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Research report

Role of TRPV1 channels of the dorsal periaqueductal gray in the modulation of nociception and open elevated plus maze-induced antinociception in mice

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HIGHLIGHTS

- Activation of the dorsal periaqueductal gray (dPAG) TRPV1 provokes antinociception.
- dPAG TRPV1 channels blockade impairs capaicin-induced antinociception.
- Open elevated plus maze (oEPM) exposure induces antinociception in mice.
- Blockade of TRPV1 channels in the dPAG attenuates the oEPM-induced antinociception.
- dPAG TRPV1 channels play a role in the modulation of fear-induced pain inhibition.

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ABSTRACT

Recent findings have identified the presence of transient receptor potential vanilloid-1 (TRPV1) channels within the dorsal portion of the periaqueductal gray (dPAG), suggesting their involvement in the control of pain and environmentally-induced antinociception. Environmentally, antinociception may be achieved through the use of an open elevated plus maze (oEPM, an EPM with 4 open arms), a highly aversive environmental situation. Here, we investigated the role of these TRPV1 channels within the dPAG in the modulation of a tonic pain and in the oEPM-induced antinociception. Male Swiss mice, under the nociceptive effect of 2.5% formalin injected into the right hind paw, received intra-dPAG injections of the TRPV1 agonist (capsaicin: 0, 0.01, 0.1 or 1.0 nmol/0.2 μL; Experiment 1) or antagonist (capsazepine: 0, 10 or 30 nmol/0.2 μL; Experiment 2) or combined injections of capsazepine (30 nmol) and capsaicin (1.0 nmol) (Experiment 3) and the time spent licking the formalin-injected paw was recorded. In Experiment 4, mice received intra-dPAG capsazepine (0 or 30 nmol) and were exposed to the oEPM or to a control situation, an enclosed EPM (eEPM; an EPM with 4 enclosed arms). Results showed that while capsaicin (1 nmol) decreased the time spent licking the formalin-injected paw, capsazepine did not change nociceptive response. Capsazepine (30 nmol) blocked pain inhibition induced by capsaicin and mildly attenuated the oEPM-induced antinociception. Our results revealed an important role of TRPV1 channels within the dPAG in the modulation of pain and in the phenomenon known as fear-induced antinociception in mice.

1. Introduction

Vanilloid type 1 channel (also known as transient receptor potential vanilloid type 1—TRPV1) is a nonselective cation channel that has been suggested to be involved in several neurobiological processes such as fear/anxiety, drug addiction and pain [1–6]. The role of TRPV1 in pain modulation has been shown through intracerebral injections of vanilloid compounds in animals exposed to various nociceptive tests (e.g., tail-flick and plantar tests [1,2,7]). Moreover, transgenic mice lacking TRPV1 have highlighted a pivotal role of these compounds in pain modulation, (e.g. [8]).

TRPV1 channels are widely localized in primary afferent neurons [8–11], where they have been implicated in the transmission of nociceptive signals (for a review see [12]). Besides their role in pain modulation on peripheral primary afferents [13,14], TRPV1 chan-

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nels have also been involved in the control of pain transmission in the central nervous system [1,2,7,15]. There is also evidence suggesting the participation of vanilloid receptors in brainstem areas involved in the modulation of defensive reactions [3,6] and on the descending pain inhibitory system [2,16,17] in which the midbrain periaqueductal gray matter (PAG) plays a crucial role (e.g. [18,19]).

Due to its involvement in the modulation of fear/anxiety states [6,20,21], and in the descending modulation of nociception [19,22], midbrain PAG has also been implicated in an important phenomenon known as fear-induced antinociception, characterized as a decrease in the perception of pain when animals are confronted with innate or learned threatening situations (e.g. [23,24]; for an update review see [25]).

Descending projections from PAG reach the rostral ventromedial medulla (RVM) and the activation of PAG–RVM pathway leads to a decrease in the perception of pain [26–28]. Initial studies conducted by Palazzo and colleagues concerning the putative role of TRPV1 channels within PAG–RVM descending nociceptive pathway showed that microinjection of capsaicin, a TRPV1 agonist, into the dorsal portion of the PAG (dPAG) increased the latency of nociceptive response in rats subjected to the plantar test, an acute nociceptive test [1]. Those authors also showed that prior local injection of capsazepine, a TRPV1 antagonist, blocked the antinociceptive effect of capsaicin [1]. Acute nociceptive tests (e.g. plantar test, tail-flick test) predominantly activate Aδ fibers of fast conduction, whereas tonic nociceptive tests (e.g., induced by intraplantar injection of a formalin solution; Formalin test) activate C-fiber polymodal nociceptors [29]. When we peripherally activate C-fiber through an inflammatory test, i.e. the formalin test, the ascending pain pathway activated is different to that recruited in an acute test, so it is subjected to different modulation. In this context, it makes the investigation of the role played by TRPV1 channels located within the dPAG in the modulation of tonic pain relevant.

This background provides us with a body of suggestive evidence about the role of TRPV1 channels in acute pain modulation within the rat PAG–RVM pathways. However, its role in tonic pain and fear-induced analgesia needs further investigation. In this context, we have shown that rodents display a high intensity antinociceptive response when confined to the open arms of the EPM or exposed to a totally open EPM (oEPM) [30–33]. In an attempt to elucidate the role of TRPV1 channels within the dPAG in the modulation of a tonic pain (i.e. assessed by the formalin test), and in oEPM-induced antinociception, this study investigated the effects of intra-dPAG injections of capsaicin and capsazepine, a TRPV1 agonist and antagonist, respectively, on nociceptive response in mice exposed to a neutral (a potentially not aversive cage) or aversive (oEPM) situation.

2. Material and methods

2.1. Animals

Subjects were male Swiss adult mice (UNESP—Univ. Estadual Paulista, SP, Brazil), weighing 28–35 g at testing. They were housed in groups of 10 per cage (41 cm × 34 cm × 16 cm) and maintained under a normal 12 h light cycle (lights on 07:00 h) in a temperature-controlled environment (23 ± 1 °C). Food and water were freely available except during the brief test periods. All mice were naïve at the beginning of experiments and each mouse was used once. All efforts were made to minimize animal suffering.

2.2. Drugs

The drugs used were capsaicin (Tocris Cookson, Ballwin, MO, USA), a TRPV1 agonist, and capsazepine (Tocris Cookson, Ballwin, MO, USA), a TRPV1 antagonist. The compounds were dissolved in undiluted DMSO (dimethylsulfoxide), which alone served as vehicle solution. The doses of capsaicin (0, 0.01, 0.1 and 1 nmol) and capsazepine (0, 10 and 30 nmol) were based on a previous study [6]. The final microinjection volume injected into the dPAG was 0.2 μL.

2.3. Surgery and microinjection

Mice received a stereotaxic (Kopf Instruments) unilateral implant of a 7 mm stainless steel guide cannula (26-gauge; Insight Equipamentos Científicos Ltda) targeted to the dPAG under ketamine + xylazine anesthesia (100 mg/kg and 10 mg/kg, i.p.). The guide cannula was fixed to the skull using dental acrylic and jeweler's screws. Stereotaxic coordinates [34] for the dPAG (dorsolateral and dorsomedial columns) were 4.1 mm posterior to bregma, 1.4 mm lateral to the midline, and 2.3 mm ventral to the skull surface, with the guide cannula angled 26° to the vertical. A dummy cannula (33-gauge stainless steel wire; Fishtex Industry and Commerce of plastics Ltd.), inserted into each guide-cannula immediately after surgery, served to reduce the incidence of occlusion. At the end of the stereotaxic surgery, each mouse received an intramuscular injection of penicillin-G benzathine (Pentabiotic, 56,7 mg/kg in a 0.1 mL volume; Fort Dodge, Campinas, SP, Brazil) and a subcutaneous injection of the anti-inflammatory analgesic Banamine (3.5 mg/kg flunixin meglumine, Intervet Schering–Plough, Rio de Janeiro, RJ, Brazil, in a volume of 0.3 mL).

Five to seven days after surgical recovery, solutions were injected into the dPAG by microinjection units (33-gauge stainless steel cannula; Insight Equipamentos Científicos Ltda), which extended 1.0 mm beyond the tips of the guide cannula. Each microinjection unit was attached to a 2 μL Hamilton microsyringe via polyethylene tubing (PE-10), and administration was controlled by an infusion pump (BI 2000, Insight Equipamentos Científicos Ltda) programmed to deliver a volume at a rate of 0.2 μL (volume injected) over a period of 45 s. The microinjection procedure consisted of gently restraining the animal, removing the dummy cannula, inserting the injection unit, infusing the solution, and keeping the injection unit in situ for a further 60 s. Confirmation of successful infusion was obtained by monitoring the movement of a small air bubble in the PE-10 tubing.

2.4. Formalin test

Nociception was assessed using the formalin test, as previously described [35]. The formalin test causes a biphasic nociceptive response [36]. The first phase begins immediately after formalin injection and lasts for approximately 5 min. It results from the direct stimulation of nociceptors [37–38]. The second phase begins 25 min after the injection and lasts for approximately 40 min [38]. This phase is caused by C-fibers activation [29–37] and also involves a period of sensitization during which inflammatory phenomena occur [29–39].

2.5. Apparatus and general procedure

The basic elevated plus-maze design was similar to that originally validated for mice [40]. The standard EPM (sEPM) consisted of two open arms (30 cm × 5 cm × 0.25 cm) and two closed arms (30 cm × 5 cm × 15 cm) connected to a common central platform (5 cm × 5 cm). The apparatus was constructed from wood (floor) and transparent glass (clear walls) and was raised to a height of 38.5 cm above floor level. In the present study, two other mazes derived from the sEPM were used. They were similarly constructed but comprised either four enclosed arms (eEPM) or four open