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Involvement of 5-$HT_{1A/1B}$ receptors in the antinociceptive effect of paracetamol in the rat formalin test

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ABSTRACT

The mechanism of analgesic action of paracetamol (acetominophen) remains still unknown. However, a relationship between serotonergic system and the effect of paracetamol has been previously demonstrated. The serotonin activity in the brainstem is primarily under the control of 5-HT_{1A} somatodendritic receptors, although some data also suggest the involvement of 5-HT_{1B} receptors. To determine whether the 5-HT_{1A} and 5-HT_{1B} receptors are involved in the antinociceptive effect of paracetamol, we evaluated the effect of paracetamol (0.125–1 g/kg i.p.) followed by different antagonists [WAY 100,635 (0.8 mg/kg s.c.) and SB 216,641 (0.8 mg/kg s.c.)] or agonists [8-OH-DPAT (0.125 mg/kg s.c.) and CP 93,129 (0.125 mg/kg s.c.)] of 5-HT_{1A} and 5-HT_{1B} receptors, respectively, in the rat model of formalin-induced pain. We demonstrated that paracetamol administration showed a dose-dependent antinociceptive effect in the formalin test. WAY 100,635 (5-HT_{1A} antagonist) induced an increase in the antinociceptive effect of paracetamol at 250 mg/kg doses. Conversely, 8-OH-DPAT (5-HT_{1A} agonist) decreased the antinociceptive effect of paracetamol at 500–1000 mg/kg doses. However, SB216641 (5-HT_{1B} antagonist) modified weakly the antinociceptive effect of paracetamol at 250 mg/kg doses and CP 93,129 (5-HT_{1B} agonist) not produce a clear effect in the antinociceptive effect of paracetamol. These results suggest that the antinociceptive effect of paracetamol can be enhanced mainly by compounds having 5-HT_{1A} antagonist properties in the formalin test and maybe by 5-HT_{1B} receptors antagonists.

Introduction

Paracetamol (acetaminophen) has been extensively studied as analgesic for pain relief in many clinical settings but its mechanism of action still is under considerable debate. Paracetamol crosses the blood brain barrier and many reports indicate that paracetamol exerts its antinociceptive activity not only peripherally, but also within the central nervous system (CNS) (Courad et al., 2001). In addition, paracetamol also exhibits antinociceptive effects in tests that are reputed to be sensitive only to central analgesics, as hot-plate test and tail-flick test (Pinardi et al., 2003; Sandrini et al., 2007), and intracerebroventricular or intrathecal administration of paracetamol have also been shown to provide antinociception (Alloui et al., 2002; Raffa et al., 2004). Paracetamol has been shown to act as a selective COX-2 inhibitor in the CNS, where the concentration of tissue peroxides is low unlike at sites of inflammation (Hinz et al., 2008; Lee et al., 2007). Also, the analgesic effects of paracetamol are attenuated by drugs that act via inhibition of serotonergic, opioid and cannabinoid systems (Pickering et al., 2006; Toussaint et al., 2010) suggesting that a number of neurotransmitter system may be involved in the central antinociceptive mechanism of paracetamol, in particular, serotonergic pathways. In support of this, different studies have shown that action of paracetamol is significantly reduced when lesions are produced in the serotonergic pathway or by inhibiting synthesis of serotonin (5-HT) in animal models (Sandrini et al., 2003; Tiippana et al., 2013). Conversely, paracetamol treatment induces a significant increase in 5-HT levels in the brainsterm (Courade et al., 2001). Another hypothesis that has surfaced is that the analgesic action of systemically administered paracetamol could be attributed to spinal 5-HT (5-HT_3 and 5-HT_7) receptors mediated the enhanced neurotransmitter release in the descending serotonergic pathway, which is responsible for modulation of pain at the spinal level (Dogrul et al., 2012). However, other studies report a serotonergic facilitatory modulation onto the spinal cord through 5-HT_3 in different pain models (Bannister et al., 2015; Sikandar et al., 2012).

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We have previously shown that the antinociceptive effect of tramadol, an analgesic that, like paracetamol is able to increase serotonin levels within CNS, is potentiated or antagonized respectively by a 5-$HT_{1A/B}$ nonspecific receptor blockade or activation (Rojas-Corrales et al., 2000). Moreover, it has been shown that the antinociceptive effect of clomipramine, 5-HT and NA re-uptake inhibitor, is also enhanced by the specific blockade of 5-HT_{1A} receptors (Ardid et al., 2001). In other study, we have shown that the selective blockade of the 5-HT_{1A} or 5-HT_{1B} potentiate the antinociceptive effect of paracetamol in the hot plate test, while this antinociceptive effect of paracetamol can antagonized by specific agonist of these autoreceptors, 5-HT_{1A} and 5-HT_{1B} (Roca-Vinardell et al., 2003). The hot plate test is one of the most commonly used tests of analgesic measure of analgesic drugs that act at the level of spine and higher centres (Vogel, 2002). As both central as well as peripheral mechanisms of paracetamol has been proposed, in the current study we employed the formalin test to assess the effect of blockade or activation of 5-HT_{1A} or 5-HT_{1B} receptors, by specific antagonist or agonist, on antinociceptive action of paracetamol in rats.

Material and methods

Animals

Experiments were carried out on adult male Wistar rats, 200–250 body weight, under standard laboratory conditions (22 °C, 12 h light/dark cycle, lights on at 08:00 AM, food and water *ad libitum*) (n = 8–11/group). All procedures and animal handling were in accordance with the guidelines of European Commission's directive (2010/63/EC) and Spanish Law (RD 53/2013) regulating animal research, and all the experimental protocols were approved by the Committee for Animal Experimentation at the University of Cadiz (Spain).

Drugs

The following drugs were used: propacetamol (provided by UPSA Laboratories Spain, Bristol-Myers-Squibb Group, Madrid, Spain), N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100,635) (Sigma, St Louis, MO, USA), N-[3-[3-(Dimethylamino)ethoxy]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1′ biphenyl]-4-carboxamide (SB 216,641) (Tocris, Bristol, U.K.), 8-Hydroxy-2-(di-*n*-propylamine) tetralin (8-OH-DPAT) (Sigma, St Louis, MO, USA) and 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrol[3,2-b]pyridin-5-one (CP 93,129) (Tocris, Bristol, U.K.). Control animals received saline (NaCl 0.9%).

Propacetamol is a prodrug which is completely hydrolysed to paracetamol by plasma esterases within 7 min after intravenous injection (2 g of propacetamol are equivalent to 1 g of paracetamol (Bannwarth et al., 1992). Therefore, 2 g of propacetamol was dissolved in saline and intraperitoneally administered at equivalent dosis of paracetamol of 125, 250, 500 and 1000 mg/kg at a volume injection of 1 ml/kg body weight. The others drugs were dissolved in saline and subcutaneously administered in a volume injection of 1 ml/kg body weight. WAY 100,635 and SB 216,641 were administered at dose of 0.8 mg/kg. 8-OH-DPAT and CP 93,129 were administered at dose of 0.125 mg/kg. The doses of WAY 100,635, SB 216,641 and 8-OH-DPAT were chosen based on published data (Rojas-Corrales et al., 2005; Rojas-Corrales et al., 2000). The doses of CP 93,129 were chosen on the basis of previous studies performed in our laboratory (data not published).

Formalin test

The formalin test was performed as described Dubbuison and Dennis (Dubuisson and Dennis, 1977). Before testing, animals were placed individually in standard cages for 15 min for three days, after these three adaptation periods, the formalin test was carried out. 50 µL of 5% formalin solution was injected subcutaneously into the dorsal surface of the right hind paw. Pain behavior was monitored for a period of 60 min; the number of flinches/shakes of the injected paw was summed at 5-min intervals starting at time 0. Two phases of spontaneous flinches behavior were observed: phase 1 began immediately after formalin injection to 10 min thereafter and phase 2 began at time 10 min. A maximum response was observed around 20–45 min after the formalin injection.

Experimental protocol

First, three adaption sessions were carried out for each animal before testing. After this, paracetamol or saline was intraperitoneally administered, and 15 min later, the antagonist (WAY 100,635 or SB 216,641) or agonist (8-OH-DPAT or CP 93,129), of 5-HT_{1A} or 5-HT_{1B} receptors respectively, or saline was subcutaneously injected. Formalin was administered 30 min after paracetamol administration and immediately the animal was placed in individual behavioural cage, the test was recorded for 60 min.

Statistical analysis

Results were expressed as mean ± SEM of the number of flinches/shakes of the phase 1 and 2 of the formalin test. The data obtained from the formalin test were statistically analyzed using two-way ANOVA. The factors of variation were paracetamol treatment and serotonin antagonist or agonist treatment. Subsequent one-way ANOVA was performed followed by Student-Newman-Keuls′ test, a value of $p < 0.05$ was considered to be significant.

Results

Antinociceptive effect of paracetamol in formalin test

The antinociceptive effect of paracetamol was evaluated in the formalin test in rats. One-way ANOVA showed a significant effect of treatment in both phases of the formalin test (Phase 1: $F_{4,34} = 10.88$, $P < 0.001$; phase 2: $F_{4,34} = 19.21$, $P < 0.001$). Paracetamol induced an increase in pain response latency in a dose-related manner in both phases of the formalin test (Fig. 1). In phase 1, paracetamol 250 mg/kg, but not 125 mg/kg, induced a non significant decrease of the number of flinches. Whereas, paracetamol 500 mg/kg and 1000 mg/kg induced a significant decreased of the number of flinches when compare to saline treated group. Also, dose of 1000 mg/kg induced significant decreased of the number of flinches compared to the doses of 125 and 250 mg/kg of paracetamol. In phase 2, paracetamol 125 mg/kg induced a non significant decreased of the number of flinches. However, paracetamol 250, 500 and 1000 mg/kg induced a significant decreased of the number of flinches compared to saline treated group, also, the decrease of number of flinches induced by paracetamol 500 and 1000 mg/kg was significant compared to paracetamol 125 and 250 mg/kg.

Therefore, paracetamol exert an antinociceptive effect in a dose-dependent manner in the formalin test.

We chose the doses of 125 and 250 mg/kg of paracetamol, with weak analgesic effect, to examine its association with specific antagonist of the serotonin receptors subtypes, 5-HT_{1A} and 5-HT_{1B}. The doses of 500 and 1000 mg/kg of paracetamol, with strong analgesic effect, were chosen to test its combination with specific agonists of the 5-HT_{1A} and 5-HT_{1B} receptors.

Involvement of 5-HT_{1A} receptors in the antinociceptive effect of paracetamol

Effect of 5-HT_{1A} antagonist on antinociceptive effect of paracetamol

The effect of WAY 100,635 0.8 mg/kg (selective 5-HT_{1A} antagonist) on the antinociceptive effect of paracetamol 125 mg/kg (a non effective analgesic dose) and 250 mg/kg (a weak antinociceptive dose) was