Brazilian Journal of Medical and Biological Research (2006) 39: 795-799
ISSN 0100-879X Short Communication

A pyrazolyl-thiazole derivative causes antinociception in mice

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Abstract

The present study investigates the antinociceptive effect of the pyrazolyl-thiazole derivative 2-(5-trichloromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-bromophenyl)-5-methylthiazole (B50) in mice. Male albino Swiss mice (30-40 g) were used in the acetic acid-induced abdominal writhes and tail-immersion tests. B50 caused dose-dependent antinociception (8, 23 and 80 μmol/kg, *sc*) in the acetic acid writhing assay (number of writhes: vehicle: 27.69 ± 6.15; B50 (8 μmol/kg): 16.92 ± 3.84; B50 (23 μmol/kg): 13.85 ± 3.84; B50 (80 μmol/kg): 9.54 ± 3.08; data are reported as means ± SEM for 9 animals per group). On the other hand, B50 did not cause antinociception in the tail immersion assay. Naloxone (2.75 μmol/kg, *sc*) prevented B50-induced antinociception (number of writhes: vehicle-saline: 31.11 ± 3.15; vehicle-naloxone: 27.41 ± 3.70; B50 (80 μmol/kg)-saline: 8.70 ± 3.33; B50 (80 μmol/kg)-naloxone: 31.84 ± 4.26; morphine-saline: 2.04 ± 3.52; morphine-naloxone: 21.11 ± 4.26; 8-9 animals per group). The removal of the methyl group of the thiazole ring of B50 or substitution of the bromo substituent with the methyl at position 4 of the phenyl group, which is attached to the thiazole ring of B50, resulted in loss of activity, suggesting that these substituents are important for antinociceptive activity. B50 had no effect on spontaneous locomotion or rotarod performance, indicating that the antinociceptive effect of B50 is not related to nonspecific motor effects. The antinociceptive profile of B50 seems to be closer to nonsteroidal anti-inflammatory drugs than to classic opioid agents, since it had no analgesic effect in a thermally motivated test.

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Research supported by PRONEX/MCT (0408660). M.A. Rubin, H.G. Bonacorso, N. Zanatta, M.A.P. Martins, and C.F. Mello are recipients of CNPq fellowships (Nos. 500096/2003-1, 303636/2002-5, 301474/2003-6, 303116/2002-0, and 500120/2003-0, respectively).

Received March 29, 2005
Accepted December 6, 2005

Key words
- Antinociception
- Pyrazolyl-thiazole derivatives
- Writhing test
- Opioid mechanism
- Naloxone

The pyrazole ring is a heterocycle compound containing two contiguous nitrogen atoms and three carbon atoms. Until 1995, in a few cases, only 5-trifluoromethyl-5-hydroxy-1*H*-pyrazolines and pyrazolidines could be synthesized, and pyrazolines other than 5-trifluoromethylated ones had been detected only by special nuclear magnetic resonance techniques (1). In 1999, Bonacorso et al. (2) described for the first time the synthesis and structural elucidation by nuclear magnetic resonance, Austin Model 1 calculations and X-ray diffraction of a novel series of twelve 3-aryl(alkyl)-5-hydroxy-1-

carboxy(thio)amide-1*H*-pyrazolines bearing a trichloromethyl group in the final stable products. These compounds served as substrates for the synthesis of novel pyrazolyl-thiazole derivatives (3) such as 2-(5-trichloromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-bromophenyl)-5-methylthiazole (B50) (Figure 1).

We have reported that the pyrazole derivative 3-methyl-5-hydroxy-5-trichloromethyl-1*H*-1-pyrazolcarboxyamide induces antinociception in the neurogenic and inflammatory phases of the formalin test, and that this effect does not involve opioid receptors (4). This compound and its 3-phenyl substituted derivative also cause antinociception in the acetic acid writhing test in mice, which seems to involve the activation of spinal α_2-adrenoceptors and 5-HT receptors (5), since their analgesic effects are prevented by the spinal administration of nonspecific serotonergic and α_2-adrenoceptor antagonists.

In the current study, we extend our investigations on the antinociceptive action of pyrazole derivatives to pyrazolyl-thiazole compounds. Thus, we investigated the antinociceptive effect of B50 and the involvement of the opioid mechanisms in this effect in adult male mice. Moreover, by introducing some modifications in the original molecule and assessing its antinociceptive activity, we obtained some structure-activity relationship information.

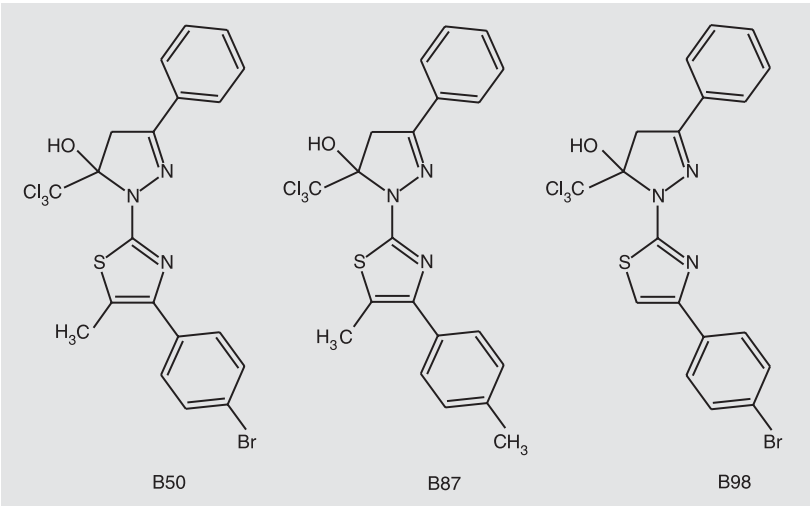

Figure 1. Chemical structure of B50: 2-(5-trichloromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-bromophenyl)-5-methylthiazole; B87: 2-(5-trichloromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-methylphenyl)-5-methylthiazole, and B98: 2-(5-trichloromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-bromophenyl)-5-thiazole.

Naloxone, morphine sulfate, acetic acid, and Tween 80 were purchased from Sigma (St. Louis, MO, USA). B50 was synthesized by our group as previously described (3). Two B50 derivatives were obtained and tested for antinociceptive activity. One of these compounds had the bromo atom substituted with the methyl substituent at position 4 of the phenyl, which is attached to the thiazole ring (2-(5-trichloromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-methylphenyl)-5-methylthiazole, B87), while the other was devoid of the methyl substituent in the thiazole ring (2-(5-trichloromethyl-5-hydroxy-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-(4-bromophenyl)-5-thiazole, B98) (Figure 1).

All drugs were dissolved in saline (0.9% sterile sodium chloride), except B50, B87 and B98 which were suspended in 5% Tween 80 in 0.9% sodium chloride (vehicle). The sterile solutions were prepared immediately before use and were injected in a volume of 10 mL/kg.

Male albino Swiss mice (30-40 g) bred in our animal house were used in the acetic acid-induced abdominal writhes, rotarod and tail-immersion tests. The animals were housed in groups of 20 per cage at controlled temperature (22 ± 1ºC) under a 12-h light/dark cycle and with standard lab chow and tap water *ad libitum*. The animals (20 per cage) were transferred to the experimental room 24 h before the experiments for habituation to the environment and handling. Each animal was used only once. The experiments were approved by the Committee on the Use and Care of Laboratory Animals of our University.

The writhing activity was evaluated by the method of Hayashi and Takemori (6).

The number of stretches or writhes (arching of the back, development of tension in the abdominal muscles, elongation of the body, and extension of the forelimbs) was counted during a period of 10 min starting 5 min after the administration of 0.8% (v/v) acetic acid (10 mL/kg, *ip*).

Animals received B50 (8, 23, 80 µmol/kg, *sc*), B87 (80 µmol/kg, *sc*) or B98 (80 µmol/kg, *sc*) 30 min before acetic acid injection. Drug doses were selected according to the maximal concentration achievable in a homogeneous suspension on an equimolar basis (maximal possible dose). Control animals received a similar volume of appropriate vehicle (5% Tween 80 in 0.9% NaCl, *sc*). Five minutes after the administration of acetic acid the animals were transferred to a separate box (28 x 18 x 12 cm), whose floor was divided into 15 equal areas. The number of abdominal writhes and areas crossed with the four paws were counted for 10 min. In order to investigate the involvement of the opioid system in the antinociceptive effects of B50, animals were pretreated with naloxone (2.75 µmol/kg, *sc*) 20 min before the administration of B50 (80 µmol/kg, *sc*), morphine (6.5 µmol/kg, *sc*) or vehicle (10 mL/kg, *sc*). The doses of naloxone and morphine were selected according to Tabarelli et al. (7).

In order to determine if B50 had nonspecific muscle-relaxant or sedative effects, the mice were tested in a rotarod, as described by Tsuda et al. (8). Each mouse was trained to run in a rotarod (3.7 cm in diameter, 8 rpm) until it could remain there for 60 s without falling. Twenty-four hours later, the animals were injected with vehicle or B50 (8, 23, 80 µmol/kg, *sc*) and, after 15 min, their latency to fall from the apparatus and the number of falls were recorded for up to 240 s.

The tail-immersion test was carried out as described by Janssen et al. (9). The animals were weighed and had the last 3.5 cm of their tail immersed in hot water (55ºC), a procedure that caused them to rapidly withdraw their tail in the absence of pharmacological treatment. Twenty-four hours after this habituation session, the animals were given B50 (80 µmol/kg, *sc*) or vehicle and, had their 3.5 cm end tail immersed again in hot water 45 min later. The latency to withdraw the tail was recorded in 0.1-s units with a stopwatch, and a maximum latency cut-off of 7 s was established to prevent tissue damage.

All data are reported as means ± SEM. The number of writhes and ambulation scores obtained from B50 dose-effect curves were analyzed by one-way ANOVA followed by the Student-Newman-Keuls test. Tail immersion and rotarod latencies were analyzed by Kruskal-Wallis H-test analysis of variance followed by the nonparametric multiple comparisons Nemenyi test. F and H values are presented only when $P < 0.05$. The dose-effect relationship was assessed by partitioning the ANOVA sum of squares into linear, quadratic or cubic trends or by nonparametric correlation analysis. Data from experiments designed to determine the role of opioid receptors in the antinociceptive action of B50 were analyzed by two-way ANOVA.

Figure 2A shows the effect of increasing doses of B50 (8, 23, 80 µmol/kg, *sc*) on the number of writhes induced by acetic acid. Statistical analysis revealed a significant effect of drug doses ($F(3,32) = 3.29$; $P < 0.05$). Partitioning of sum of squares into trend components revealed a significant linear trend ($F(1,32) = 5.99$; $P < 0.05$), indicating that the number of writhes decreased linearly with the dose of B50. *Post hoc* comparisons showed that B50 at doses of 23 and 80 µmol/kg produced a significant decrease in the mean number of writhes, as compared to the vehicle-treated group. Figure 2B shows that the antinociceptive effects of morphine (6.5 µmol/kg, *sc*) and B50 (80 µmol/kg, *sc*) were fully prevented by previous injection of naloxone (2.75 µmol/kg, *sc*) in the ab-

dominal writhing test ($F(1,30) = 6.36$; $P < 0.02$). Naloxone (0.27, 2.75, and 27.5 μmol/kg) had no effect *per se* on the total number of writhes induced by 0.8% acetic acid ($F(3,28) = 0.065$; $P = 0.978$, data not shown).

B50 did not alter spontaneous locomotor activity measured by the number of areas crossed with all paws: vehicle: 28.5 ± 5.1; B50 (8 μmol/kg): 22.2 ± 6.0; B50 (23 μmol/kg): 25.2 ± 3.6; B50 (80 μmol/kg): 27.3 ± 4.4. B50 did not alter rotarod performance measured by the latency for the first fall(s): vehicle: 154.5 ± 36.9; B50 (8 μmol/kg): 82.1 ± 26.5; B50 (23 μmol/kg): 122.5 ± 37.5; B50 (80 μmol/kg): 109.0 ± 33.6, and total number of falls in a 4-min session: vehicle: 1.3 ± 0.6; B50 (8 μmol/kg): 1.8 ± 0.5; B50 (23 μmol/kg): 1.4 ± 0.2; B50 (80 μmol/kg): 3.1 ± 1.0, or tail immersion latencies: vehicle: 3.2 ± 1.1; B50 (80 μmol/kg): 3.0 ± 0.6. B87 and B98 did not alter the number of writhes induced by acetic acid or spontaneous locomotor activity (data not shown).

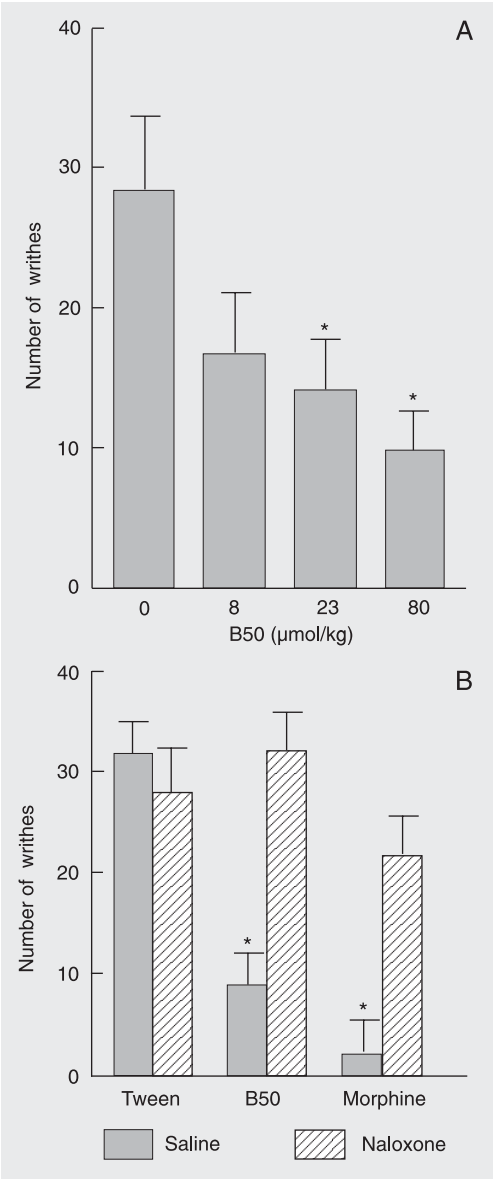

Figure 2. Effect of B50 on the number of writhes induced by acetic acid (A) and effect of naloxone on the antinociception induced by B50 and morphine (B). The dose of B50 was 80 μmol/kg, *sc*, naloxone 2.75 μmol/kg, *sc*, and morphine 6.5 μmol/kg, *sc*. Data are reported as means ± SEM for 8-9 animals per group. *P < 0.05 compared with vehicle (5% Tween 80 in 0.9% sodium chloride; Student-Newman-Keuls test).

The results presented here show that the subcutaneous administration of B50 elicited a significant and dose-dependent antinociception in a chemical model of nociception in mice. In contrast, B50 did not alter tail withdrawal latencies in the tail immersion test.

This profile is similar to that of 3-methyl-5-hydroxy-5-trichloromethyl-1*H*-1-pyrazolcarboxyamide, which causes antinociception in the formalin test in mice, but has no effect in the tail immersion test (4). As a matter of fact, mild analgesics such as non-steroidal anti-inflammatory drugs seem to have little or no analgesic effect in standard thermally motivated tests such as the hot-plate and tail-flick tests (10). Therefore, in this context, B50 seems to be closer to non-steroidal anti-inflammatory drugs than to classic opioid agents. On the other hand, B50-induced antinociception was prevented by the non-selective opioid antagonist naloxone at a dose that completely prevented morphine-induced antinociception in the writhing test. The unusual antinociceptive profile of B50 (a compound whose antinociceptive action is prevented by naloxone and that does not cause antinociception in the tail immersion test) may be important but at this time it does not allow us to identify a precise mechanism of action, and further studies assessing its anti-inflammatory action are needed to clarify this point.

We also determined whether selective substitutions in the moiety of the compound altered its antinociceptive activity. The re-

moval of the methyl group in the thiazole ring and the substitution of the Br in the phenyl-thiazole group with a methyl radical completely abolished its antinociceptive action. These results suggest that both the methyl group in the thiazole ring and the Br in the phenyl-thiazole group are important to determine its antinociceptive action. Regarding this point, it is interesting that while the anti-inflammatory properties of pyrazole-derived compounds have been associated with the presence of carbonyl groups at positions 3 and/or 5 of the pyrazole moiety (11), a limited amount of information is available about the effect of substitutions in the pyrazole ring on the antinociceptive action of pyrazole derivatives. Indeed, pyrazole-derived compounds lacking such carbonyl groups have little or no anti-inflammatory activity, but some of them present a significant antinociceptive action (4,5,12,13). Accumulated evidence in the literature and in studies by our group suggests that the presence of a phenyl group at the 1 position of the pyrazole ring increases antinociceptive activity (7). In the present study, we show that a pyrazoline derivative with a phenyl-methylthiazole at the 1 position of the pyrazole ring may have significant antinociceptive activity, which is abolished by the removal of the methyl group in the thiazole ring.

In summary, we report here that the systemic injection of the pyrazolyl-thiazole derivative B50 causes antinociception in a chemically stimulated test. B50-induced antinociception was reversed by the nonselective opioid antagonist naloxone, suggesting that opioid mechanisms may be involved in this effect.

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