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Diacerein decreases visceral pain through inhibition of glutamatergic neurotransmission and cytokine signaling in mice

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ABSTRACT

The present study evaluated the antinociceptive effect of the pro-inflammatory cytokines inhibitor diacerein in mice and its possible mechanism of action. The antinociception produced by diacerein was tested at different sites of action, moreover selective antagonists or agonists were used to identify the mechanism that may be involved in its antinociceptive action against acetic acid-induced visceral pain. Diacerein administered systemically (intraperitoneal [i.p.] or intra-gastric [i.g.] routes), supra-spinally (i.c.v.), spinally (i.t.) or peripherally (in association with the irritant agent) inhibited the visceral nociception induced by acetic acid in mice. Interestingly, diacerein treatment (25 mg/kg, i.p. or 50 mg/kg, i.g.) produced long-lasting (for up to 4 h) inhibition of acetic acid-induced nociception. Intraperitoneal treatment of mice with diacerein (25.0 mg/kg) inhibited somatic nociception induced by i.t. injection of glutamate, NMDA, kainate, and trans-ACPD but not that caused by AMPA. Diacerein (5.0–25.0 mg/kg) also produced dose related inhibition of interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) induced nociception. These results indicate that diacerein produces antinociception by inhibiting glutamatergic transmission through both ionotropic and metabotropic receptors as well as activity of pro-inflammatory cytokines.

1. Introduction

Diacerein (4,5-diacetoxy-9,10-dihydro-9,10-dioxo-2 anthracenecarboxylicacid) is a purified anthraquinone that is entirely transformed during absorption into rhein, the active metabolite found in plasma and synovial fluid. It belongs to a new class of anti-osteoarthritis drugs, known as "disease modifying osteoarthritis drugs (DMOAD)" or "chondroprotective agents" (Verbruggen, 2006; Pelletier and Martel-Pelletier, 2007). For its actions both *in vivo* and *in vitro*, diacerein has been used in the treatment of osteoarthritis and demonstrated benefits in alleviating joint pain in humans and in rodent models of the disease (Pavelka et al., 2007; Pelletier et al., 2000; Tamura et al., 1999, 2001, 2002) (for review see, Hunter and Wise, 2007). Recent data from our group showed that diacerein inhibits neuropathic pain induced by partial ligation of the sciatic nerve and also produced anti-allodynic effects against carrageenan- and complete Freund's adjuvant (CFA)-induced inflammatory nociception (Quintão et al., 2005).

Although the mechanisms involved on those actions are still incompletely understood, several studies have shown that diacerein and rhein decrease pro-inflammatory cytokines (such as interleukin-1β [IL-1β] and tumor necrosis factor alpha [TNF-α]) release and production either *in vitro* (Martel-Pelletier et al., 1998; Yaron et al., 1999) or *in vivo* (Martel-Pelletier et al., 1998; Moldovan et al., 2000), as well as inhibit *in vitro* NF-κβ activation (Mendes et al., 2002). In fact, NF-κβ, a transcription factor that acts as a central mediator of the immune response, is one of the most important regulators of gene expression among the family of pro-inflammatory mediators (Bonizzi and Karin, 2004; Yoshida et al., 1999). Moreover, diacerein has been found to prevent cartilage breakdown (Moore et al., 1998) and protects against Baker's yeast-induced fever and peritoneal leukocyte migration (Pasin et al., 2010) due to inhibition of the release of pro-inflammatory cytokines.

Although the analgesic effect of diacerein and its action to inhibit the production and release of pro-inflammatory cytokines is well established, there is a lack of a broader perspective of the extent to what mechanisms of action may be applied in well-established models of acute antinociception. Therefore, we designed the present study to analyze the antinociceptive effects of diacerein against acetic

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acid-induced visceral pain as well as glutamate agonists- or pro-inflammatory cytokines-induced somatic pain. Here, we shown that diacerein exhibited antinociceptive effect in different sites of action and that this effect may be related to an inhibition of the glutamate-pro-inflammatory cytokines signaling pathway.

2. Materials and methods

2.1. Animals

Experiments were conducted using male Swiss mice (25–35 g), housed at 22 ± 2 °C under a 12-h light/12-h dark cycle (lights on at 06:00) and with access to food and water *ad libitum*. Mice were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments. The experiments were performed after approval of the protocol (PP00651) by the Institutional Ethics Committee and were carried out in accordance both with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983). The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects observed from diacerein treatment.

2.2. Intrathecal injections

Intrathecal injections were given to fully conscious mice using the method previously described by Hylden and Wilcox (1980). Briefly, the animals were manually restrained, and a 30-gauge needle connected by a polyethylene tube to a 25 μl Hamilton syringe (Hamilton, Birmingham, UK) was inserted through the skin and between the vertebrae into the subdural space of the L5–L6 spinal segments. Intrathecal injections were given over a period of 5 s.

2.3. Intracerebroventricular injections

Intracerebroventricular (i.c.v.) administration was performed under ether anesthesia as previously described (Budni et al., 2007). Briefly, a 0.4 mm external diameter hypodermic needle attached to a cannula, which was linked to a 25 μl Hamilton syringe was inserted perpendicularly through the skull and no more than 2 mm into the brain of the mouse. A volume of 5 μl was then administered in the left lateral ventricle. The injection was given over 30 s and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. The injection site was 1 mm to left from the mid-point on a line drawn through to the anterior base of the ears.

2.4. Abdominal constriction response caused by intraperitoneal injection of acetic acid

The abdominal constrictions resulting from intraperitoneal (i.p.) injection of acetic acid (0.6%, 450 μl) consist of a contraction of the abdominal muscle together with a stretching of the hind limbs and were induced according to procedures described previously (Santos et al., 2005). The mice were individually placed into glass cylinders of 20 cm in diameter and the abdominal constrictions were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions, i.e. the difference between control animals (mice pre-treated with vehicle) and animals pre-treated with drugs. Mice were pre-treated with diacerein (5–200 mg/kg) or with vehicle (10 ml/kg) by i.p. or intra-gastric (i.g.) routes, 30 and 60 min before the irritant injection, respectively. In an attempt to evaluate the possible spinal or supra-spinal antinociceptive action of diacerein, animals were treated with diacerein (5–100 μg/site) or with vehicle (5 μl/site) by intrathecal (i.t.) or intracerebroventricular (i.c.v.) routes, 10 min prior to injection of acetic acid.

In another set of experiments, we also evaluated the effect of diacerein (100–1000 μg/cavity) co-injected with the irritant agent. In addition, we investigated the time course of the antinociceptive effect of diacerein given i.p. (25 mg/kg) or i.g. (50 mg/kg) 0.5, 1, 2, 4 and 6 h before acetic acid administration. Control mice received an equal volume of vehicle and were observed at the same time intervals.

2.5. Nociception induced by glutamatergic agonists and pro-inflammatory cytokines

Animals received an i.p. injection of diacerein (25 mg/kg) or vehicle (10 ml/kg) 30 min before i.t. injection of 5 μl of algogen. Injections were given to fully conscious mice using the method described by Hylden and Wilcox (1980) (see Section 2.2) with minor modifications. The nociceptive response was elicited by glutamate (175 nmol/site), AMPA (a selective agonist of AMPA-subtype of glutamatergic ionotropic receptors, 135 pmol/site), NMDA (450 pmol/site, a selective agonist of the N-methyl-D-aspartic acid [NMDA] glutamatergic ionotropic receptor), kainate (a selective agonist of kainate-subtype of glutamatergic ionotropic receptors, 110 pmol/site) or trans-ACPD (50 nmol/site, a selective agonist for metabotropic glutamate receptors, that is active at both group I (mGlu1, mGlu5) and group II (mGlu2, mGlu3) receptors) (Scheidt et al., 2002; Gadotti et al., 2006; Martins et al., 2011); IL-1β (1 pg/site) and TNF-α (0.1 pg/site) (Choi et al., 2003; Lapa et al., 2009).

Moreover, to verify the link played by the NMDA receptor on behavioral nociception induced by pro-inflammatory cytokines, we assessed the effect of MK-801 (0.05 mg/kg) given i.p., 0.5 h before test, on TNF-α (1 pmol/site)- and IL-1β (1 pmol/site)-induced nociception to the mice. Test duration was 15 min for trans-ACPD and each of the cytokines, 1 min for AMPA, 5 min for NMDA, 4 min for kainate and 3 min for glutamate. Nociceptive behaviors after i.t. delivery were defined as a single head movement directed at the flanks or hind limbs, resulting in contact of the animal's snout with the target organ. The amount of time (seconds) animals spent licking/biting their hind paws, tail or abdomen was determined with a chronometer and considered as nociceptive response (Scheidt et al., 2002; Gadotti et al., 2006; Martins et al., 2011).

2.6. Reagents

The following substances were used: diacerein (Trb Pharma, São Paulo, Brazil), acetic acid, Tween 80 and morphine hydrochloride (Merck, A.G., Darmstadt, Germany), L-glutamic acid hydrochloride (Sigma Chemical Co, St. Louis, USA). Kainic acid (kainate), (±)-1-Aminocyclopentane-trans-1,3-dicarboxylic acid (trans-ACPD), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartic acid (NMDA) and MK-801 (Tocris, Cookson Inc., Ellisville, USA), tumor necrosis factor alpha (TNF-α), Interleukin-1β (IL-1β) (R&D Systems, Minneapolis, USA). All the drugs were dissolved in isotonic saline solution (0.9% NaCl, pH = 5.5) with the exception of diacerein, which was dissolved in saline plus Tween 80. The corresponding pH values for the diacerein solution were 5.4, 5.5, and 5.0 for the 20 mg/ml (200 mg/kg), 10 mg/ml (100 mg/kg), and 2.2 mg/ml (1000 μg/site) solutions, respectively. The final concentration of Tween 80 did not exceed 5% and did not cause any effect *per se*. Also, control groups for each route of delivery received isotonic saline with 5% of Tween 80. The choice of the doses of each drug was based on previous data from our laboratory (Quintão et al., 2005; Gadotti et al., 2006; Martins et al., 2011).

2.7. Statistical analysis

The results are presented as mean ± S.E.M., except the ID_{50} values (i.e., the dose of diacerein that reduce the nociceptive response by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. The