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A novel, potent, oral active and safe antinociceptive pyrazole targeting kappa opioid receptors

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ABSTRACT

Pyrazole compounds are an intriguing class of compounds with potential analgesic activity; however, their mechanism of action remains unknown. Thus, the goal of this study was to explore the antinociceptive potential, safety and mechanism of action of novel 1-pyrazole methyl ester derivatives, which were designed by molecular simplification, using in vivo and in vitro methods in mice. First, tree 1-pyrazole methyl ester derivatives (DMPE, MPFE, and MPCIE) were tested in the capsaicin test and all presented antinociceptive effect; however the MPClE (methyl 5-trichloromethyl-3-methyl-1*H*-pyrazole-1-carboxylate) was the most effective. Thus, we selected this compound to assess the effects and mechanisms in subsequent pain models. MPCIE produced antinociception when administered by oral, intraperitoneal, intrathecal and intraplantar routes and was effective in the capsaicin and the acetic acid-induced nociception tests. Moreover, this compound reduced the hyperalgesia in diverse clinically-relevant pain models, including postoperative, inflammatory, and neuropathic nociception in mice. The antinociception produced by orally administered MPClE was mediated by κ-opioid receptors, since these effects were prevented by systemically pre-treatment with naloxone and the κ-opioid receptor antagonist nor-binaltorphimine. Moreover, MPCIE prevented binding of the κ-opioid ligand [^{3}H]-CI-977 in vitro (IC_{50} of 0.68 (0.32–1.4) μM), but not the TRPV1 ([^{3}H]-resiniferatoxin) or the α_2-adrenoreceptor ([^{3}H]-idazoxan) binding. Regarding the drug-induced side effects, oral administration of MPClE did not produce sedation, constipation or motor impairment at its active dose. In addition, MPCIE was readily absorbed after oral administration. Taken together, these results demonstrate that MPClE is a novel, potent, orally active and safe analgesic drug that targets κ-opioid receptors.

Abbreviations: CLog*P*, coefficient of partition octanol/water; [^{3}H]-IDZ, [^{3}H]-idazoxan; MPCA, 5-hydroxy-3-methyl-5-(trichloromethyl)-1*H*-pyrazole-1-carboxamide; MPF4, methyl 5-hydroxy-4-methyl-5-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazole-1-carboxylate; DMPE, methyl 3,5-dimethyl-1*H*-pyrazole-1-carboxylate; MPCIE, methyl 3-methyl-5-(trichloromethyl)-1*H*-pyrazole-1-carboxylate; MPFE, Methyl 3-methyl-5-(trifluoromethyl)-1*H*-pyrazole-1-carboxylate; [^{3}H]-CI-977, [^{3}H]-(5*R*)-(5a,7a,8b)-(−)-*N*-methyl-*N*-(7-[1-pyrrolidinyl]-1-oxaspiro[4,5]dec-8-yl)-4-benzofuranacetamide hydrochloride; nor-BNI, nor-binaltorphimine; [^{3}H]-RTX, [^{3}H]-resiniferatoxin.

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1. Introduction

Two main classes of analgesic drugs are commonly used to treat pain: opioids, such as morphine, which directly abolish nociceptive transmission in the nervous system by binding to opioid receptors (Jordan and Devi, 1998), and non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and ibuprofen, which reduce prostanoid formation due to cyclooxygenase inhibition (Vane and Botting, 1998). Whereas opioid analgesics are limited by drug-induced tolerance, dependence and constipation (Ballantyne, 2007), the use of NSAIDs is not limited by these factors; however, gastrointestinal irritation and renal function abnormalities are associated with NSAIDs use (Fernandez et al., 1995; Lichtenberger et al., 1995; Suleyman et al., 2007).

Pyrazole-derived compounds, such as metamizol, gained popularity because of their analgesic and antipyretic efficacy (Borne, 1995; Edwards et al., 2001; Tewari et al., 2010). Afterwards, several groups have synthesised new pyrazoles derivatives with analgesic and antipyretic activities (Ochi et al., 1999a, 1999b). Similarly, our group studied pyrazole compounds for their analgesic effects and ability to not induce side effects. These compounds induce antinociceptive effects in thermal and chemical models of pain (de Souza et al., 2001; Sauzem et al., 2008; Souza et al., 2002; Tabarelli et al., 2004). From these studies, two pyrazole derivatives have been identified as lead-like compounds (Fig. 1A). 5-Hydroxy-3-methyl-5-(trichloromethyl)-1*H*-pyrazole-1-carboxamide (MPCA) has antinociceptive and antipyretic activity due to its action on noradrenergic α_2 receptors (de Souza et al., 2001, 2002; Godoy et al., 2004). On the other hand, the antinociceptive effects elicited by methyl 5-hydroxy-4-methyl-5-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazole-1-carboxylate (MPF4) involved opioid receptors (Milano et al., 2008a, 2008b). Since previous studies from our group have shown that pyrazole derivatives have antinociceptive activity with limited adverse effects, these compounds are an interesting source for novel analgesic drugs.

In the present study, we examined the antinociceptive effects of novel 1*H*-pyrazole derivatives (Fig. 1A), namely, methyl 3,5-dimethyl-1*H*-pyrazole-1-carboxylate (DMPE), methyl 3-methyl-5-(trifluoromethyl)-1*H*-pyrazole-1-carboxylate (MPFE) and methyl 3-methyl-5-(trichloromethyl)-1*H*-pyrazole-1-carboxylate (MPClE), which were designed using molecular simplification approach. This concept has used as a drug design strategy to shorten synthetic routes and/or to simplify molecular structures, while keeping or enhancing the biological activity of the lead drugs (Barreiro, 2002). We investigated whether these compounds have enhanced analgesic activity compared with the lead-like compounds (MPF4 and MPCA). Furthermore, we determined the analgesic and toxic potential in diverse pain models and the mechanism of action of these compounds.

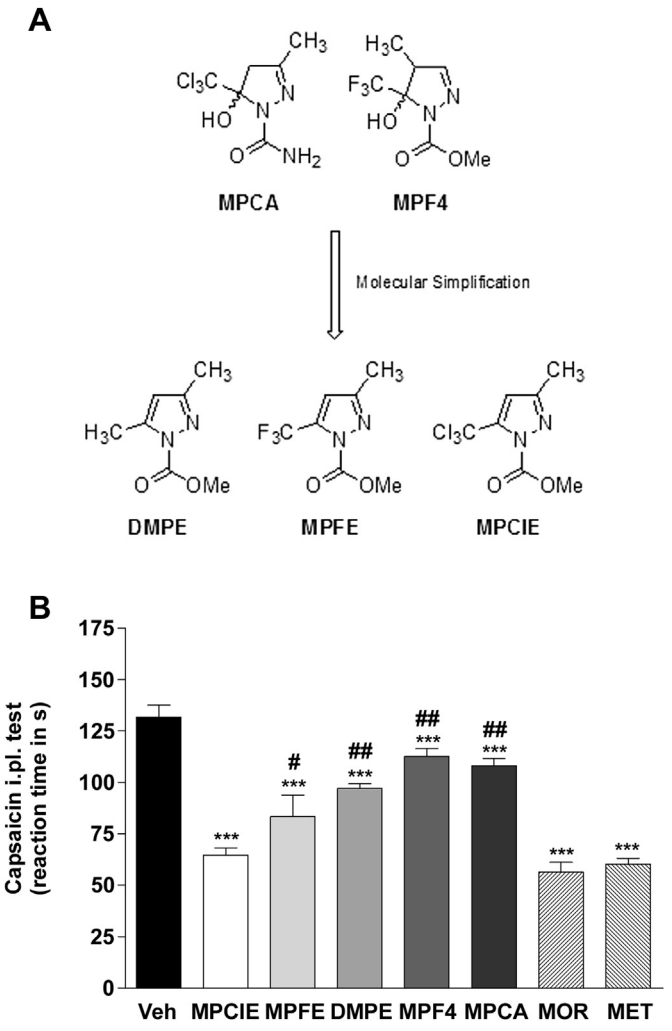

Fig. 1. Antinociceptive effects of MPCIE, MPFE and DMPE on capsaicin-induced nociception. (A) Design and scaffold evolution of the methyl 1*H*-pyrazole-1-carboxylate derivatives, methyl 3,5-dimethyl-1*H*-pyrazole-1-carboxylate (DMPE), methyl 3-methyl-5-(trifluoromethyl)-1*H*-pyrazole-1-carboxylate (MPFE) and methyl 3-methyl-5-(trichloromethyl)-1*H*-pyrazole-1-carboxylate (MPClE), based on molecular simplification of the lead-like compounds 5-hydroxy-3-methyl-5-(trichloromethyl)-1*H*-pyrazole-1-carboxamide (MPCA) and methyl 5-hydroxy-4-methyl-5-(trifluoromethyl)-4,5-dihydro-1*H*-pyrazole-1-carboxylate (MPF4). (B) Evaluation of antinociceptive effects of MPCIE, MPFE, DMPE, MPF4 and MPCA (6 μmol/kg, p.o.), morphine (MOR, 13 μmol/kg, p.o.), and metamizol (MET, 1 mmol/kg, p.o.) in the mouse capsaicin intraplantar (i.pl., 1 nmol/paw, 20 μL) test 1 h after administration. Data represent the mean ± S.E.M. of nociception time observed for 5 min after the i.pl. administration of capsaicin ($n = 7$–10). $^{***}P < 0.001$, when compared with vehicle (veh), $^{\#\#}P < 0.001$, $^{\#}P < 0.05$ when compared with MPCIE; one-way ANOVA followed by the Bonferroni's post-hoc test.

2. Methods

2.1. Animals

Male albino Swiss mice (20–30 g) bred in-house were used in all experiments. Animals were kept in a controlled environment (22 ± 2 °C) with a 12 h light/dark cycle (lights on 6:00 a.m. to 6 p.m.) and fed standard lab chow and tap water *ad libitum*. Before the experiments, the animals were acclimatized to the laboratory room for at least 1 h. All experiments were carried out between 08:00 a.m. and 5:00 p.m. Each animal was used only once. Animal care and experiments were conducted in accordance with the ethical principles for animal research (Scientific procedures) Act (UK, 1986) and were approved by the Ethics Committee of the Federal University of Santa Maria (protocol no. 23081.018371/2006-94). In addition, the number of animals and the intensity of noxious stimulus used were the minimum necessary to demonstrate the consistent effects of the drug treatments (Zimmermann, 1983).

2.2. Drugs and reagents

If not otherwise indicated, all reagents were from Sigma (Sigma, St Louis, MO, USA) and were dissolved in the appropriate vehicle solutions. Morphine sulphate was purchased from Cristália (São Paulo, Brazil). Arabic gum and activated charcoal were purchased from Vetec (Rio de Janeiro, Brazil). Radiolabeled [^{3}H]-resiniferatoxin ([^{3}H]-RTX) was purchased from Perkin Elmer (Boston, USA). [^{3}H]-idazoxan ([^{3}H]-IDZ) and [^{3}H]-CI-977 were obtained from Amersham International (Buckinghamshire, UK).

Methyl 3,5-dimethyl-1*H*-pyrazole-1-carboxylate (DMPE), Methyl 3-methyl-5-(trifluoromethyl)-1*H*-pyrazole-1-carboxylate (MPFE), and Methyl 3-methyl-5-(trichloromethyl)-1*H*-pyrazole-1-carboxylate (MPClE) (Fig. 1A) were synthesised using the cyclocondensation reaction of hydrazine methyl carboxylate with pentane-2,4-dione or 1,1,1-trifluoro[chloro]-4-methoxy-3-penten-2-one, as described previously (Martins et al., 2006; Moura et al., 2008). Furthermore, MPCA and MPF4 were synthesised in accordance with the described procedures (Bonacorso et al., 1999;