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Hypothermic and antipyretic effects of 3-methyl- and 3-phenyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-pyrazole-1-carboxyamides in mice

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Abstract

The effect of novel pyrazolines, 3-methyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-pyrazole-1-carboxyamide (MPCA) and 3-phenyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-pyrazole-1-carboxyamide (PPCA) on body temperature and endotoxin-induced fever was investigated in mice. The subcutaneous (s.c.) administration of 1.5 mmol/kg dipyrone, MPCA or PPCA and the intracerebroventricular (i.c.v.) administration of 225 nmol dipyrone reduced basal rectal temperature. Intracerebroventricular administration of 225 nmol MPCA or PPCA did not alter basal rectal temperature. The administration of 0.15 mmol/kg (s.c.) or 25 nmol (5 μl) dipyrone (i.c.v.), MPCA or PPCA had no effect on basal rectal temperature, but reversed lipopolysaccharide-induced fever. These results suggest that MPCA and PPCA cause antipyresis, which is similar to that caused by dipyrone, and may be useful antipyretic agents.

Keywords: Lipopolysaccharide; Pyrazole; Antipyresis; Fever; Hypothermia; Mouse

1. Introduction

The discovery of pyrazole derivatives as antipyretic agents dates back to 1884, when the German chemist Ludwig Knorr attempted to synthesize quinoline derivatives with antipyretic activity and accidentally obtained antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one), which has analgesic, antipyretic and antirheumatic activity. Aminopyrine, a more potent analogue, was synthesized thereafter and these drugs were widely used in the United States and Europe as antipyretics until the appearance of reports of fatal agranulocytosis and aplastic anemia associated with the use of these compounds. Due to these side effects, interest in the pyrazolones decreased, and they were replaced with acetaminophen and acetylsalicylic acid as first choice antipyretics in the United States (Borne, 1995).

In 1921, the Hoechst laboratory synthesized dipyrone (Fig. 1) and launched this compound as a useful analgesic and a potent antipyretic agent (Lecannelier, 1976). However, the clinical use of this pyrazolone has been prohibited in the United States since it has also been linked to agranulocytosis (Insel, 1996). Nevertheless, in Europe and in developing countries, this compound gained popularity (Arellano and Sacristan, 1990), especially due to its low cost, effectiveness, and the demonstration that it is not linked to aplastic anemia and that the incidence of agranulocytosis among dipyrone users is low (3–19 per million), around twice the annual incidence of agranulocytosis in the general population (2–10 per million) (The International Agranulocytosis and Aplastic Anemia Study, 1986; Laporte and Carné, 1987). In addition, the other commonly prescribed antipyretics, acetylsalicylic acid and paracetamol, are not completely safe since they seem to cause Reye syndrome (in children) and acute hepatic failure, respectively (Adam and Stankov, 1994). Therefore, it is important to search for new effective antipyretic compounds with potentially fewer side effects.

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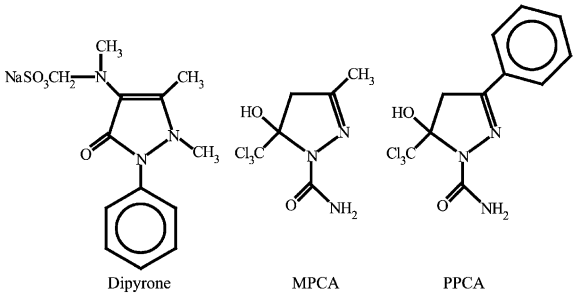

Fig. 1. Molecular structures of dipyrone, MPCA and PPCA.

The mechanism of action of antipyretics is not completely elucidated, but accumulating evidence suggests that they interfere within the host response to endogenous pyrogens such as cytokines and to exogenous pyrogens such as lipopolysaccharide, which is a constituent of Gram-negative bacteria (Elsbach, 2000). Exposure to lipopolysaccharide activates the immune system to release key cytokines into the plasma, the cerebrospinal fluid or the brain, which seem to elevate the set-point for body temperature by activating phospholipase A_2 (Cao et al., 1997) and cyclooxygenase (Elmquist et al., 1997). These enzymes are involved in the synthesis of the endogenous thermogenic substance prostaglandin E_2 (Rowsey and Gordon, 2000; Tsushima and Mori, 2000). In fact, systemic administration of lipopolysaccharide to experimental animals causes a so-called sickness syndrome, which manifests as malaise, anorexia, leukocytosis and fever, similar to the human condition (Tolchard et al., 1996; Elmquist et al., 1997; Li et al., 1999; Benamar et al., 2000; Dogan et al., 2000; Rowsey and Gordon, 2000; Tsushima and Mori, 2000; Yang and Krukoff, 2000), and which responds to classical antipyretics, representing a reliable animal model for the screening of antipyretic agents.

In the present study, we investigated the effects of novel pyrazole derivatives, 3-methyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-pyrazole-1-carboxyamide (MPCA—Fig. 1) and 3-phenyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-1-pyrazole-1-carboxyamide (PPCA—Fig. 1), on basal rectal temperature and on lipopolysaccharide-induced fever in mice.

2. Materials and methods

2.1. Drugs

Escherichia coli lipopolysaccharide (serotype K-235) was purchased from Sigma (Saint Louis, MO). Lipopolysaccharide was dissolved in pyrogen-free 0.9% NaCl, aliquoted under aseptic conditions, and stored at − 20 °C until use. On the day of the experiment, lipopolysaccharide was diluted to 12.5–1250 μg/10 ml of 0.9% NaCl. Dipyrone (Hoechst) was diluted in 0.9% NaCl. 3-Methyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-pyrazole-1-carboxyamide (MPCA) and 3-phenyl-5-hydroxy-5-trichloromethyl-4,5-dihydro-1*H*-pyrazole-1-carboxyamide (PPCA) were synthesized by the NUQUIMHE, as reported elsewhere (Bonacorso et al., 1999), and were suspended in 0.5–5% Tween 80 depending on the experiment. All other reagents were of analytical grade and were purchased from local suppliers.

2.2. Animals

Three-month-old male albino 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. The animals were transferred to the experimental room 2 h before the experiments for acclimation to the environment. All experiments were started between 09:00 and 11:00 h in order to minimize the effect of circadian variation on body temperature. 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. Rectal temperature measurement

Rectal temperature (T_R) was measured by inserting a lubricated thermistor probe (external diameter: 3 mm) 2.8 cm into the rectum of the animal. The probe was linked to a digital device, which displayed the temperature at the tip of the probe with a 0.1 °C precision. The values displayed were manually recorded. In order to minimize the effects of the stress associated with handling and injecting during the experiments on rectal temperature, all mice were habituated to the measuring procedure on three consecutive days. In these sessions, the animals were subjected to the same temperature measuring procedure described above, and were injected intraperitoneally (i.p.) with 0.9% NaCl (10 ml/kg).

2.4. Effect of lipopolysaccharide on rectal temperature

Immediately after measurement of their basal rectal temperature, the animals were injected with lipopolysaccharide (12.5, 25, 50, 250 or 1250 μg/kg, i.p.) or 0.9% NaCl (10 ml/kg). T_R changes were recorded every 30 min up to 5.5 h, and expressed as the difference from the basal value.

2.5. Effect of pyrazoles on basal rectal temperature

Immediately after measurement of their basal T_R, the animals were subcutaneously (s.c.) injected with vehicle (5% Tween 80), dipyrone (0.15, 0.5 or 1.5 mmol/kg), MPCA (0.15, 0.5 or 1.5 mmol/kg) or PPCA (0.15, 0.5 or 1.5 mmol/kg). T_R was recorded every 30 min up to 5.5 h after the drug injections. The same protocol was used to investigate the effects of central administration of these pyrazoles on rectal temperature, except that the animals were injected intracerebroventricularly (i.c.v.) with vehicle

(0.5% Tween 80), dipyrone (25 or 225 nmol), MPCA (25 or 225 nmol) or PPCA (25 or 225 nmol). The volume of the i.c.v. injections was 5 μl, delivered over 30 s. The initial doses of MPCA (s.c.) were selected according to the maximal concentration achievable in a homogeneous suspension (maximal dose). The doses of dipyrone and PPCA were selected in an equimolar basis, compared to MPCA.

2.6. Effect of pyrazoles on lipopolysaccharide-induced fever

The animals had their basal T_R measured and were injected with a pyrogenic dose of lipopolysaccharide (25 μg/kg, i.p.). T_R was monitored at 1-h intervals during the following 3 h, when the animals were injected with vehicle [Tween 80 (5%, s.c. or 0.5%, i.c.v.)], dipyrone (0.15 mg/kg, s.c. or 25 nmol, i.c.v.), MPCA (0.15 mg/kg, s.c. or 25 nmol, i.c.v.) or PPCA (0.15 mg/kg, s.c. or 25 nmol, i.c.v.). T_R was monitored over the following 3 h. These doses were chosen because they had no effect per se on basal T_R.

2.7. Statistical analysis

Changes in rectal temperature were expressed as means ± S.E.M. of the differences from basal rectal temperature immediately before injection of lipopolysaccharide or of the drugs (vehicle, dipyrone, MPCA or PPCA). Data were analyzed by two- or three-way analysis of variance (ANOVA), with time treated as within subject factor, depending on the experimental design. Post hoc analysis was carried out by the F-test on simple effect or by the Student–Neuman–Keuls test, depending on the experimental design. A value of $P<0.05$ was considered statistically significant.

3. Results

3.1. Thermal response induced by lipopolysaccharide

Statistical analysis of the data from experiments with 50, 250 and 1250 μg/kg lipopolysaccharide revealed a significant lipopolysaccharide dose by time interaction [$F(30, 280)=1.97$; $P<0.005$]. Post hoc analysis showed that i.p. injection of 250 μg/kg lipopolysaccharide [$F(10, 280)=2.61$; $P<0.01$] and 1250 μg/kg lipopolysaccharide [$F(10,280)=2.61$; $P<0.01$] altered T_R along time compared with the control group (Fig. 2). This occurred because the animals presented a slight hypothermia 1 h after the injection of lipopolysaccharide, which lasted about 3 h, altering the shape of the temperature variation curve along time. Statistical analysis of data from experiments with low doses of lipopolysaccharide (12.5, 25 and 50 μg/kg) also revealed a significant lipopolysaccharide dose by time interaction [$F(30,380)=2.55$; $P<0.0001$]. Post hoc analysis showed that i.p. injection of 25 μg/kg lipopolysaccharide altered T_R along time compared with the control group [$F(10,380)=5.71$; $P<0.001$]. This effect occurred because lipopolysaccharide-injected animals presented a sustained increase in rectal temperature after 3 h (Fig. 3).

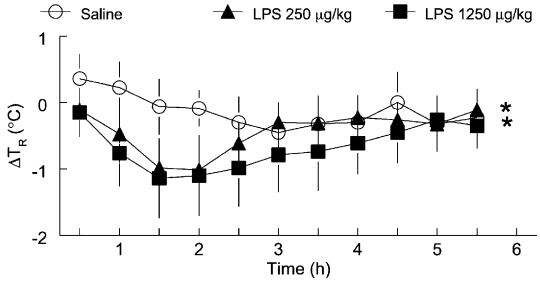

Fig. 2. Effect of intraperitoneal administration of 0.9% NaCl, 250 or 1250 μg/kg lipopolysaccharide on rectal temperature change (ΔT_R) along time. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n=8$ animals per group. $^*P<0.01$ compared with saline (F values in the text). For the sake of clarity, 50 μg/kg lipopolysaccharide data were omitted.

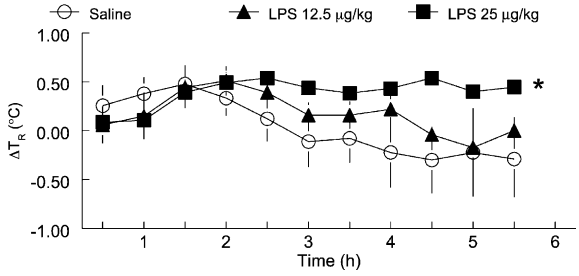

Fig. 3. Effect of intraperitoneal administration of 0.9% NaCl, 12.5 or 25 μg/kg lipopolysaccharide on rectal temperature change (ΔT_R) along time. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n=9–13$ animals per group. $^*P<0.001$ compared with saline (F values in the text). For the sake of clarity, 50 μg/kg lipopolysaccharide data were omitted.

3.2. Effect of pyrazoles on basal rectal temperature

The effect of s.c. administration of dipyrone (0, 0.15, 0.5 or 1.5 mmol/kg) on basal rectal temperature along time is shown in Fig. 4. Statistical analysis revealed a significant dose by time interaction [$F(30,190)=4.52$; $P<0.0001$]. Post hoc analysis showed that 1.5 mmol/kg dipyrone

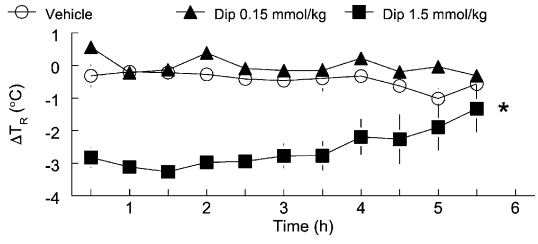

Fig. 4. Effect of subcutaneous administration of 0.9% NaCl, 0.15 or 1.5 mmol/kg dipyrone on rectal temperature change (ΔT_R) along time. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n=5–6$ animals per group. $^*P<0.001$ compared with saline (F values in the text). For the sake of clarity, 0.5 mmol/kg dipyrone data were omitted.

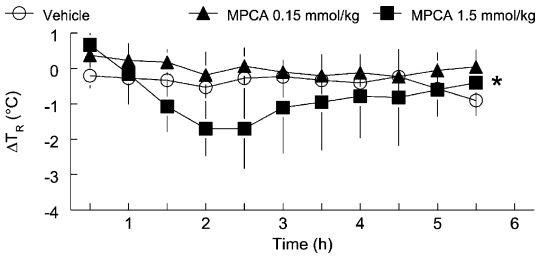

Fig. 5. Effect of subcutaneous administration of vehicle (5% Tween 80), 0.15 or 1.5 mmol/kg MPCA on rectal temperature change (ΔT_R) along time. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n=6$ animals per group. * $P<0.001$ compared with vehicle (F values in the text). For the sake of clarity, 0.5 mmol/kg MPCA data were omitted.

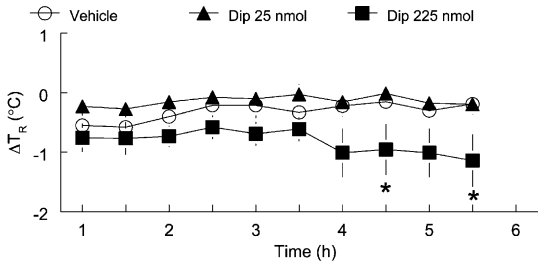

Fig. 7. Effect of intracerebroventricular administration of 0.9% NaCl, 25 or 225 nmol dipyrone on rectal temperature change (ΔT_R) along time. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n=9-12$ animals per group. * $P<0.05$ compared with saline (F values in the text).

decreased T_R along time compared with the control group [$F(10,190)=9.29$; $P<0.001$].

The effect of s.c. administration of MPCA (0, 0.15, 0.5 or 1.5 mmol/kg) on basal rectal temperature along time is shown in Fig. 5. Statistical analysis revealed a significant dose by time interaction [$F(30,200)=2.05$; $P<0.002$]. Post hoc analysis revealed that 1.5 mmol/kg MPCA decreased T_R along time compared with the control group [$F(10,200)=4.64$; $P<0.001$].

The effect of s.c. administration of PPCA (0, 0.15, 0.5 or 1.5 mmol/kg) on basal rectal temperature along time is shown in Fig. 6. Statistical analysis revealed a significant dose by time interaction [$F(30,200)=2.12$; $P<0.001$]. Post hoc analysis showed that 1.5 mmol/kg PPCA decreased T_R along time compared with the control group [$F(10,200)=5.03$; $P<0.001$].

Since s.c. injection of dipyrone, MPCA and PPCA reduced T_R along time (Figs. 4–6), we decided to test whether the central administration of these pyrazoles affected T_R. The effect of i.c.v. administration of dipyrone (0, 25 or 225 nmol) on basal rectal temperature along time is shown in Fig. 7. Statistical analysis revealed a significant effect of treatment [$F(2,27)=3.92$; $P<0.05$], indicating that dipyrone treatment altered T_R values. Post hoc analysis (Student–Newman–Keuls test) showed that 225 nmol dipyrone significantly decreased T_R at 4.5 and 5.5 h. Intracerebroventricular administration of MPCA (0, 25 or 225 nmol) and PPCA (0, 25 or 225 nmol) had no effect on basal rectal temperature (data not shown).

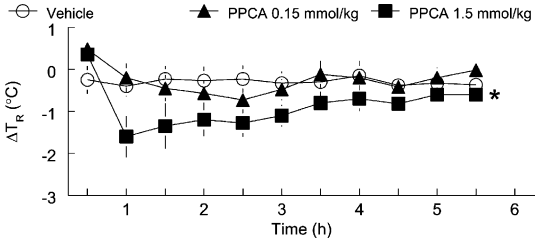

Fig. 6. Effect of subcutaneous administration of vehicle (5% Tween 80), 0.15 or 1.5 mmol/kg PPCA on rectal temperature change (ΔT_R) along time. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n=6$ animals per group. * $P<0.001$ compared with vehicle (F values in the text). For the sake of clarity, 0.5 mmol/kg MPCA data were omitted.

3.3. Effects of pyrazoles on lipopolysaccharide-induced fever

The effect of s.c. dipyrone, MPCA or PPCA on lipopolysaccharide-induced fever is shown in Fig. 8. Three hours after the injection of 25 μg/kg lipopolysaccharide (i.p.), the mice were injected with 0.15 mmol/kg dipyrone, MPCA, PPCA or vehicle (5% Tween 80) and had their T_R followed up for 3 h. Statistical analysis of T_R change showed a significant treatment by time interaction [$F(15,120)=3.57$; $P<0.0001$]. Post hoc analysis revealed that dipyrone [$F(5,120)=4.37$; $P<0.005$], MPCA [$F(5,120)=7.34$; $P<0.001$] and PPCA [$F(5,120)=7.24$; $P<0.001$] reversed lipopolysaccharide-induced fever. This antipyretic effect was progressive and persisted for 3 h after dipyrone, MPCA or PPCA injection.

The effect of i.c.v. dipyrone, MPCA and PPCA on lipopolysaccharide-induced fever is depicted in Fig. 9. Three hours after the injection of 25 μg/kg lipopolysaccharide (i.p.), the mice were injected with 25 nmol dipyrone, MPCA, PPCA or vehicle (0.5% Tween 80). Statistical

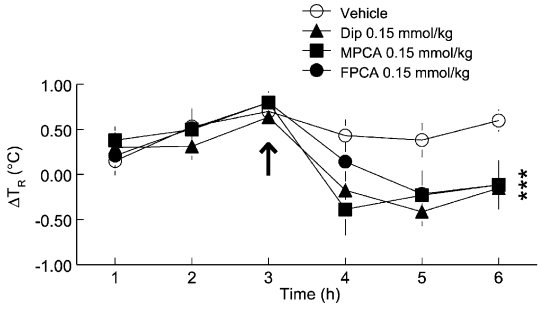

Fig. 8. Effects of subcutaneous administration of vehicle (1.6% Tween 80), dipyrone (0.15 mmol/kg); MPCA (0.15 mmol/kg) or PPCA (0.15 mmol/kg) on fever induced by lipopolysaccharide. The arrow indicates time of injection of pyrazoles or vehicle. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n=6-8$ animals per group. * $P<0.005$ compared with vehicle (F values in the text).

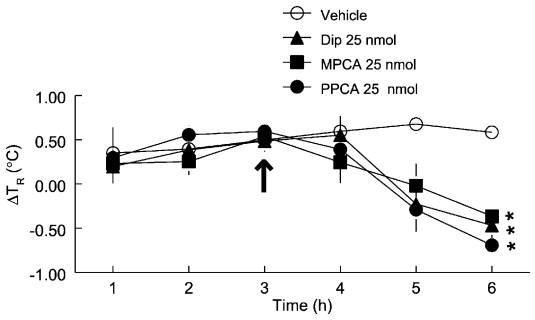

Fig. 9. Effects of intracerebroventricular administration of vehicle (0.5% Tween 80), dipyrone (25 nmol); MPCA (25 nmol) or PPCA (25 nmol) on fever induced by lipopolysaccharide. The arrow indicates time of injection of pyrazoles or vehicle. Values represent mean ± S.E.M. change from baseline rectal temperature. Data are reported as means for $n = 5$ animals per group. * $P < 0.05$ compared with vehicle (F values in the text).

analysis revealed a significant treatment by time interaction [$F(15,80) = 4.19$; $P < 0.0001$]. Post hoc analysis showed that dipyrone [$F(5,80) = 3.20$; $P < 0.025$], MPCA [$F(5,80) = 2.57$; $P < 0.05$] and PPCA [$F(5,80) = 4.45$; $P < 0.005$] reversed lipopolysaccharide-induced fever.

4. Discussion

There are various reports in the literature showing that thermal responses to lipopolysaccharide vary from hypothermia to fever (Shemi and Kaplanski, 1995; Elmquist et al., 1997; Blanqué et al., 1999; Li et al., 1999; Benamar et al., 2000; Rowsey and Gordon, 2000; Tsushima and Mori, 2000; Josa et al., 2001), and this discrepancy has been attributed to different environmental (Shemi and Kaplanski, 1995; Li et al., 1999) and methodological factors, including the source of lipopolysaccharide (Dogan et al., 2000).

In the present study, we report that the i.p. administration of lipopolysaccharide (250, 1250 μg/kg) caused significant hypothermia, while the administration of 12.5 and 25 μg/kg lipopolysaccharide had no effect on basal T_R or fever, respectively. The presently reported biphasic effect of lipopolysaccharide on body temperature of mice depending on the dose administered is, to some extent, similar to those reported by others (Feldberg and Saxena, 1975; Dogan et al., 2000) in rats. It has been suggested that lipopolysaccharide-induced hypothermia may be a consequence of the vascular changes induced by toxic levels of lipopolysaccharide (Feldberg and Saxena, 1975), but it has also been argued that lipopolysaccharide-induced hypothermia may be observed with low "nontoxic" doses of the lipopolysaccharide, depending on its serotype (Dogan et al., 2000). Another possible explanation may be related to the experimental stress of the temperature measuring procedure. The exposure to novel environments, handling processes or restraining may cause a stress response (Soszynski, 2001), and it has been suggested that stressed animals might not be able to develop fever in response to a pyrogenic stimulus due to the antipyretic effectiveness of the various humoral factors released during stress (Kluger, 1991). Nevertheless, stress-induced hyperthermia has also been reported (Briese and Cabanac, 1990; Long et al., 1991; Watanabe et al., 1997), and no agreement regarding this point has been reached in the literature. Therefore, the lipopolysaccharide dose–effect curve showed that the dose of 25 μg/kg was pyrogenic under our experimental conditions, and this concentration was used to induce fever in subsequent experiments.

In the present study, we demonstrated that systemically administered dipyrone and the new pyrazole compounds MPCA and PPCA, at the dose of 1.5 mmol/kg, induce hypothermia (Figs. 4–6). To our knowledge, this is the first report on the hypothermic effect of pyrazoles in mice. It has been previously reported that dipyrone potentiates the hypnosis and hypothermia induced by pentobarbital, barbital and chloral hydrate (Bansinath et al., 1982), but no hypothermic effect of dipyrone per se has been described to date in mice. In fact, it is generally believed that classical antipyretics such as dipyrone do not affect basal temperature (Beutler and Beutler, 1992). It is important to point out, however, that 1.5 mmol/kg of MPCA and PPCA corresponded to the maximal concentration achievable in a homogeneous suspension (and the highest dose possible). Such a high dose of MPCA, which is twofold the dose required to cause antinociception (Souza et al., 2001), might cause some degree of toxicity to the animals, manifested by hypothermia.

Interestingly, only dipyrone (225 nmol/mouse) caused hypothermia when administered by the i.c.v. route, suggesting that dipyrone-induced hypothermia involves centrally mediated mechanisms. On the other hand, it is rather difficult to define whether MPCA and PPCA-induced hypothermia involves central mechanisms since these compounds were devoid of hypothermic effects when injected by the i.c.v. route. It is worth pointing out, however, that such a lack of i.c.v. effect may suggest a peripheral mechanism of action for these new pyrazole compounds or, alternatively, that their central hypothermic effect requires prior systemic metabolization.

In order to investigate whether MPCA and PPCA presented antipyretic activity, we selected, from dose–effect curves, the doses of pyrazoles that had no effect per se on basal T_R. Figs. 4–6 show that s.c. (1.5 mmol/kg) and i.c.v. (25 nmol/mouse) administration of dipyrone, MPCA and PPCA reversed the fever induced by lipopolysaccharide. A remarkable result of these experiments was that the systemic administration of all compounds reversed lipopolysaccharide-induced fever within 1 h, while the central administration of the same compounds took about 2 h to reverse it. These results indicate that the antipyretic effect of these pyrazoles requires prior metabolization, and that they are slowly converted to an active metabolite by the cerebral tissue. This is in agreement with the view that dipyrone is basically a prodrug, and that its effects occur

after its conversion to other main active metabolites (probably 4-methylaminoantipyrine and 4-aminoantipyrine) (Zylber-Katz et al., 1992; Shimada et al., 1994; Campos et al., 1999), and suggest that MPCA and PPCA behave similarly. It is important to point out that the generation of such an active metabolite occurs despite the presence of methyl or phenyl groups as substituents in the 3-pyrazole ring of the new pyrazoles. Finally, it does not seem that a difference in the ability to cross the blood–brain barrier accounts for the differences between the central and the peripheral effects of pyrazoles. If this were the case, the antipyretic effect of the tested compounds should be delayed after the systemic, but not after the central administration, which bypasses blood–brain barrier. Since we found the opposite (i.e., that the subcutaneously administered pyrazoles caused prompt antipyresis), this possibility sounds unlikely.

The neurochemical mechanisms underlying the antipyretic effect of dipyrone (and of other pyrazole compounds) are not fully understood, but there is experimental evidence supporting the notion that they involve the inhibition of prostaglandin synthesis (or a step before prostaglandin E_2 formation) in the central nervous system since dipyrone attenuates interleukin-1β-induced but not prostaglandin E_2-induced fever (Shimada et al., 1994). Moreover, it has recently been described that dipyrone selectively inhibits cyclooxygenase-2 in intact cells (Campos et al., 1999), and that selective cyclooxygenase-2 inhibition potently reverses fever in rats (Taniguchi et al., 1997) and human beings (Schwartz et al., 1999). The relative paucity of underlying mechanisms of antipyretic drugs in the literature may be related to the fact that pyrexia, per se, is a poorly understood phenomenon. Therefore, it remains to be determined if other proposed mechanisms of action for pyrazoles at the peripheral level, such as glutamate binding displacement (Beirith et al., 1998), and alterations in the nitric oxide-GMPc pathway (Lorenzetti and Ferreira, 1996; Aguirre-Banuelos and Granados-Soto, 1999) are also involved in the antipyretic effects of this compound.

In conclusion, in the present study, we report the antipyretic action of MPCA and PPCA, which proved to be similar to that of the well-known pyrazole compound, dipyrone. These compounds seem to act centrally, as prodrugs, since their systemic administration causes a rapid antipyresis, while intracerebroventricular administration induces antipyresis only after a 2-h lag. We suggest that MPCA and PPCA may be useful as antipyretic agents.

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