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α_2-Adrenoceptors and 5-HT receptors mediate the antinociceptive effect of new pyrazolines, but not of dipyrone

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Abstract

In this study, we investigated whether spinal noradrenergic and serotonergic systems are involved in the antinociception induced by the novel pyrazolines 3-methyl- and 3-phenyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-pyrazole-1-carboxyamide (MPCA and PPCA, respectively), and the pyrazolinone dipyrone in the acetic acid writhing (stretching) test in mice. Intrathecal (i.t.) administration of methysergide (3 and 10 μg) and yohimbine (3 μg), but not of prazosin (0.3 and 1 μg) prevented the antinociceptive action of MPCA and PPCA (500 μmol/kg, s.c.). Dipyrone-induced antinociception (500 μmol/kg, s.c.) was not affected by methysergide or adrenoceptor antagonists. These results suggest that spinal 5-HT receptors and α_2-adrenoceptors are involved in the antinociception induced by MPCA and PPCA, but not in that elicited by dipyrone.

Keywords: Dipyrone; Antinociception; Serotonin; Methysergide; Yohimbine; Pyrazoline

1. Introduction

Pyrazole-derived compounds are synthetic molecules with antiinflammatory, antipyretic and analgesic actions (Borne, 1995). These compounds, particularly dipyrone, gained popularity in Europe and in developing countries due to their low cost and good effectiveness as analgesics and antipyretics (Arellano and Sacristan, 1990), although some concern regarding their safety appeared in the literature (The International Agranulocytosis And Aplastic Anemia Study, 1986). Since pain and fever are the most common complaints in clinical practice and the arsenal of effective analgesics and antipyretics is relatively small, we have been investigating the antinociceptive and antipyretic potential of new pyrazole derivatives (de Souza et al., 2001; Souza et al., 2002).

We have recently described the antipyretic activity of 3-methyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-pyrazole-1-carboxyamide (MPCA; Fig. 1A, inset) and 3-phenyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-pyrazole-1-carboxyamide (PPCA; Fig. 1B, inset) in mice (Souza et al., 2002). MPCA is known to cause antinociception in the formalin test, an effect that does not involve opioid mechanisms (de Souza et al., 2001). However, the mechanisms underlying the antinociceptive effect of MPCA have not been elucidated and we do not know whether its phenyl-substituted congener causes antinociception. Therefore, in the present study we investigated whether MPCA and PPCA cause antinociception in the acetic acid writhing test (stretching test) in mice, and whether spinal serotonergic and adrenergic mechanisms are involved in the antinociceptive effect of these compounds. The effects of bioactive pyrazoles on other behavioral measures, such as

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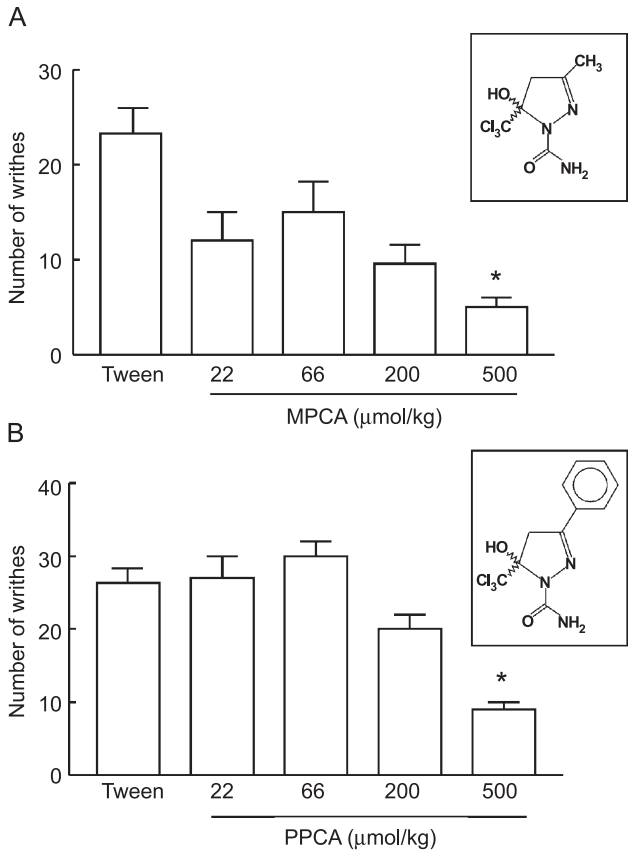

Fig. 1. Effect of MPCA (A) and of PPCA (B) on the number of writhes induced by acetic acid. Data are reported as means ± S.E.M., $n = 8–9$ per group. *$P < 0.05$ compared with 5% Tween 80. Insets A and B: Chemical structures of MPCA and PPCA, respectively. We determined, in a pilot experiment, that Tween-injected animals were not different from saline-injected animals.

spontaneous and forced locomotion (rotarod testing) were also evaluated.

2. Materials and methods

2.1. Drugs

MPCA and PPCA were synthesized as reported elsewhere (Bonacorso et al., 1999) and were suspended in 5% Tween 80. Methysergide, prazosin and yohimbine were purchased from Sigma (St. Louis, MO) and were solubilized in 5% ethanol, 1.5% ethanol plus 0.3% dimethyl sulfoxide and 0.9% NaCl, respectively. Dipyrone (Hoechst, São Paulo, Brazil) was diluted in 0.9% NaCl. All other reagents were of analytical grade and were purchased from local suppliers.

2.2. Animals

Three-month-old male albino Swiss mice (30–40 g) bred in our animal house were used. The animals were housed in groups of 20 to a cage at controlled temperature (23 ± 1 °C) with a 12-h light/dark cycle and with standard lab chow and tap water ad libitum. Each animal was used only once. The experiments were approved by the Committee on the Use and Care of Laboratory Animals of our University.

2.3. Rotarod

Twenty-four hours before the experiments, all animals were trained in the rotarod (3.7 cm in diameter, 8 rpm) until they could remain in the apparatus for 60 s without falling. On the day of the experiment, the animals were injected with vehicle or the pyrazole derivative (22, 66, 200 or 500 μmol/kg, s.c.) and subjected to the rotarod 15 min thereafter. In the experiments designed to evaluate the participation of spinal serotonergic and adrenergic mechanisms in the antinociceptive effect of the pyrazolines, the animals were injected with the pyrazole derivative (500 μmol/kg, s.c.) and with methysergide (1, 3 or 10 μg/5 μl, i.t.), yohimbine (3 μg/5 μl, i.t.), prazosin (0.3 or 1 μg/5 μl, i.t.) or the respective vehicle. Fifteen minutes thereafter, each mouse was tested in the rotarod. The latency to fall from the apparatus was recorded with a stopwatch up to 240 s, as also was the number of falls in a 4-min session (Tsuda et al., 1996). The intrathecal injection was performed as described by Hylden and Wilcox (1980).

2.4. Writhing test

Ten minutes after rotarod evaluation, the effect of the pyrazolines on the number of writhing responses induced by the intraperitoneal administration of acetic acid was evaluated (Hayashi and Takemori, 1971). The animals were injected with acetic acid (0.8% in distilled water–10 ml/kg body weight, i.p.), and 5 min later were transferred to an open field (28 × 18 × 12 cm) with a floor divided into 15 equal areas. The number of writhes, rearing responses and areas crossed with the four paws was counted over a period of 10 min by an observer who was not aware of the animals' treatment.

2.5. Statistical analysis

Data concerning the number of writhes and ambulation scores of dose–effect curves were analyzed statistically by one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. Dose–effect relationships were assessed by partitioning ANOVA total sum of squares into trend components. Data from the experiments evaluating the role of spinal 5-HT receptors and α-adrenoceptors in pyrazole derivative-induced analgesia were analyzed by two-way ANOVA, followed by the Student–Newman–Keuls test.

3. Results

Fig. 1A shows the effect of MPCA on the number of writhes induced by acetic acid. Statistical analysis revealed

that MPCA decreased writhing activity [$F(4,37)=3.14$; $P<0.05$] linearly with its dose [$F(1,37)=7.00$; $P<0.05$]. The 3-phenyl substituted pyrazole derivative (PPCA) also decreased writhing scores [$F(4,37)=4.93$; $P<0.005$–Fig. 1B] linearly with its dose [$F(1,37)=17.21$; $P<0.001$]. MPCA and PPCA did not alter rotarod performance or spontaneous locomotor activity during writhing evaluation (data not shown), suggesting that PPCA- and MPCA-induced antinociception is not related to motor impairment or sedation.

The involvement of spinal α_2-adrenoceptors in the antinociception induced by dipyrone, MPCA and PPCA was investigated by pretreating the animals with yohimbine (3 μg/5 μl, i.t.). Statistical analysis revealed a significant pretreatment (vehicle or yohimbine) by treatment (vehicle, MPCA, PPCA or dipyrone) interaction [$F(3,51)=5.18$; $P<0.005$; Fig. 2A]. Post hoc analysis revealed that yohimbine prevented MPCA- and PPCA-, but not dipyrone-induced antinociception. Prazosin (0.3 and 1 μg/5 μl, i.t.) had no effect on the antinociception induced by the pyrazole derivatives (data not shown).

Fig. 2B shows the effect of the nonselective 5-HT receptor antagonist methysergide (1, 3 or 10 μg/5 μl, i.t.) on MPCA-, PPCA- and dipyrone-induced antinociception. Statistical analysis revealed a significant pretreatment (vehicle, 1, 3 or 10 μg methysergide) by treatment (vehicle, MPCA, PPCA or dipyrone) interaction [$F(9,148)=2.32$; $P<0.05$]. Post-hoc analysis revealed that intrathecal administration of 3 and 10 μg methysergide prevented MPCA- and PPCA-, but not dipyrone-induced antinociception and that 10 μg methysergide caused antinociception per se. These results suggest the involvement of serotonergic mechanisms in the antinociceptive effect of MPCA and PPCA. Methysergide had no effect on animals' performance in the rotarod or on spontaneous locomotor behavior during writhing evaluation (data not shown).

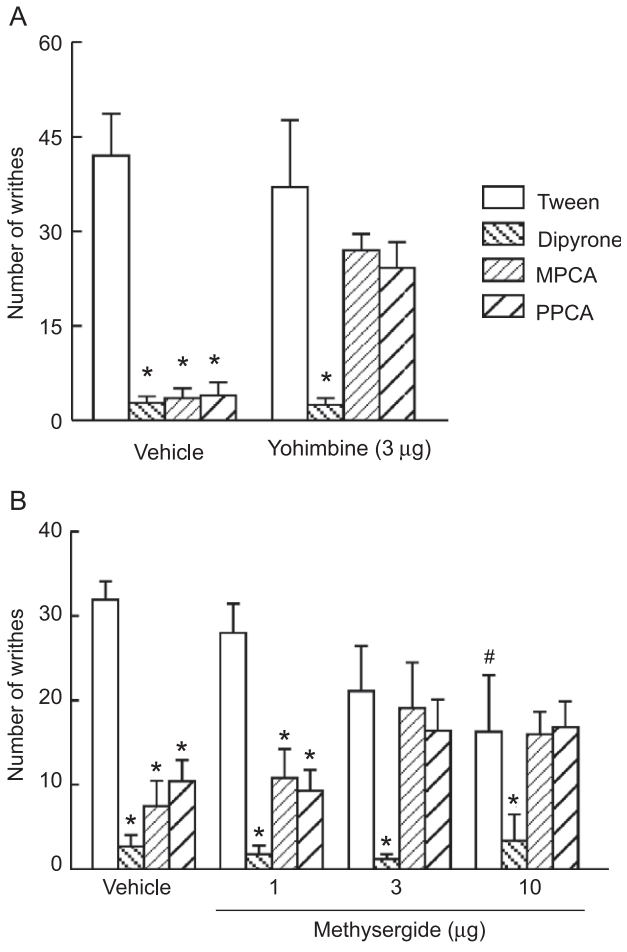

Fig. 2. Effect of yohimbine (A) and methysergide (B) on the antinociception induced by MPCA, PPCA and dipyrone (500 μmol/kg, s.c.). Data are reported as means ± S.E.M., $n=8$–9 per group. *$P<0.05$ compared with 5% Tween 80. #$P<0.05$ compared with vehicle in the absence of methysergide.

4. Discussion

It is well known that adrenergic and serotonergic descending spinal pathways modulate nociception (Basbaum and Fields, 1984; Millan, 2002), and there is evidence that these pathways are activated by some analgesics (Yaksh, 1985; Pini et al., 1996; Ochi and Goto, 2000b, 2001). In fact, the periaqueductal gray and its efferent descending serotonergic pathway have been implicated in the antinociceptive effect of centrally administered dipyrone (Tortorici et al., 1996; Carlsson and Jurna, 1987) and 3-(difluoromethyl)-1-(4-methoxyphenyl)-5-[4-(methyl sulfinyl) phenyl] pyrazole (FR140423), a pyrazole derivative (Ochi and Goto, 2000a, 2001). Interestingly, the spinal noradrenergic system has also been implicated in the antinociceptive effect FR140423, suggesting that the activation of descending inhibitory systems may be a common mechanism of action for pyrazole derivatives (Ochi and Goto, 2000b).

In the present study, we describe for the first time the antinociceptive effect of the pyrazole derivative PPCA and present evidence that spinal serotonergic and α_2-adrenergic mechanisms are involved in the nociception induced not only by PPCA, but also by MPCA. Moreover, we report that adrenoceptor antagonists and 5-HT receptor antagonists do not alter the antinociception induced by the systemic administration of dipyrone, which was used as an internal control in our experiments. The currently reported lack of participation of serotonergic mechanisms in dipyrone-induced antinociception agrees with data reported by Beirith et al. (1998), who have described that serotonin-depleted animals present dipyrone-induced analgesia, but disagrees with studies that have proposed the involvement of the periaqueductal gray (and the descendent serotonergic pathway) in the antinociceptive action of dipyrone (Carlsson et al., 1986; Carlsson and Jurna, 1987; Vanegas et al., 1997). It is worth noting, however, that the studies that have sug-

gested a role for periaqueductal gray and nucleus raphe magnus in dipyrone-induced analgesia have injected this compound centrally (Tortorici et al., 1996; Jones, 1996; Hernández and Vanegas, 2001). Therefore, one should consider that centrally and systemically administered dypirone might have different mechanisms of action. In fact, spinal rats do respond to systemically injected dipyrone, but do not respond to the intrathecal injection of this pyrazolinone (Carlsson et al., 1986). Moreover, the ED_{50} for dipyrone (given i.p.) determined in spinal rats does not differ from the one obtained from experiments on intact animals (Carlsson et al., 1986), indicating that the participation of the descending spinal systems in the antinociception induced by systemic dipyrone is minimal, and may involve peripheral mechanisms (Alves and Duarte, 2002). On the other hand, a role for on- and off-cells of the rostral ventral medulla in systemic dipyrone-induced analgesia has been proposed based on the temporal coincidence between the inhibition and activation of these cells within this structure, and the occurrence of analgesia (Tortorici and Vanegas, 1994). However, it is worth noting that such a temporal coincidence suggests but does not imply a cause–effect relationship between these events.

In summary, the literature indicates that the periaqueductal gray is involved in the antinociceptive action of centrally, but not systemically administered dipyrone, and our results suggest that spinal adrenergic and serotonergic mechanisms are not involved in the antinociceptive action of this pyrazolinone. Moreover, the pharmacological profiles of dipyrone and the new pyrazole derivatives tested were markedly different, suggesting that they do not share a common mechanism of action.

In the present study, MPCA and PPCA interacted similarly with α_2-adrenoceptor and 5-HT receptor antagonists. These results suggest that the substitution of a methyl for a phenyl group in position 3 in the pyrazole ring does not alter the antinociceptive properties of these compounds or, alternatively, that their metabolism generates a common bioactive metabolite, which interacts with the spinal noradrenergic and serotonergic systems. Although the exact mechanisms by which MPCA and PPCA alter serotonergic and adrenergic functions are unknown, this is the first study implying the descendent inhibitory systems in the antinociceptive effect of pyrazole carboxyamides. In addition, it provides the first positive evidence for a mechanism of action for these compounds, since it has been previously demonstrated that opioid mechanisms are not involved in the antinociceptive action of MPCA, and that this compound lacks antiinflammatory activity (de Souza et al., 2001).

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