaccid paralysis of the hind limbs indicated that the catheter tip was in the correct spinal position.

2.1.2. Intracerebroventricular surgery

The method of implanting i.c.v. cannulae into the right ventricular space and the stereotaxic coordinates (Anterior–Posterior: −0.7 mm from bregma; Medial–Lateral: −1.5 mm from bregma; Dorsal–Ventral: −3.5 mm from the top of the skull) were adopted from a method described elsewhere (Taylor et al., 1994). The guide cannula was secured in place with screws and dental cement. (Cannula parts were obtained from Plastics One, Inc., Roanoke, VA. Dental cement was obtained from Stoelting, Wood Dale, IL.) A dummy cannula was inserted into the guide cannula to keep it patent. Five microliters of either drug or vehicle was injected into the ventricular space with an injection cannula that extended 1 mm below the guide cannula. At the end of the experiment, prior to euthanasia, 5 μl of methylene blue was injected into the right ventricular space to confirm proper placement of the guide cannula.

2.2. Testing procedures

2.2.1. Hot plate test

Hind paw sensitivity of rats to a noxious heat stimulus was assessed using the hot plate test. Rats were placed on a heated (54.5 °C) surface encased by a Plexiglas chamber. When the rat licked its hind paw, the rat was removed from the apparatus. The duration of time between placement of the rat on the heated surface and the hind paw lick was recorded as the response latency (in seconds). Prior to injection of either drug or vehicle, the baseline response latency was measured. Rats were tested once every 30 min up to 120 min post-injection. To prevent possible plantar skin damage, a cut-off of 45 s was used. Following completion of the test, rats were euthanized.

2.2.2. Formalin test

Ten minutes after injection (either i.t. or i.c.v.) of either drug or vehicle, 50 μl of 5% formalin was subcutaneously injected into the left plantar hind paw and rats were immediately placed in clear Plexiglas chambers (Dubuisson and Dennis, 1977). The number of hind paw flinching and licking occurring in one min were counted in 5 min intervals up to 60 min post-formalin injection (Abbott et al., 1995; Amodel and Paxinos, 1980; Tjolsen et al., 1992). Phase 1 ("acute" phase) was defined as the first min following formalin injection (0–1 min) and phase 2 ("tonic" phase) was defined as 15–61 min post-formalin injection (Hama et al., 2006; Hama and Sagen, 2009). Rats were used only once in this test.

2.2.2.1. Antagonism of intrathecal WIN 55,212-2 in the formalin test

To test for a possible CB1 receptor-mediated antagonist effect of HE on WIN 55,212-2-induced antinociception, rats were i.t. pre-treated with 1 μg HE (or vehicle), followed 10 min later by i.t. injection of 30 μg WIN 55,212-2 (or vehicle). Formalin was injected into the hind paw 10 min following the second i.t. injection. In a separate comparator group, i.t. injection of 30 μg rimonabant (or vehicle) was followed 10 min later by 30 μg WIN 55,212-2.

Thus, there were four treatment groups in the antagonist arm of the study (pre-treatment/post-treatment): *i*) vehicle/vehicle, *ii*) vehicle/WIN 55,212-2, *iii*) antagonist/vehicle and *iv*) antagonist/WIN 55,212-2.

2.3. Drugs

A volume of 5 μl was used for i.t. and i.c.v. injections of drugs. A 5 μl vehicle flush followed i.t. drug injection. Hemopressin (PVNFKFLSH) was obtained from 21st Century Biochemicals (Marlboro, MA) and dissolved in saline. WIN 55,212-2 mesylate was obtained from Sigma-Aldrich, Co. (St. Louis, MO) and was dissolved in a vehicle of 45% 2-hydroxypropyl-ß-cyclodextrin in saline. Rimonabant was obtained from Cayman Chemical, Co. (Ann Arbor, MI) and dissolved in a vehicle of 10% DMSO: 10% Tween-80: 80% saline.

In the current study, the highest tested i.t. dose of WIN 55,212-2 was 30 μg. Higher doses, e.g. 100 μg, led to hind limb flaccid paralysis and it has been reported that hind paw withdrawal thresholds are elevated well beyond normal levels (Martin et al., 1999). Doses of HE tested in the current study were based on i.t. doses used by Heimann et al. (2007). Additional doses (3, 10 μg), higher than those tested by Heimann et al. (2007) were used as well. The i.t. dose of rimonabant (30 μg) was chosen from studies that reported no observable side-effects and demonstrated in vivo antagonism of CB receptor agonists (Kang et al., 2007; Khodayar et al., 2006; Martin et al., 1999; Welch et al., 1998; Yoon and Choi, 2003).