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3-Methyl-5-hydroxy-5-trichloromethyl-1*H*-1-pyrazolcarboxyamide induces antinociception

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Abstract

The antinociceptive action of a novel pyrazole-derived compound, 3-methyl-5-hydroxy-5-trichloromethyl-1*H*-1-pyrazolcarboxyamide (MPCA) was evaluated using the formalin and tail-immersion tests in mice. Anti-inflammatory activity was assessed by paw plethysmometry in adult rats using the carrageenin-induced paw edema test. Subcutaneous administration of MPCA (22, 66, and 200 mg/kg) induced a dose-dependent decrease in the time spent licking during the neurogenic and inflammatory phases of the formalin test, and preadministration of naloxone (1 mg/kg, sc) did not prevent MPCA-induced (200 mg/kg, sc) antinociception. Naloxone decreased the spontaneous locomotor activity of mice, while MPCA had no effect on locomotion. In contrast, administration of the opioid antagonist caused a significant increase in the locomotor behavior of mice previously injected with MPCA. MPCA was devoid of antinociceptive action by the tail-immersion test and of anti-inflammatory activity. Moreover, MPCA had no effect on the motor performance of mice in the rotarod test. These results suggest that MPCA induces antinociception in the neurogenic and inflammatory phases of the formalin test, an effect that does not involve opioid receptors.

Keywords: Pyrazole-derived compounds; Antinociception; Formalin test; Rotarod test; Tail-immersion test; Carrageenin-induced paw edema

1. Introduction

In addition to caffeine and ethyl alcohol, nonsteroidal anti-inflammatory drugs (NSAIDs) are probably the most widely used drugs in the world (Verderame, 1986). The prototype agent of this class is acetylsalicylic acid, which has therapeutically useful analgesic, antipyretic, and anti-inflammatory actions. Besides salicylates, other compounds such as aminophenols, arylalkanoic acid-derived compounds, and pyrazole derivatives are classified as NSAIDs, since they probably share the same mechanism of action, i.e., the inhibition of cyclooxygenase, a key enzyme in the metabolism of arachidonic acid (Hunskaar et al., 1986; Swerdlow et al., 1985; Verderame, 1986).

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, 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 for those purposes until the appearance of reports of fatal agranulocytosis associated with the use of these compounds (Borne, 1995). Due to this side effect, interest in the pyrazolones decreased until the mid-1940s, when a series of safer pyrazolidinediones, including phenylbutazone, with anti-inflammatory activity were synthesized. Since then, phenylbutazone and related compounds have been used as anti-inflammatory and analgesic agents. However, phenylbutazone is poorly tolerated by many patients. Nausea, vomiting, epigastric discomfort, and skin rashes are the most frequently reported

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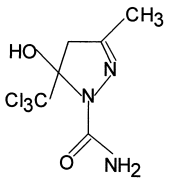

Fig. 1. 3-Methyl-5-hydroxy-5-trichloromethyl-1*H*-1-pyrazolcarboxyamide.

untoward effects and some cases of agranulocytosis have been reported (Insel, 1996).

Due to the need for more effective analgesic and anti-inflammatory compounds with fewer side effects, a number of heterocyclic compounds have been synthesized and evaluated for biological activity (Goel & Madan, 1995). In the present study, we investigated the potential analgesic and anti-inflammatory activity of a novel pyrazole-derived compound, 3-methyl-5-hydroxy-5-trichloromethyl-1*H*-1-pyrazolcarboxyamide (MPCA, Fig. 1).

2. Material and methods

2.1. Drugs

Naloxone, indomethacin, morphine sulfate, and Tween 80 were purchased from Sigma (St. Louis, MO). MPCA was synthesized by H.G.B. and M.R.O. as previously described (Bonacorso et al., 1999). The other reagents were of analytical grade and were acquired from local suppliers.

2.2. Subjects

Three-month-old male albino mice (30–40 g) bred in our animal house were used in the formalin and tail-immersion tests. The animals were housed in groups of 20 per cage at controlled temperature (22 ± 1°C) on a 12-h light/dark cycle and with standard lab chow and tap water ad libitum. The animals were transferred to the experimental room 24 h before the experiments for habituation to the environment and handling. Each animal was used only once.

Male Wistar rats (300–330 g) bred in our animal house were used in the plethysmography test. The animals were housed in groups of five per cage at controlled temperature (22 ± 1°C) on a 12-h light/dark cycle. Standard lab chow and tap water were freely available except during the experiments. Each animal was used only once.

2.3. Formalin test

The formalin test was carried out as described by Hunskaar and Hole (1987). Animals were injected subcutaneously with 20 μl of 1.5% formalin (v/v) into the dorsal right hindpaw. The time spent licking or biting the injected paw or leg was recorded for 30 min at 5-min intervals. Vehicle (5% Tween 80) or MPCA (22, 66, and 200 mg/kg) was injected subcutaneously into the dorsal region 15 min before formalin injection into the paw.

Subcutaneous administration of 1.5% formalin causes a typical biphasic response of licking behavior. During the first 5 min the animals lick the injected paw because formalin probably causes direct stimulation of nerve terminals and this phase is termed ''neurogenic.'' A second burst of licking behavior occurs between 15 and 30 min after the injection and seems to be related to the inflammatory response elicited by formalin. For this reason, this second phase is termed ''inflammatory'' (Leventhal et al., 1996).

In those experiments designed to evaluate the involvement of opioid mechanisms in MPCA-induced antinociception, animals were preinjected subcutaneously into the dorsal region with naloxone (1 mg/kg) or saline (0.85% NaCl) 15 min before vehicle (5% Tween 80), MPCA (200 mg/kg), or morphine (5 mg/kg) administration (Shibata et al., 1989).

2.4. Tail-immersion

The tail-immersion test was carried out as described by Janssen et al. (1963). The lower 3.5 cm portion of the tail was marked and the animals were then injected with MPCA (200 mg/kg, sc), morphine (5 mg/kg, sc), or vehicle (5% Tween 80, sc). After 15 min, the reaction time of each animal was determined by immersing the lower 3.5 cm of the tail into a cup freshly filled with water from a large constant-temperature (55°C) bath until the typical tail withdrawal response was observed. The cut-off time was 10 s. The reaction time was measured in 0.5-s units with a stopwatch.

2.5. Carrageenin-induced paw edema

Paw edema was induced with carrageenin as described by Winter et al. (1962). Adult male Wistar rats were injected subcutaneously with indomethacin (5 mg/kg), MPCA (22, 66, and 200 mg/kg), or equivalent volumes of 0.85% saline (1 ml/kg) or 5% Tween 80 (1 ml/kg). The plantar region of the right hindpaw was injected with 50 μl of carrageenin (1% in sterile saline) 30 min later and the left paw was injected with the same volume of saline. The volume of the injected paws was measured 0, 1, 2, 3, and 4 h after injection with a plethysmometer (Plethysmometer 7150, Ugo Basile, Italy). Paw edema was calculated according to the following equation at each time point:

$$\text{Edema score} = \frac{V_1 \text{ right hind paw}}{V_0 \text{ right hind paw}} - \frac{V_1 \text{ left hind paw}}{V_0 \text{ left hind paw}} \times 100\%$$

where V_0 is the initial volume of the paw and V_1 is the final volume of the paw.

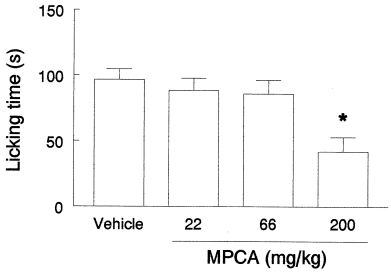

Fig. 2. Effect of MPCA (vehicle, 22, 66, and 200 mg/kg, sc) on the time spent licking during the neurogenic phase (0–5 min) of the formalin test in mice. Data are reported as means ± S.E.M. for $n = 8$ per group. *$P < 0.05$, compared to vehicle (SNK test).

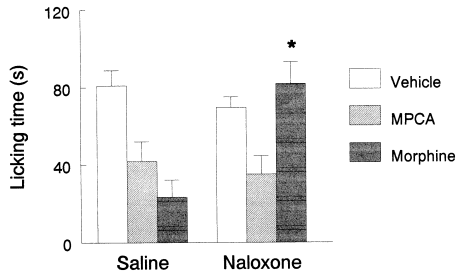

Fig. 4. Effect of preadministration (subcutaneous) of naloxone or 0.85% saline to mice treated with morphine (5 mg/kg, sc) and MPCA (vehicle and 200 mg/kg, sc) on the licking time during the neurogenic phase (0–5 min) of the formalin test. Data are reported as means ± S.E.M. for $n = 15$ per group. *$P < 0.05$, compared to saline + morphine (SNK test).

2.6. Rotarod performance test

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 (5% Tween 80) or MPCA (22, 66, or 200 mg/kg) and after 15 min they were placed in the rotarod and their latency to fall from the apparatus was recorded with a stopwatch up to 240 s (Tsuda et al., 1996).

2.7. Statistical analysis

Formalin test data were analyzed by two-way ANOVA with time treated as a within-subject factor. When necessary, data were square root-transformed before analysis in order to meet the assumptions for ANOVA. In those experiments designed to evaluate the involvement of opioid receptors in the antinociceptive effects of MPCA, data were analyzed by three-way ANOVA, with time treated as a within-subject factor.

Rotarod data were analyzed by one-way ANOVA.

Tail-immersion and plethysmometry data (edema score) were analyzed by two-way ANOVA with time treated as a within-subject factor.

In those experiments designed to evaluate the dose–response relationship, the between group sum of squares of ANOVA was partitioned into trend components (linear, quadratic, or cubic).

3. Results

The effect of MPCA on the time spent licking during the neurogenic (0–5 min) phase of the formalin test is depicted in Fig. 2. Statistical analysis (one-way ANOVA partitioned into trend components) of the data revealed that MPCA linearly decreased the time spent licking in a dose-dependent manner [$F(1,31) = 17.86$, $P < .002$ for linear trend]. MPCA had no effect on the spontaneous locomotor activity of the animals (data not shown). These results indicate that the observed decrease in licking behavior in the neurogenic phase of the test was not related to gross motor impairment, since locomotion was not affected.

The effect of MPCA on the time spent licking during the inflammatory (15–30 min) phase of the formalin test is shown in Fig. 3. Statistical analysis (two-way ANOVA with the time factor treated as within subject factor) of square root-transformed data revealed a significant dose by time interaction [$F(9,93) = 2.15$, $P < .05$] indicating that MPCA has an antinociceptive activity during the inflammatory phase of the formalin test. MPCA also had no effect on the spontaneous locomotor activity of the animals during the

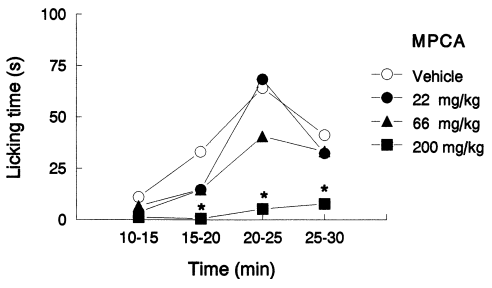

Fig. 3. Effect of MPCA (vehicle, 22, 66, and 200 mg/kg, sc) on the time spent licking during the inflammatory phase (15–30 min) of the formalin test in mice. Data are reported as means ± S.E.M. for $n = 8$ per group. *$P < 0.05$, compared to respective vehicle (SNK test).

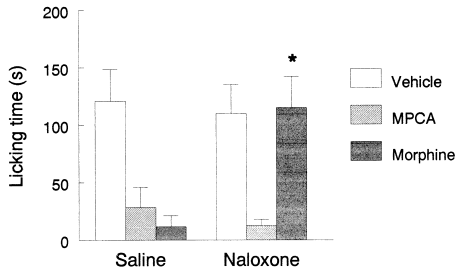

Fig. 5. Effect of the preadministration (subcutaneous) of naloxone or 0.85% saline to mice treated with morphine (5 mg/kg, sc) and MPCA (vehicle and 200 mg/kg, sc) on the licking time during the inflammatory phase (15–30 min) of the formalin test. Data are reported as means ± S.E.M. for $n = 15$ per group. *$P < 0.05$, compared to saline + morphine (SNK test).

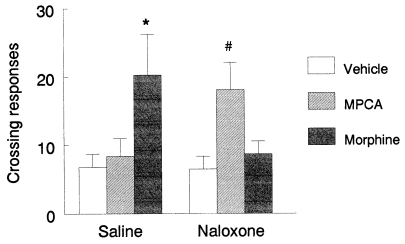

Fig. 6. Effect of the preadministration (subcutaneous) of naloxone or 0.85% saline to mice treated with morphine (5 mg/kg, sc) and MPCA (vehicle and 200 mg/kg, sc) on the number of crossings during the neurogenic phase (0–5 min) of the formalin test. Data are reported as means ± S.E.M. for $n = 15$ per group. *$P < 0.05$ compared with naloxone + morphine. *$P < 0.05$, compared to saline + MPCA (SNK test).

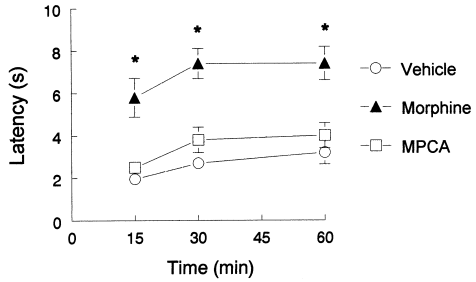

Fig. 8. Effect of morphine (5 mg/kg, sc) and MPCA (vehicle and 200 mg/kg, sc) on the latency to withdrawing the tail at 15, 30, and 60 min after administration of drugs in the tail-immersion test in mice. Data are reported as means ± S.E.M. for $n = 16$ per group. *$P < 0.05$, compared to respective vehicle (SNK test).

inflammatory phase of the test, corroborating the data obtained in the neurogenic phase (data not shown).

The involvement of opioid mechanisms in the antinociceptive effect of MPCA in the neurogenic (Fig. 4) and inflammatory (Fig. 5) phases of the formalin test was evaluated by the preadministration of naloxone. Naloxone prevented the morphine-induced antinociception in both phases of the test, but had no effect on the MPCA-induced antinociception [significant Pretreatment (saline or naloxone) × Treatment (vehicle, morphine, or MPCA) interaction: $F(2,42) = 9.39$, $P < .001$, for the neurogenic phase and $F(2,42) = 8.24$, $P < .001$, for the inflammatory phase]. The effect of naloxone on the locomotor response of the animals during the neurogenic (Fig. 6) and inflammatory phases (Fig. 7) of the test was also examined. Statistical analysis revealed significant Pretreatment (saline or naloxone) × Treatment (vehicle, morphine, or MPCA) interactions: $F(2,42) = 9.39$, $P < .001$, for neurogenic phase data and $F(2,42) = 9.47$, $P < .001$, for inflammatory phase data. Post hoc analysis (Duncan's multiple range test) of neurogenic phase data revealed that naloxone prevented the opioid-induced increase of locomotor activity. Moreover, naloxone increased the locomotor activity of animals previously injected with MPCA. Although the locomotion scores of MPCA-injected animals were increased three times by naloxone preadministration during the inflammatory phase, the difference between means did not achieve statistical significance in the post hoc analysis.

The effect of MPCA and morphine on the latency to tail withdrawal in the tail-immersion test is depicted in Fig. 8. Statistical analysis revealed a significant effect of drug [$F(2,45) = 13.92$, $P < .001$]. Post hoc analysis (Student-New Keuls test) showed that morphine increased and MPCA had no effect on tail withdrawal latencies.

The effect of MPCA and indomethacin on the carrageenin-induced paw edema is shown in Fig. 9 (for the sake of clarity, 0.22, 0.66 mg/kg MPCA, and 5% Tween 80 data were omitted from the figure). Statistical analysis revealed a significant effect of drug by time interaction [$F(20,124) = 5.73$, $P < .001$]. Post hoc analysis (F test on simple effect) showed that indomethacin decreased [$F(4,100) = 3.84$, $P < .05$] and MPCA had no effect [$F(4,100) = 0.10$, $P = .85$] on the carrageenin-induced paw edema along time.

MPCA caused no impairment in the rotarod test [$F(3,31) = 2.07$, $P = .10$, data not shown] and this finding

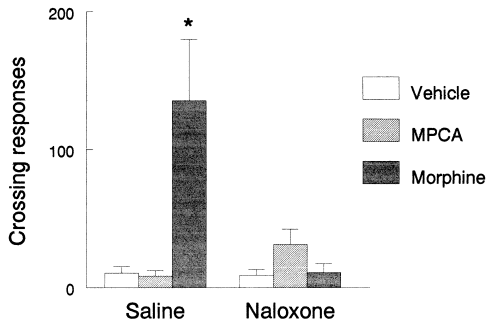

Fig. 7. Effect of the preadministration (subcutaneous) of naloxone or 0.85% saline to mice treated with morphine (5 mg/kg, sc) and MPCA (vehicle and 200 mg/kg, sc) on the number of crossings during the inflammatory phase (15–30 min) of the formalin test. Data are reported as means ± S.E.M. for $n = 15$ per group. *$P < 0.05$, compared to the other groups (SNK test).

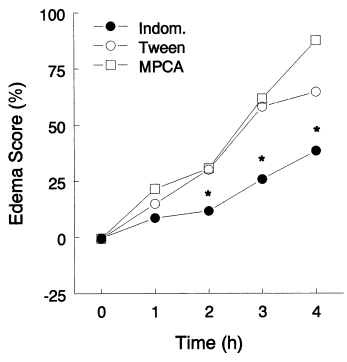

Fig. 9. Effect of indomethacin (5 mg/kg, sc) and MPCA (vehicle and 200 mg/kg, sc) on the paw edema induced by carrageenin (50 μl, ipl) during a period of 4 h after paw edema-induction in rats. Data are reported as means ± S.E.M. for $n = 8$ per group. *$F(4,100) = 3.84$, P, .05, compared to vehicle (5% Tween 80).

further suggests that this compound does not cause gross motor incoordination.

4. Discussion

The present study demonstrates the antinociceptive properties of the pyrazoline MPCA in the neurogenic and inflammatory phases of the formalin test. Although MPCA caused significant antinociception in the inflammatory phase of the formalin test, it was devoid of anti-inflammatory activity in the carrageenin-induced paw edema test. Moreover, MPCA had no antinociceptive effect in the tail-immersion test.

MPCA presents a pyrazole ring, which is similar to the pyrazole ring of dipyrone, aminopyrine, and phenylbutazone. The anti-inflammatory properties of pyrazoline compounds have been associated with the presence of carbonyl groups at positions 3 and/or 5 of the pyrazole moiety (Ferreira and Vane, 1974). Indeed, pyrazole-derived compounds lacking such carbonyl groups have little or no anti-inflammatory activity, but some of them present a significant antinociceptive action (Beirith et al., 1998; Kuo et al., 1984). This seems to be the case for MPCA, which does not present such carbonyl groups, but does present significant antinociceptive activity in the formalin test. An interesting finding is that MPCA, similarly to dipyrone and unlike most of the nonsteroidal anti-inflammatory drugs (NSAIDs), was effective in preventing the neurogenic pain response (first phase) induced by formalin (Corrêa and Calixto, 1993; Malmberg and Yaksh, 1992; Shibata et al., 1989; Vaz et al., 1993). Such antinociceptive action on the neurogenic phase of the formalin test has been argued to involve a different mechanism of action for dipyrone compared to other NSAIDs (Beirith et al., 1998; Carlsson and Jurna, 1987; Lorenzetti and Ferreira, 1985) and, in this respect, MPCA seems to behave like dipyrone.

One interesting finding of the present study was the lack of antinociceptive effect of MPCA in the tail-immersion test. As a matter of fact, mild analgesics, like NSAIDs, seem have little or no analgesic effects in standard thermally motivated tests (Hunskaar et al., 1986) such as hot-plate and tail-flick. According to this view, it has been reported that systemically administered dypirone is devoid of antinociceptive effects in the hot-plate and tail-flick tests (Akman et al., 1996; Beirith et al., 1998). Hunskaar et al. (1986) tried to overcome this problem and described a modified hot-plate test in which temperature was slowly increased from nonnoxious levels, which proved to be more sensitive to NSAIDs. Ten years later, Yeomans and colleagues (Yeomans and Proudfit, 1996; Yeomans et al., 1996), in an elegant study, provided both behavioral and electrophysiological evidence that the relative contribution of A-delta mechanothermal nociceptive and C-polymodal nociceptive afferents in mediating noxious heat-evoked responses is critically dependent upon the rate of heating. They suggested that nociceptive responses to high skin heating rates are mediated predominantly by A-delta nociceptors, and that the ratio of activation of A-delta to C fibres covaries directly with heating (McCormack et al., 1998). Taking these studies into consideration it would be tempting to propose that systemically administered MCPA, like dipyrone and most NSAIDs, may cause its antinociceptive action by acting predominantly at afferent C fibres, and that the high rate of heating was responsible for the presently observed lack of effect of MCPA in the tail-immersion test. However, this statement is speculative in nature, and specific studies are needed to confirm or not this hypothesis.

It has long been known that the antinociceptive effects of systemically injected pyrazole derivatives (i.e., dipyrone) are not blocked by naloxone (Beirith et al., 1998; Taylor et al., 1998). Accordingly, naloxone preadministration at doses capable of preventing morphine-induced antinociception did not prevent the MPCA-induced antinociception, suggesting that opioid mechanisms may not underlie such effects. However, when administered together, these drugs increased the locomotor activity of the animals. General opioid antagonists decrease spontaneous locomotor activity in rodents (Rocha and Mello, 1994; Rotta et al., 1988), an effect that seems to involve mu, delta, and kappa opioid receptors (Leventhal et al., 1996) and the dopaminergic system (Toyoshi et al., 1992 Kimmel & Holzman, 1997). Moreover, opioid agonists stimulate locomotor activity (Anagnostakis et al., 1992; Beleslin et al., 1982). Most of the studies in the literature that investigate the combined use of opioid antagonists and other compounds on locomotor behavior have reported that opioid antagonists decrease or do not modify the stimulant actions of such compounds (Holzman and Jewett, 1973; Swerdlow et al., 1985). The presently observed increase in locomotor activity induced by MPCA and naloxone, at doses at which neither drug alone has any effect, somewhat resembles the already described interaction of apomorphine and naloxone on the locomotion of rats (Adams et al., 1981). At the moment, however, very little can be said about the physiological mechanisms underlying such interaction, except that it probably reflects a central effect of MPCA, since it is well known that opioid antagonist-mediated alterations in locomotor behavior involve the basal ganglia (Anagnostakis et al., 1992; Holzman, 1974; Holzman and Jewett, 1973; Winter et al., 1962). It is important to point out, however, that such central effect does not mean that the antinociceptive effects of MPCA are mediated by central mechanisms, and further investigation is necessary to clarify this point.

The results of the present study suggest that MPCA may be a useful drug as a mild analgesic. We are currently studying the effects of the substitution of some radicals in order to increase the presently observed analgesic effect of the drug and, if possible, determine its mechanism of action.

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