

Antinociceptive effect of novel pyrazolines in mice

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Abstract

The antinociceptive effect of six novel synthetic pyrazolines (3-ethoxymethyl-5-ethoxycarbonyl-1*H*-pyrazole (Pz 1) and its corresponding 1-substituted methyl (Pz 2) and phenyl (Pz 3) analogues, and 3-(1-ethoxyethyl)-5-ethoxycarbonyl-1*H*-pyrazole (Pz 4) and its corresponding 1-substituted methyl (Pz 5) and phenyl (Pz 6) analogues) was evaluated by the tail immersion test in adult male albino mice. The animals (N = 11-12 in each group) received vehicle (5% Tween 80, 10 ml/kg, *sc*) or 1.5 mmol/kg of each of the pyrazolines (Pz 1-Pz 6), *sc*. Fifteen, thirty and sixty minutes after drug administration, the mice were subjected to the tail immersion test. Thirty minutes after drug administration Pz 2 and Pz 3 increased tail withdrawal latency (vehicle = 3.4 ± 0.2 ; Pz 2 = 5.2 ± 0.4 ; Pz 3 = 5.9 ± 0.4 s; mean \pm SEM), whereas the other pyrazolines did not present antinociceptive activity. Dose-effect curves (0.15 to 1.5 mmol/kg) were constructed for the bioactive pyrazolines. Pz 2 (1.5 mmol/kg, *sc*) impaired motor coordination in the rotarod and increased immobility in the open-field test. Pz 3 did not alter rotarod performance and spontaneous locomotion, but increased immobility in the open field at the dose of 1.5 mmol/kg. The involvement of opioid mechanisms in the pyrazoline-induced antinociception was investigated by pretreating the animals with naloxone (2.75 μ mol/kg, *sc*). Naloxone prevented Pz 3- but not Pz 2-induced antinociception. Moreover, naloxone pretreatment did not alter Pz 3-induced immobility. We conclude that Pz 3-induced antinociception involves opioid mechanisms but this is not the case for Pz 2.

Key words

- Pyrazole-derived compounds
- Antinociception
- Tail immersion test
- Rotarod test
- Open-field test
- Immobility

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Introduction

Some pyrazole-derived compounds such as pyrazolidinediones possess a number of pharmacological and biochemical properties, which include oxidative phosphorylation

uncoupling, stabilization of lysosomal membranes, inhibition of the biosynthesis of various mucopolysaccharides, and, perhaps most importantly, inhibition of prostaglandin biosynthesis in the cyclooxygenase step (1).

Due to their ability to inhibit cyclooxy-

genase activity and the arachidonic acid cascade, pyrazole-derived compounds, including dipyrone, have anti-inflammatory, antipyretic and analgesic properties (2-6). Because of these properties, these drugs have been classified as nonsteroidal anti-inflammatory drugs (NSAIDs), and their administration results in a reduced synthesis of peripherally and centrally formed prostaglandins (2,4,7-9), which physiologically enhance the sensitivity of the primary afferent to algogenic substances (10). However, other mechanisms of action have been proposed for the antinociception induced by pyrazole-derived compounds, such as decrease of on/off cell firing in the periaqueductal gray (11) and activation of opioid mechanisms (12). It is worth noting, however, that the pharmacological profile of pyrazole-derived compounds is not homogeneous, since lack of anti-inflammatory activity has also been described for different pyrazole-derived compounds, as well as independence of opioid mechanisms (13).

In the present study, we describe the antinociceptive action of a new series of pyrazole-derived compounds and the involvement of opioid mechanisms in this effect. The effects of bioactive pyrazolines on other behavioral measures, such as spontaneous and forced locomotion (rotarod testing) were also evaluated.

Material and Methods

The present study was conducted in accordance with the ethical guidelines established for investigations of experimental pain in conscious animals (14) and was approved by the Ethics Committee for the Use of Animals in Research of Universidade Federal de Santa Maria, RS, Brazil.

Animals

Male albino mice weighing 25-35 g were used. The animals had free access to water

and food and were maintained on a 12-h light/dark cycle under conditions of controlled temperature ($23 \pm 1^\circ\text{C}$) and humidity (40-60%).

Drugs

3-Ethoxymethyl-5-ethoxycarbonyl-1*H*-pyrazole (Pz 1), and its corresponding 1-substituted methyl (Pz 2) and phenyl (Pz 3) analogues were obtained from the cyclocondensation reaction of 5-bromo-1,1,1-trichloro-4-methoxy-3-penten-2-one (15,16) with hydrazine (methyl hydrazine or phenyl hydrazine) using ethanol as a solvent. 3-(1-Ethoxyethyl)-5-ethoxycarbonyl-1*H*-pyrazole (Pz 4) and its corresponding 1-substituted methyl (Pz 5) and phenyl (Pz 6) analogues were obtained from the cyclocondensation reaction of 5-bromo-1,1,1-trichloro-4-methoxy-3-hexen-2-one (15,16) with methyl hydrazine or phenyl hydrazine using ethanol as a solvent. These cyclocondensation reaction proceeds with simultaneous transformation of the trichloromethyl group into an ethoxycarbonyl group and of the bromomethyl group into an ethoxymethyl group, according to methodologies developed in our laboratory (16). Tween 80 and naloxone were purchased from Sigma (St. Louis, MO, USA).

Pyrazolines were suspended in 5% Tween 80 (0.5, 1.0 or 1.5 mmol/10 ml); naloxone (2.75 $\mu\text{mol}/10\text{ ml}$) and morphine sulfate (6.6 $\mu\text{mol}/10\text{ ml}$) were dissolved in saline. All solutions were administered subcutaneously (*sc*, 10 ml/kg).

Tail immersion test

The tail immersion test was carried out to determine whether one or more of the novel pyrazolines caused antinociception. Twenty-four hours before the experiment, all mice were habituated to the experimental procedure (subcutaneous injection and measurement of tail withdrawal latency) in order to minimize novelty-induced antinociception

(17). On the day of the experiment the animals were injected *sc* with vehicle (5% Tween 80) or Pz 1, Pz 2, Pz 3, Pz 4, Pz 5, or Pz 6 at the dose of 1.5 mmol/kg, and subjected to the tail immersion test 15, 30 and 60 min thereafter. Drug doses were selected according to the maximal concentration achievable in a homogeneous suspension on an equimolar basis (maximal possible dose).

The tail immersion test was carried out as described by Janssen et al. (18). The tail-flick latency of each animal was determined by immersing the lower 3.5 cm of the tail into a cup filled with water from a large constant temperature (55°C) bath and recording the tail withdrawal latency (in seconds) with a manual stopwatch. A cut-off time of 7 s was established in order to prevent tissue damage.

When the compounds which caused antinociception were identified, a dose-response curve was constructed for each one. Pz 2 and Pz 3 (0.15-1.5 mmol/kg) were administered 30 min before the tail immersion test, since the first set of experiments showed that after 60 min their antinociceptive effect had completely vanished. Five minutes after the tail immersion test the animals were subjected to the rotarod test.

In the experiments designed to evaluate the participation of opioid mechanisms in Pz 2- and Pz 3-induced antinociception, animals were injected with naloxone (2.75 µmol/kg, *sc*) or saline (0.9% NaCl, 10 ml/kg, *sc*) 20 min before the injection of vehicle (5% Tween 80, 10 ml/kg) or of the pyrazolines (Pz 2 or Pz 3 at 1.5 mmol/kg), and subjected to the same experimental protocol as described above.

Rotarod evaluation

The rotarod test was used to assess whether Pz 2 and Pz 3 caused gross motor impairment in the animals. Twenty-four hours before the experiment, all mice were habituated to running in a rotarod (3.7 cm in diam-

eter, 8 rpm) until they could remain there for 60 s without falling. Five minutes after the measurement of tail withdrawal (35 min after *sc* injection of vehicle or pyrazolines), each mouse was tested in the rotarod. The latency to fall, as well as the number of falls from the apparatus, were recorded with a stopwatch up to 240 s (19-21).

Open-field evaluation

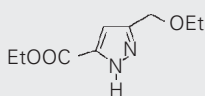
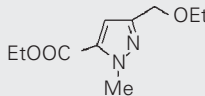
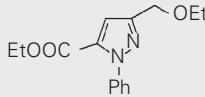
The effect of the compounds on spontaneous locomotor activity and exploratory behavior was assessed by the open-field test, as previously reported (19). The apparatus was a round arena (34 cm in diameter) with the floor divided into 21 equal areas. Immediately after rotarod evaluation (40 min after *sc* injection of vehicle or pyrazolines), the animals were transferred to the apparatus and observed for 5 min. The number of rearing responses, number of areas crossed with all paws, and total time spent immobilized (immobility) were recorded.

Statistical analysis

The tail withdrawal latencies, the latency to the first fall from the rotarod, the number of falls from the rotarod, and the duration of immobility were analyzed by one-way ANOVA. Data were square root transformed before analysis in order to meet the assumptions for ANOVA. *Post hoc* tests (Student-Newman-Keuls test) were carried out when appropriate.

In those experiments designed to evaluate the dose-response relationships, the ANOVA between groups sum of squares test was partitioned into trend components (linear, quadratic or cubic). Data from experiments designed to evaluate the participation of opioid mechanisms in pyrazoline-induced analgesia were analyzed by two-way ANOVA. The level of significance was set at $P < 0.05$ and F values are presented in the text only when $P < 0.05$. All behavioral

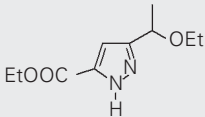
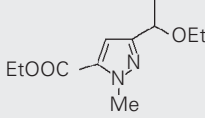
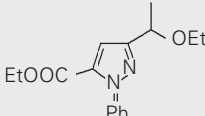
Table 1. Effects of pyrazoles 1, 2 and 3 and morphine on mouse tail withdrawal latency (in seconds) at 15, 30 and 60 min after injection.

Structure	Molecular formula/MW	Name	15 min	30 min	60 min
Vehicle	Tween 80 (5%) 1309.68	Polyoxyethylene (20) sorbitan monooleate	3.1 ± 0.5	3.4 ± 0.2	3.4 ± 0.4
 Pz 1	C ₉ H ₁₄ N ₂ O ₃ 198.21	3-ethoxymethyl-5-ethoxycarbonyl-1H-pyrazole (Pz 1)	2.6 ± 0.3	3.2 ± 0.3	2.5 ± 0.3
 Pz 2	C ₁₀ H ₁₆ N ₂ O ₃ 212.24	3-ethoxymethyl-5-ethoxycarbonyl-1-methyl-1H-pyrazole (Pz 2)	5.0 ± 0.5*	5.2 ± 0.4*	3.9 ± 0.4
 Pz 3	C ₁₅ H ₁₈ N ₂ O ₃ 274.31	3-ethoxymethyl-5-ethoxycarbonyl-1-phenyl-1H-pyrazole (Pz 3)	4.3 ± 0.4	5.9 ± 0.4*	4.2 ± 0.3
Morphine	C ₃₄ H ₄₀ N ₂ O ₁₀ ·5H ₂ O 758.78	Morphine hemi[sulfate pentahydrate]	5.1 ± 0.5*	6.5 ± 0.3*	6.2 ± 0.4*

The structures of Pz 1 to 3 are given in the first column. The pyrazoles (1.5 mmol/kg) and morphine (6.6 μmol/kg) were injected subcutaneously. Data are reported as mean ± SEM for N = 11-12 animals per group.

*P < 0.001 compared with vehicle (5% Tween 80) group (Student-Newman-Keuls test).

Table 2. Effects of pyrazoles 4, 5 and 6 and morphine on mouse tail withdrawal latency (in seconds) at 15, 30 and 60 min after injection.

Structure	Molecular formula/MW	Name	15 min	30 min	60 min
Vehicle	Tween 80 (5%) 1309.68	Polyoxyethylene (20) sorbitan monooleate	3.0 ± 0.3	3.6 ± 0.2	3.6 ± 0.4
 Pz 4	C ₁₀ H ₁₆ N ₂ O ₃ 212.24	3-(1-ethoxyethyl)-5-ethoxycarbonyl-1H-pyrazole (Pz 4)	3.1 ± 0.2	3.6 ± 0.2	3.2 ± 0.3
 Pz 5	C ₁₁ H ₁₈ N ₂ O ₃ 226.26	3-(1-ethoxyethyl)-5-ethoxycarbonyl-1-methyl-1H-pyrazole (Pz 5)	3.4 ± 0.3	4.2 ± 0.3	4.2 ± 0.4
 Pz 6	C ₁₆ H ₂₀ N ₂ O ₃ 288.34	3-(1-ethoxyethyl)-5-ethoxycarbonyl-1-phenyl-1H-pyrazole (Pz 6)	3.5 ± 0.4	4.0 ± 0.3	4.2 ± 0.3
Morphine	C ₃₄ H ₄₀ N ₂ O ₁₀ ·5H ₂ O 758.78	Morphine hemi[sulfate pentahydrate]	5.1 ± 0.5*	6.6 ± 0.3*	6.1 ± 0.4*

The structures of Pz 4 to 6 are given in the first column. The pyrazoles (1.5 mmol/kg) and morphine (6.6 μmol/kg) were injected subcutaneously. Data are reported as mean ± SEM for N = 11-12 animals per group.

*P < 0.001 compared with vehicle (5% Tween 80) group (Student-Newman-Keuls test).

data were scored by two observers who were not aware of the animals' treatment, with >90% agreement between them.

Results

Tail immersion test

Table 1 shows the effect of Pz 1 and its corresponding 1-substituted methyl (Pz 2) and phenyl (Pz 3) analogues (1.5 mmol/kg) on the latency of tail withdrawal from hot water. Morphine (6.6 μ mol/kg) was included in the experiment as an internal standard. Statistical analysis (one-way ANOVA) of tail withdrawal latencies revealed a significant effect of treatment at 15 [F(4,53) = 6.39, $P < 0.001$], 30 [F(4,53) = 16.16, $P < 0.001$] and 60 [F(4,53) = 19.68, $P < 0.001$] min. *Post hoc* analysis (Student-Newman-Keuls test) revealed that Pz 2 and morphine increased tail withdrawal latencies 15 min after their administration, while Pz 2, Pz 3 and morphine increased withdrawal latencies 30 min after their administration. However, only morphine induced significant antinociception 1 h after its administration. Pz 1 did not alter tail withdrawal latencies at any time tested.

Table 2 shows the effect of Pz 4 and its corresponding 1-substituted methyl (Pz 5) and phenyl (Pz 6) analogues (1.5 mmol/kg) on the latency of tail withdrawal from hot water. Statistical analysis (one-way ANOVA) revealed that Pz 4, Pz 5 and Pz 6 did not alter tail withdrawal latencies, while morphine increased withdrawal latencies at all times tested, 15 [F(4,49) = 5.12, $P < 0.002$], 30 [F(4,49) = 19.87, $P < 0.001$] and 60 [F(4,49) = 8.79, $P < 0.001$] min (*post hoc* analysis by the Student-Newman-Keuls test).

Once the pyrazolines that presented antinociceptive activity were identified, a dose-response curve was constructed for each bioactive compound. Figure 1A shows the effects of increasing amounts of Pz 2 on the latency of tail withdrawal from a hot water

bath. Statistical analysis (one-way ANOVA followed by the Student-Newman-Keuls test) revealed that Pz 2 (1.5 mmol/kg) administration significantly increased tail withdrawal latency [F(3,28) = 12.40, $P < 0.001$]. Partitioning of the total sum of squares into trend components revealed that Pz 2 increased withdrawal latencies linearly in a dose-dependent manner [F(1,28) = 34.00, $P < 0.001$].

Figure 1B shows the effect of increasing

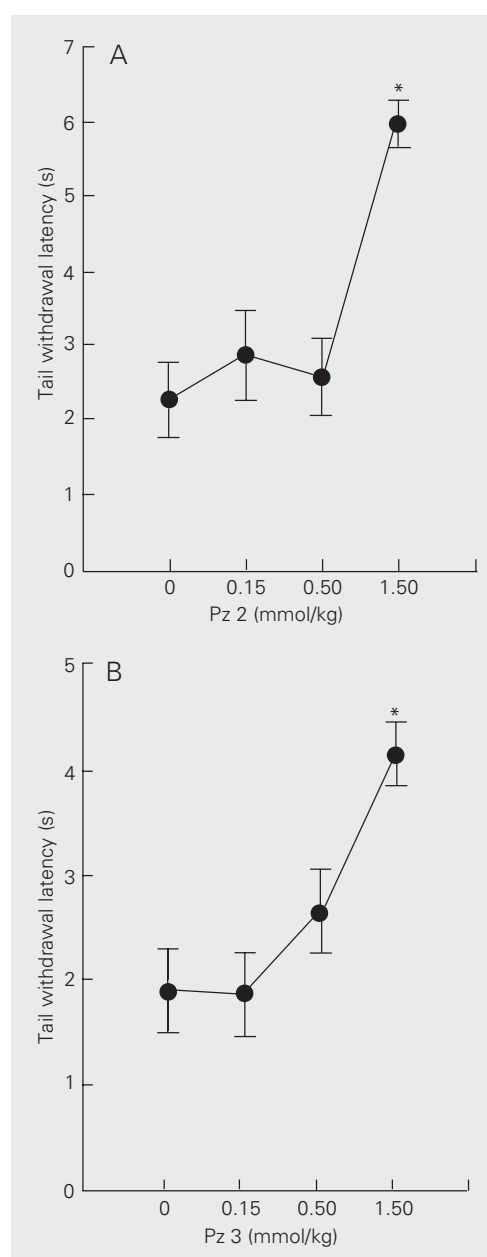


Figure 1. Dose-response curve for Pz 2 (A) and Pz 3 (B) acting on the latency of mouse tail withdrawal from a hot water bath (55°C). Data are reported as mean \pm SEM for N = 6 animals per group. * $P < 0.05$ compared with vehicle group (Student-Newman-Keuls test).

Pz 3 doses on the latency of tail withdrawal from a hot water bath. Statistical analysis revealed that Pz 3 linearly increased tail withdrawal latencies [$F(3,26) = 6.30$, $P < 0.002$] in a dose-dependent manner [significant linear trend: $F(1,26) = 8.94$, $P < 0.01$].

Rotarod evaluation

Figure 2A,B shows the effect of Pz 2 on the rotarod performance of mice. Statistical

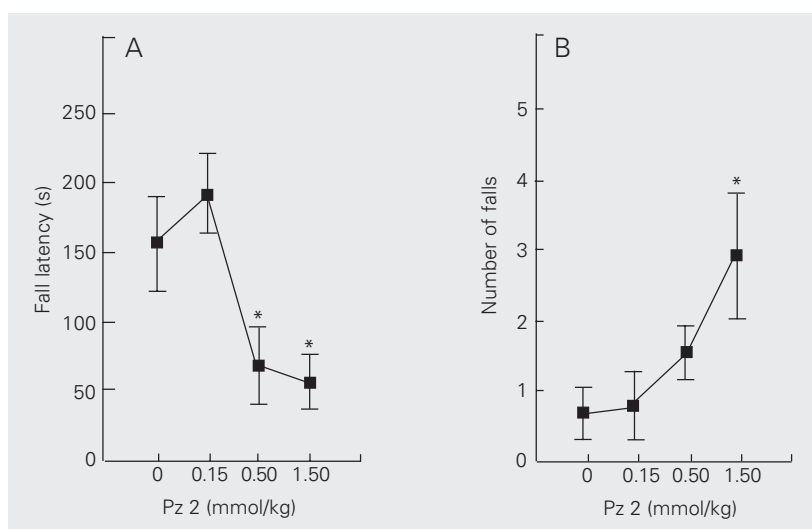


Figure 2. Effects of Pz 2 administration on the latency for the first fall (A) and on the number of falls (B) from the rotarod. Data are reported as means \pm SEM for $N = 8$ animals per group. * $P < 0.05$ compared with vehicle group (Student-Newman-Keuls test).

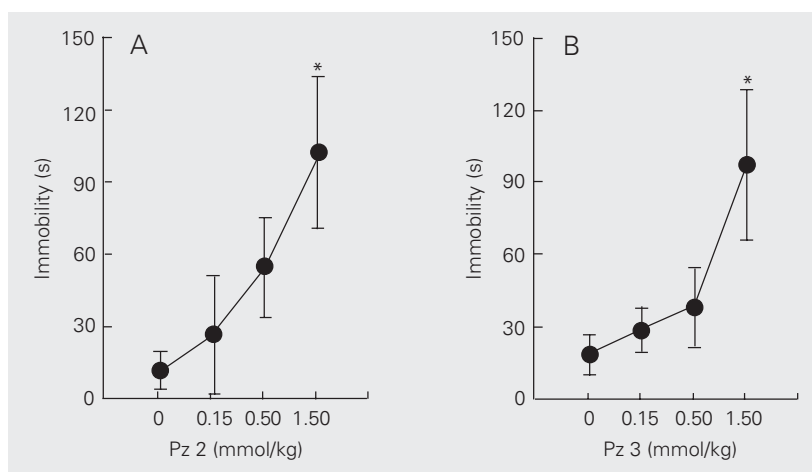


Figure 3. Effects of Pz 2 (A) and Pz 3 (B) on time spent immobilized (immobility) in the open field. Data are reported as means \pm SEM for $N = 8$ animals per group. * $P < 0.05$ compared with vehicle group (Student-Newman-Keuls test).

analysis (one-way ANOVA followed by the Student-Newman-Keuls test) showed that Pz 2 (0.5 and 1.5 mmol/kg) decreased the latency for the first fall from the rotarod [$F(3,28) = 5.80$, $P < 0.05$] and that Pz 2 (1.5 mmol/kg) increased the number of falls from the rotarod [$F(3,28) = 3.22$, $P < 0.03$] in a 4-min session, suggesting that Pz 2 causes some degree of motor impairment.

In contrast to Pz 2, Pz 3 did not alter rotarod performance (data not shown).

Open-field evaluation

Pz 2 and Pz 3 did not alter the number of crossings or the number of rearing responses in the open-field test (data not shown), but increased immobility in the open field [$F(3,28) = 3.80$, $P < 0.03$ and $F(3,26) = 3.55$, $P < 0.03$ - for Pz 2 (Figure 3A) and Pz 3 (Figure 3B), respectively]. Taken together, these data suggest that some degree of motor impairment occurs in the presence of Pz 2 and Pz 3. Due to the deleterious effect of Pz 2 in the rotarod and in the open-field test, it is not possible to definitely rule out a motor effect in the antinociceptive effect of Pz 2 and Pz 3. Pz 3 also seems to cause some degree of motor impairment, but only if immobility in the open field is considered.

Involvement of opioid mechanisms

The involvement of opioid mechanisms in Pz 2- and Pz 3-induced antinociception was investigated by pre-administering naloxone, a nonselective opioid antagonist. Figure 4A shows that naloxone pre-administration prevented Pz 3-, but not Pz 2-induced antinociception (significant pretreatment (saline or naloxone) \times treatment (Tween, Pz 2 or Pz 3) interaction - [$F(2,66) = 3.90$, $P < 0.05$], suggesting the involvement of opioid mechanisms in the presently reported antinociceptive effect of Pz 3. Naloxone, on the other hand, did not alter Pz 3-induced immobility in the open field (Figure 4B).

Discussion

The present study reports the antinociceptive effect of two novel pyrazole-derived compounds (Pz 2 and Pz 3) and the involvement of opioid mechanisms in Pz 3-induced antinociception. Some preliminary conclusions about the structure-activity relationship can be drawn from the comparison of the antinociceptive activity between pyrazole derivatives. It is clear from the structures given in Tables 1 and 2 that the presence of a methyl or a phenyl substituent group at the 1-position of the pyrazole ring increases antinociceptive activity, while the ramification of the lateral chain at the 3-position of the pyrazole ring decreases it. Apart from possible pharmacokinetic differences that could account for the differential antinociceptive effect of these compounds, it is remarkable that a similar structure-activity relationship has been described for the anti-inflammatory activity of pyrazole derivatives, since the presence of a phenyl group at the 1-position of the ring also increases the anti-inflammatory activity of 3,5-pyrazolinediones (1).

Anti-inflammatory agents (including 3,5-pyrazolinediones) are usually devoid of antinociceptive activity in thermal stimulation tests (22). Therefore, although it seems unlikely that a putative anti-inflammatory action underlies the presently reported antinociceptive action of Pz 2 and Pz 3, further specific studies about the anti-inflammatory potential of these compounds are necessary to clarify this point. Opioid agonists are known to cause antinociception in thermal stimulation tests (23-26), a fact that suggests an opioid-like action for the tested pyrazole derivatives. As will be discussed below, the present suggestion of the involvement of opioid mechanisms in Pz 3-induced antinociception was corroborated by the finding that naloxone prevented Pz 3-induced antinociception.

A major concern in experiments designed

to evaluate the analgesic action of new agents is whether pharmacological treatment causes other behavioral alterations, such as motor incoordination and sedation, which might be misinterpreted as analgesia. In the present study, we demonstrate that Pz 2 causes motor incoordination in the rotarod test (as judged by the decreased latency to fall from the apparatus and increased number of falls in a 4-min session). Accordingly, Pz 2 increased immobility in the open-field test, suggesting that this compound causes some degree of motor impairment (or sedation), which might underlie its antinociceptive effect. Therefore, our data do not permit us to conclude that Pz 2 is an analgesic.

However, Pz 3, the other pyrazoline that had antinociceptive activity (see Table 1 and Figure 1B), had no effect on rotarod performance (data not shown), suggesting that this compound does not cause gross motor impairment. Nevertheless, despite the lack of effect on the rotarod test, Pz 3, at doses that caused antinociception in the tail immersion test, increased immobility in the open field (Figure 3B).

It has been suggested that an altered exploratory behavior in the open field may be

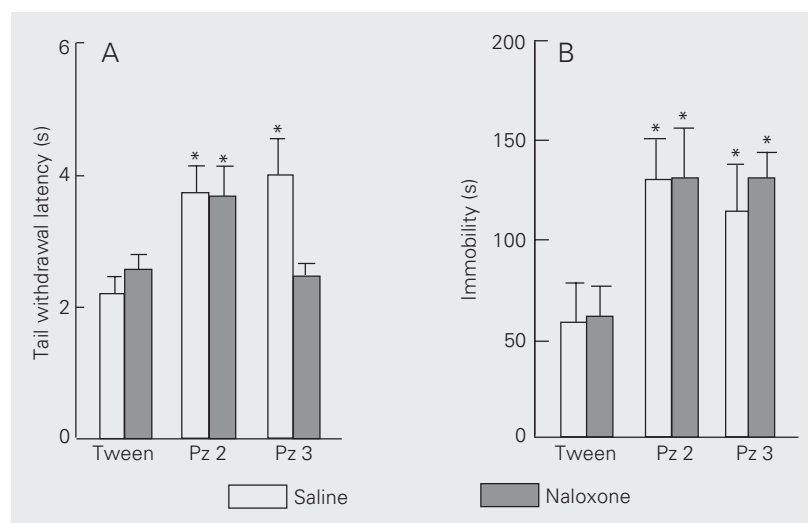


Figure 4. Effect of naloxone on the antinociception (A) and immobility (B) induced by Pz 2 and Pz 3. Naloxone (2.75 μ mol/kg) and Pz 2 and 3 (1.5 mmol/kg) were injected sc. Data are reported as means \pm SEM for N = 7-9 animals per group. *P < 0.05 compared with vehicle (Tween) group (Student-Newman-Keuls test).

caused by alterations in the anxiety state and/or sedation (27). Since it is not known whether Pz 3 modifies the animals' anxiety state, it is not possible to rule out the involvement of these factors in the antinociceptive effect of this compound. On the other hand, Pz 3-induced antinociception was prevented by the injection of naloxone at a dose that had no effect *per se* (Figure 4A).

Naloxone, a nonselective opioid antagonist, has been widely used to demonstrate the involvement of opioid mechanisms of selected compounds in various behavioral tasks, including exploratory behavior and antinociceptive tests (13,28-32). The prevention of Pz 3-induced antinociception by naloxone supports the involvement of opioid mechanisms in this effect. Moreover, although the reversal of the Pz 3 antinociceptive activity by naloxone does not guarantee an analgesic role for this compound, it is rather possible, due to the fairly known selective role of opioid mechanisms in analgesia, that the antinociceptive effects of Pz 3 are, in fact, related to opioid analgesia. Moreover, it is worth mentioning that the reversal of Pz 3 effects by naloxone was restricted to the antinociceptive action of this compound (Figure 4B), suggesting that the antinociceptive effects of Pz 3 are not related to the concomitant increase in immobility (that was not altered by naloxone pretreatment).

This dissociation of the effects of naloxone on motor behavior and on antinociception is an effect of this opioid antagonist that has not been sufficiently addressed. A great deal of the difficulties in elucidating the effect of naloxone and of opioid agonists on locomotor behavior is certainly due to the significant controversy that has appeared in the literature regarding the contribution of each opioid receptor subtype to the locomotor stimulation induced by opioids in mice. A large number of studies have proposed that the opioid-induced increase of locomotor activity in mice is related to μ and δ , but not κ receptor stimulation (33-35), since κ

agonists, in general, cause a decrease in locomotion, while μ and δ agonists increase it. The effects of these opioid agonists on locomotion have been attributed to a modulation of the dopaminergic mesolimbic and nigrostriatal pathways by opioid receptors (36-38). The inhibitory effect of κ agonists on locomotion is reverted by the specific κ antagonist nor-binaltorphimine (nor-BNI) (39). 'Nevertheless, stimulatory effects of κ -agonists at low doses have also been described (39). To further complicate the interpretation of these pharmacological data, it has been demonstrated that the increase of locomotor activity induced by low doses of κ agonists is not reversed by nor-BNI, but is completely eliminated by naloxone (39). Therefore, these studies (and others, related to opioid analgesia) reveal a complex interaction between μ and κ receptors (38), indicating that further investigation is needed to explain these conflicting data.

Nevertheless, it is fairly well known that while naloxone decreases, morphine increases motor activity (13,28-32). Moreover, naloxone (at doses capable of preventing morphine-induced analgesia) prevents the increase of locomotor behavior induced by morphine (13). In this respect, Pz 3 seems to be similar to κ agonists and different from μ agonists such as morphine, since instead of increasing, Pz 3 decreases locomotor activity. Therefore, a selective effect of Pz 3 on κ receptors cannot be ruled out if we consider that a decrease in locomotor activity is often associated with κ receptor activation (but see the above comments on the conflicting role of κ receptor agonists on locomotion).

We have described an interesting interaction of the effects of naloxone and another pyrazoline derivative, 3-methyl-5-hydroxy-5-trichlormethyl-1*H*-1-pyrazolcarboxamide (MPCA) (13). In that study we showed that naloxone (at a dose that has no effect on locomotion or antinociception *per se*) does not alter MPCA-induced antinociception but causes, in the presence of MPCA, a para-

doxical increase in the locomotor behavior of mice. Therefore, the currently reported dissociation of the antinociceptive and motor effects of naloxone is an incompletely understood, but complex event, which merits further investigation.

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