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Research paper

Regulation of neuropathic pain behavior by amygdaloid TRPC4/C5 channels

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HIGHLIGHTS

- Blocking of amygdaloid TRPC4/C5 attenuated neuropathic hypersensitivity.
- Blocking of amygdaloid TRPC4/C5 attenuated affective-like neuropathic pain.
- In healthy rats, blocking amygdaloid TRPC4/C5 failed to influence pain behavior.
- Amygdaloid TRPC4/C5 contributes to maintenance of neuropathic pain.

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ABSTRACT

Pain *per se* may increase anxiety and conversely, anxiety may increase pain. Therefore, a positive feedback loop between anxiety and pain possibly contributes to pain and suffering in some pathophysiological pain conditions, such as that induced by peripheral nerve injury. Recent results indicate that transient receptor channels 4 and 5 (TRPC4/C5) in the amygdala have anxiogenic effects in rodents, while their role in chronic pain conditions is not known. Here, we studied whether the amygdaloid TRPC4/C5 that are known to have anxiogenic properties contribute to the maintenance of sensory or affective aspects of pain in an experimental model of peripheral neuropathy. Rats with a spared nerve injury (SNI) model of neuropathy in the left hind limb had a chronic cannula for microinjections of drugs into the right amygdala or the internal capsule (a control site). Sensory pain was assessed by determining mechanical hypersensitivity with calibrated monofilaments and affective pain by determining aversive place-conditioning. Amygdaloid treatment with ML-204, a TRPC4/C5 antagonist, produced a dose-related (5–10 μg) antihypersensitivity effect, without obvious side-effects. Additionally, amygdaloid administration of ML-204 reduced affective-like pain behavior. In the internal capsule, ML-204 had no effect on hypersensitivity or affective-like pain in SNI animals. In healthy controls, amygdaloid administration of ML-204 failed to influence pain behavior induced by mechanical stimulation or noxious heat. The results indicate that the amygdaloid TRPC4/C5 contribute to maintenance of pain hypersensitivity and pain affect in neuropathy.

1. Introduction

Amygdala has an important role in primary emotions, such as fear [10]. Amygdala, particularly its lateral capsular subdivision within the central nucleus (CeA), the main output pathway to the brainstem, is also involved in processing and regulation of emotional aspects of pain and, through its efferent brainstem projections, in descending control of spinal pain-relay neurons [14,16]. Recently, it was demonstrated that among multiple amygdaloid mechanisms involved in the control of anxiety-driven behaviors are transient potential channels 4 and 5 (TRPC4/C5) that are members of the TRPC1/4/5 subfamily and that gate afferent amygdaloid inputs in the lateral nucleus of the amygdala [17,18]. The lateral amygdala has direct and indirect projections through the basolateral nucleus to the main amygdaloid output nucleus CeA [10]. It may be hypothesized that TRPC4/C5-expressing amygdaloid neurons through projections to the CeA may regulate amygdaloid processing of nociception and thereby also amygdaloid outputs to brainstem pain regulation centers. Since, the amygdala is involved

Abbreviations: ANOVA, analysis of variance; DMSO, dimethylsulfoxide; CeA, central nucleus of the amygdala; i.c., internal capsule; i.t., intrathecal; SNI, spared nerve injury; TRPC4/C5, transient receptor channel 4/5.

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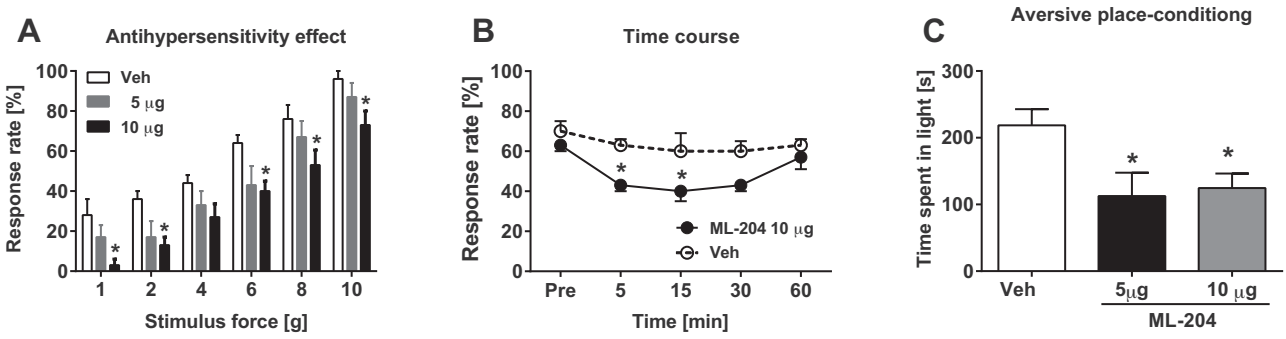

Fig. 1. Effect of amygdaloid administration of ML-204, a TRPC4/C5 antagonist, on pain behavior in neuropathic animals. (A) Mechanical hypersensitivity 15 min following administration of ML-20. (B) Time course of mechanical antihypersensitivity effect by ML-204. (C) Affective-like pain behavior assessed using aversive place-conditioning paradigm following administration of ML-204.
In all graphs, vehicle (Veh) or ML-204 was administered into the right amygdala at a dose shown in the graphs. Error bars represent S.E.M. ($n = 5–6$). $^{*}P < .05$ (Tukey's test; reference: the corresponding Veh-value).

both in pain and affect and has reciprocal connections with the pain system [14,16], it may be speculated that amygdala in general and amygdaloid TRPC4/C5 in particular might be among neural substrates contributing to the comorbidity between affect, such as anxiety, and chronic pain (e.g., see Ref. [2]). To test this hypothesis, we assessed whether microinjection of a TRPC4/C5 antagonist ML-204 [12] into the amygdala or a control injection site in the brain influences pain hypersensitivity in animals with an experimental model of chronic neuropathy. The effect of blocking the amygdaloid TRPC4/C5 was separately assessed on the sensory component of neuropathic pain by determining mechanical hypersensitivity and on the affective component of neuropathic pain by determining aversive place-conditioning. For comparison, we determined whether blocking the amygdaloid TRPC4/C5 influences pain behavior in healthy controls.

2. Materials and methods

2.1. Animals

The experiments were performed with adult male Hannover-Wistar rats (Harlan, Horst, The Netherlands) weighing 180–280 g. The experimental protocols were approved by the Experimental Animal Ethics Committee of the Provincial Government of Southern Finland (Hämeenlinna, Finland), and the experiments were performed according to the guidelines of European Communities Council Directive of 22 September 2010 (2010/63/EU). All efforts were made to limit distress and to use only the number of animals necessary to produce reliable scientific data. Rats were housed in a 12-h light/dark cycle with food and water access ad libitum.

2.2. Surgical procedures for producing neuropathy

For inducing neuropathy, the spared nerve injury (SNI) model, as described by Decosterd and Woolf [5], was adopted. Prior to surgery, the rat was anesthetized with sodium pentobarbital (Orion Pharma, Espoo, Finland), administered intraperitoneally at the dose of 60 mg/kg. Further, doses of pentobarbitone were given at the dose of 15–20 mg/kg as needed to keep the depth of anesthesia deep enough so that the animal did not react to noxious stimulation. An incision was subsequently made into the skin on the lateral surface of the left thigh, followed by a section through the biceps femoris muscle to expose the sciatic nerve and its terminal branches: the sural, common peroneal and tibial nerves. The common peroneal and tibial nerves were then tightly ligated with 4–0 silk, sectioned distal to the ligation and 3–4 mm of the distal nerve stump was removed. The sural nerve was left intact. To prevent postoperative pain, animals were treated subcutaneously with 0.01 mg/kg of buprenorphine twice daily for 2–3 days and they were allowed to recover for at least a week before the experiments. Only animals with tactile allodynia-like hypersensitivity (hind limb withdrawal threshold to monofilament stimulation in the operated side $\leq$4 g, which is below the lower 95% confidence limit of the threshold in unoperated control animals) were selected for this study. SNI model produced mechanical hypersensitivity in all animals of the present study.

2.3. Cannula insertion and drug injection procedure

The animals had a guide cannula for drug administration into the right amygdala (contralateral to the peripheral nerve injury) as described in detail earlier [19]. The rationale for choosing the right amygdala was that earlier results have suggested that the right amygdala has a more important role in processing of pain-related signals than the left amygdala [4,6], although not in all conditions [20]. Moreover, another reason for choosing the right amygdala as the treatment target was that in the present study mechanical hypersensitivity was induced in the left hind limb and the amygdala-induced descending control of mechanically evoked pain is expected to be stronger in the contra- than ipsilateral limb [3,8]. For placement of the guide cannula (26 gauge; Plastics One, Roanoke, VA, USA), the skull was exposed and a hole drilled for its placement under sodium pentobarbital anesthesia (60 mg/kg i.p.). The desired center of injection in the right amygdala was in the capsule lateral of the CeA: 2.1 mm posterior from the bregma, 4.3 mm lateral from the midline, and 7.8 mm ventral from the dura mater [15]. The control injection site was in the right internal capsule: 2.1 mm posterior from bregma, 3.6 mm lateral from the midline, and 5.0 mm ventral from the dura mater. The tip of the guide cannula was positioned 2 mm above the desired injection site. The cannula was fixed into the skull using a dental screw and dental cement. Drug administration to the brain and experimental protocols started 1 week after fixation of the guide cannula to the skull. A dummy cannula was placed in the guide cannula, except when drug administrations were performed.

2.4. Drugs and their administration procedure

ML-204, a selective TRPC4/C5 antagonist [12], and dimethylsulfoxide (DMSO), the vehicle, were purchased from Sigma–Aldrich (St. Louis, MO, USA). ML-204 was dissolved in DMSO (100%) and administered at the dose of 5 μg or 10 μg. Unilateral infusions of ML-204, or an equivalent volume of vehicle, were made by using 33 gauge injection needles (Plastics One) connected to a 10 μl