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A new sigma ligand, (±)-PPCC, antagonizes kappa opioid receptor-mediated antinociceptive effect

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Abstract

The compound (1*R*,2*S*/1*S*,2*R*)-2-[4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl) cyclopropanecarboxylate [(±)-PPCC] is a ligand with high affinity for sigma (σ) sites of which the selectivity towards several other receptor systems has been demonstrated. Given the existence of a relationship between the σ system and the kappa opioid (KOP)-mediated analgesia, to characterize the pharmacological properties of (±)-PPCC we analyzed its influence on the analgesic effect of the systemic injected kappa agonist (−)-U-50,488H comparing the effects with those shown by (+)-pentazocine and BD1047. The results demonstrate that the systemic administration of (±)-PPCC (1 mg/kg s.c.) does not modify basal tail-flick latency. Pre-treatment with (±)-PPCC, at the same dose, significantly decreased the antinociceptive effect of (−)-U-50,488H, analogously to the σ compounds used. This study confirms that (±)-PPCC plays the role of σ agonist in this model and strengthens the hypothesis of the σ receptor modulatory role on KOP-mediated analgesia.

Keywords: Sigma; KOP receptor; Analgesia; (±)-PPCC; Rat

Introduction

Sigma (σ) receptors have been studied since the mid 1970s (Martin et al., 1976), however, their physiological role remains unclear (Leonard, 2004). Although there is evidence supporting the existence of an endogenous ligand for these receptors (Contreras et al., 1987; Su et al., 1986, 1988), to date a conclusive identification has not been established (Guitart et al., 2004). It has been defined that σ receptors are distinct pharmacological entities reclassified as non-opioid (Su, 1982) and non-phencyclidine (PCP) unique sites (Wong et al., 1988). Biochemical and pharmacological studies suggest that there are multiple subtypes of σ receptors (Guitart et al., 2004) but the best characterized are σ_1 and σ_2 (Quirion et al., 1992). In situ hybridization and immunohistochemical studies have demonstrated σ receptor localization especially in the central nervous system (CNS) with a different distribution between σ_1 and σ_2 (Bouchard and Quirion, 1997). The σ_1 subtype is present in brain areas implicated in motor and endocrine functions, in limbic areas and in sites involved in modulation of pain processing, such as periaqueductal central gray and spinal dorsal horn (Alonso et al., 2000). A role for the σ_1 receptor in pain modulation was suggested by studies that demonstrate a relationship with opioids. In fact, Chien and Pasternak (1995) have shown that a tonically active antiopioid σ_1 system markedly influences the sensitivity of mice toward opioid analgesia, especially when it is kappa opioid (KOP)-receptor-mediated. We have previously reported (Ronsisvalle et al., 2001) that (+)-MR200, a σ ligand structurally related to the putative σ_1 antagonist haloperidol, increases the analgesic effect induced by the systemic KOP agonist (−)-U-50,488H, which shows antiopioid activity. Our group has recently developed a new σ ligand (1*R*,2*S*/1*S*,2*R*)-2-[4-hydroxy-4-phenylpiperidin-1-yl)methyl]-1-(4-methylphenyl) cyclopropanecarboxylate [(±)-PPCC] (Fig. 1) with high affinity for σ sites (σ_1, K_i= 1.5 nM, σ_2, K_i=50.8 nM). In binding studies, (±)-PPCC shows marked σ selectivity over several tested receptors such as *N*-

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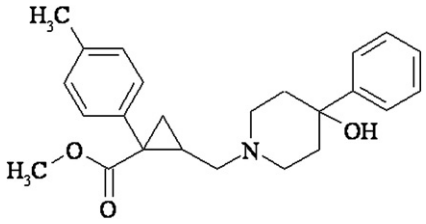

Fig. 1. Chemical structure of (±)-PPCC.

methyl-D-aspartate (NMDA), dopaminergic (D_1, D_2, D_3), muscarinic, histaminergic H_1, adrenergic (σ_1, α_2), serotoninergic (5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4, 5-HT_6), dopamine and serotonin transporters (DAT, SERT) (Prezzavento et al., 2007). Here we investigated the effect of in vivo administration of (±)-PPCC, with respect to σ reference compounds, (+)-pentazocine and BD1047, on the analgesic response of (−)-U-50,488H (the most active enantiomer that has previously been used to investigate the action of σ compounds on KOP analgesia) (Chien and Pasternak 1994; Mei and Pasternak 2002), in order to better define the pharmacological activity of this new selective $\sigma_{1/2}$ compound.

Materials and methods

Animals

Male Sprague-Dawley rats (Morini, S. Polo d'Enza, Reggio Emilia, Italy), weighing 180–200 g, were used.

Animals were kept at a constant room temperature (25 ± 1 °C) under a 12:12 h light and dark cycle with free access to food and water. Each rat was used for only one experiment. Experimental procedures were approved by the local ethical committee (IACUC) and conducted in accordance with international guidelines as well as European Communities Council Directive and National Regulations (CEE Council 86/609 and DL 116/92).

Nociceptive test

Nociception was evaluated by the radiant heat tail-flick test (Marrazzo et al., 2006). Briefly, this consisted of irradiation of the lower third of the tail with an infrared source (Ugo Basile, Comerio, Italy). The day before the experiment, rats were habituated to the procedure for measuring nociception threshold. Experiments were performed at room temperature (25 ± 1 °C). The basal pre-drug latency was established between 3 and 4 s, calculated as the average of the first three measurements, performed at 5 min intervals. A cut-off latency of 10 s was established to minimize damage to the tail. Post-treatment tail-flick latencies (TFLs) were determined at 10, 20, 30, 40, 50 and 60 min after subcutaneous (s.c.) injection. TFLs obtained in succeeding tests, after each pharmacological treatment, were also expressed as percentage changes from basal level and we have reported the results as mean areas under the curve (MAUC) over a 60 min testing session. For the dose–response curve, antinociceptive response was calculated as maximum possible effect percentage (MPE%) where MPE% = [(test latency − baseline latency)/(cut-off − baseline latency)] × 100. Animals were divided into groups each consisting of 8–10 rats.

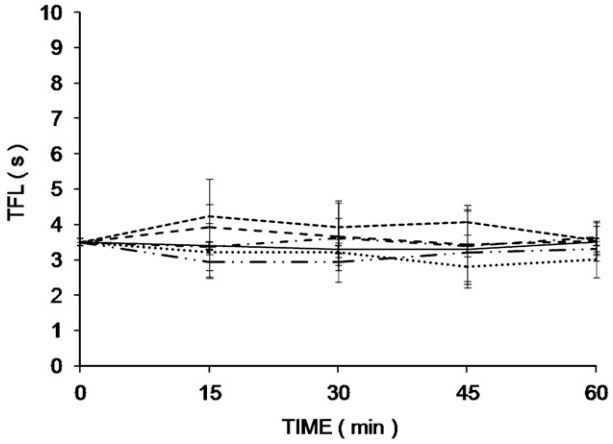

Fig. 2. Effect of different doses of s.c. administered (±)-PPCC on tail flick latency (TFL), expressed in seconds (*s*). The different groups of rats were treated, respectively, with saline (____), 0.25 mg/kg s.c. (±)-PPCC (·····), 0.5 mg/kg s.c. (±)-PPCC (– – –), 1 mg/kg s.c.(±)-PPCC (– · –), 1.5 mg/kg s.c. (±)-PPCC (____) and 3 mg/kg s.c. (±)- PPCC (– · ·). TFLs were determined at 15, 30, 45 and 60 min after treatment. Results are expressed as the mean ± SE.

Drugs and experimental procedures

Two groups of rats received, respectively, saline and selective KOP agonist (−)-U-50,488H (5 mg/kg s.c.) (Tocris, Bristol, UK). Five groups of animals were treated with different doses (0.25, 0.5, 1, 1.5, 3 mg/kg s.c.) of (±)-PPCC, synthesized in our laboratory (Prezzavento et al., 2007). For dose–response curve determination, five groups of rats were pre-treated with (±)-PPCC, at the same doses previously used, followed, after 45 min, by (−)-U-50,488H (5 mg/kg s.c.). The other four groups of animals were injected, respectively, with (+)-pentazocine

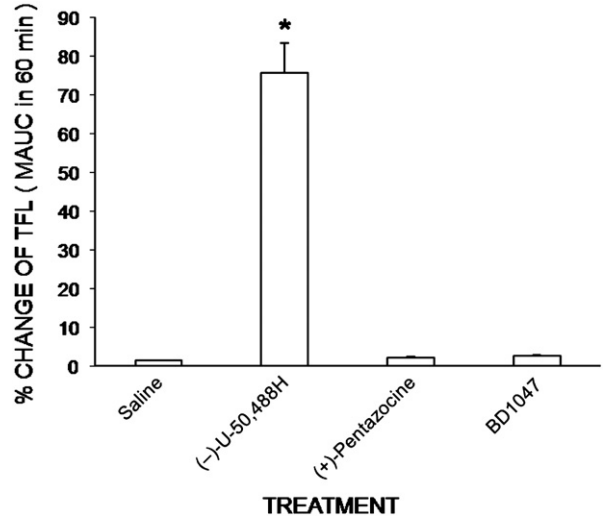

Fig. 3. Effect of (−)-U-50,488H (5 mg/kg s.c.), (+)-pentazocine (5 mg/kg s.c.) and BD1047 (1 mg/kg s.c.). Results are expressed as the mean area under the curve (MAUC) after the last injection over the 60 min testing period. Columns represent the mean ± SE (n = 8 – 10). *P<0.05 vs. normal saline-treated rats.

(5 mg/kg s.c.), synthesized in our laboratory; BD1047 (1 mg/kg s.c.) (Tocris, Bristol, UK); (+)-pentazocine (5 mg/kg s.c.), followed, after 45 min, by (−)-U-50,488H and BD1047 (1 mg/kg s.c.) followed, after 45 min, by (−)-U-50,488H.

Statistical analysis

Data are expressed as means ± SE. Intergroup comparisons were assessed using an initial two-way analysis of variance (ANOVA) followed by Duncan's multiple range post hoc test. Differences were considered significant at $P<0.05$.

Regarding the dose–response curve, the ED_{50} value was calculated from linear regression of the dose–effect functions and means and the 95% confidence interval was obtained (for each group of rats). Linear regression was determined using GraphPad Prism (GraphPad, San Diego, CA, USA).

Results

Our results showed that (±)-PPCC, for each dose administered (0.25, 0.5, 1, 1.5, 3 mg/kg), did not affect tail withdrawal latencies during the time of observation (Fig. 2).

Injection of KOP agonist (−)-U-50,488H, at a dose of 5 mg/kg s.c., significantly increased the nociceptive latency following thermal stimulation (tail-flick), which demonstrated a clear analgesic effect (Fig. 3). Compared with the group of rats treated with saline, the percentage changes from basal level of TFL (MAUC over 60 min of observation) was increased from 1.55 to 75.77%. Fig. 3 also shows the MAUC value obtained after treatment with (+)-pentazocine (5 mg/kg s.c) and BD1047 (1 mg/kg s.c.), which did not modify basal withdrawal latency.

At the same doses used in the previous experiments (0.25, 0.5, 1, 1.5, 3 mg/kg), (±)-PPCC was followed, after 45 min, by (−)-U-50,488H (5 mg/kg s.c.). We chose this interval time between the two treatments because of the better effect of

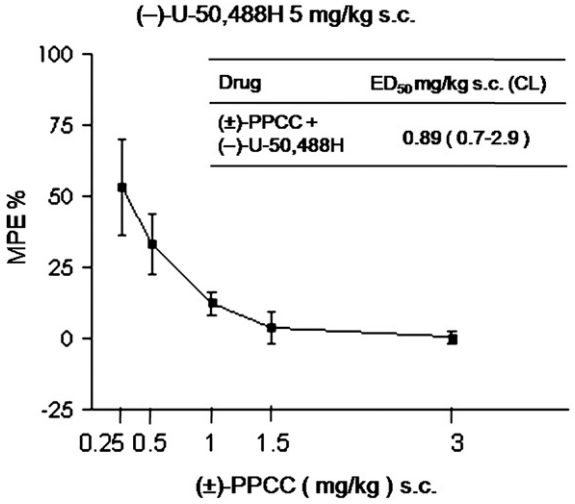

Fig. 4. Dose–response curve of the functional antagonism of (±)-PPCC toward analgesia induced by (−)-U-50,488H. Five groups of rats were given the indicate doses of (±)-PPCC in combination with a fixed dose of (−)-U-50,488H (5 mg/kg s.c.). Results are presented as MPE% ± SEM. The (±)-PPCC ED_{50} was 0.89 mg/kg (0.7–2.93).

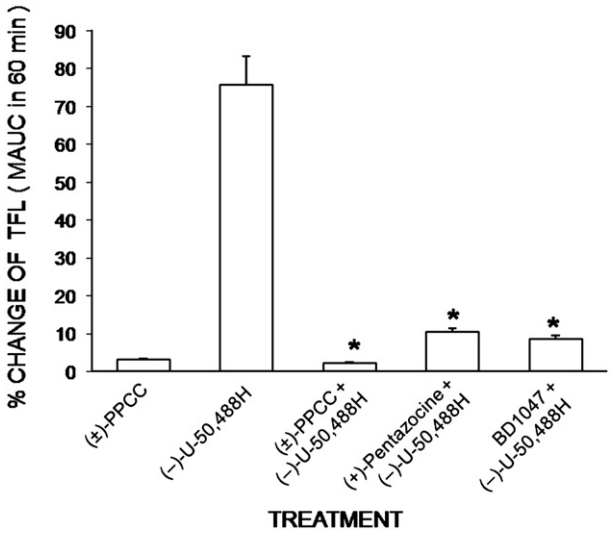

Fig. 5. Effect of (±)-PPCC (1 mg/kg s.c.), (+)-pentazocine (5 mg/kg s.c.) and BD1047 (1 mg/kg s.c.) on (−)-U-50,488H (5 mg/kg s.c.) analgesia. Results are expressed as the mean area under the curve (MAUC) after the last injection over the 60 min testing period. Columns represent the mean ± SE (n = 8–10). *$P<0.05$ vs. (−)-U-50,488H-treated rats.

(±)-PPCC toward (−)-U-50,488H analgesia when compared with the time intervals of 30 or 60 min (data not shown). After the combined treatment, the rats were tested at the time of maximal analgesic effect of (−)-U-50,488H (40 min) (Ronsisvalle et al., 2001). The dose–response curve (Fig. 4) showed a reduction of the (−)-U-50,488H analgesic effect with different doses of (±)-PPCC (0.25–3 mg/kg s.c.). The table insert in Fig. 4 shows the ED_{50} value (0.89 mg/kg s.c.) with its confidence limits (CL) (0.7–2.9).

Pre-treatment with (±)-PPCC, 1 mg/kg s.c., followed by (−)-U-50,488H, at the same dose as used in the single treatment, caused a well-defined antagonism of opioid analgesia: the MAUC was significantly lower than that in rats treated with opioid alone (MAUC of 2.14% versus 75.77%) (Fig. 5). The KOP analgesia was also prevented by the pre-treatment with (+)-pentazocine (MAUC of 10.33% versus 75.77%) and BD1047 (MAUC of 8.55% versus 75.77%).

Discussion

In this study we found evidence that the systemically administered $\sigma_{1/2}$ ligand (±)-PPCC, which alone did not modify the basal TFL, was able to antagonize the analgesic response induced by the KOP selective agonist (−)-U-50,488H. The (±)-PPCC pharmacological effect was similar to that obtained with the σ_1 selective agonist (+)-pentazocine, and the $\sigma_{1/2}$ ligand BD1047. For our experiments we chose BD1047 as a possible σ_1 antagonist (Matsumoto et al., 1995) although it has been reported that this compound may act as a partial agonist (Zambon et al., 1997). The results, however, showed that BD1047 caused the same effect as (±)-PPCC and (+)-pentazocine, and suggested that the σ_1 agonist activity of BD1047, at least at the dose used in our study, was prevalent (Fig. 5). Nevertheless, BD1047, as a partial agonist, would be expected to

have lower efficacy than the full agonist (+)-pentazocine, therefore, possible additional mechanisms may be involved in the removal of the (−)-U-50,488H analgesic response, for example modulation of Ca^{2+} influx and $[Ca^{2+}]_i$ (Novakova et al., 1998).

These data suggest that our compound possesses a σ_1 agonist profile, as it shows an antiopioid action. This is probably a functional antagonism that may be justified by the co-presence of σ and KOP receptors in areas involved in modulation of pain transmission (Guitart et al., 2004; Bodnar and Klein 2006). Moreover, the effect of (±)-PPCC on (−)-U-50,488H analgesia was prevented by pre-treatment with the σ_1 putative antagonists (+)-MR200 (Ronsisvalle et al., 2001) and haloperidol, (Chien and Pasternak, 1995) (data not shown), which confirms its action on the σ receptor.

This pharmacological effect correlates with that in biological studies that have assessed the correlation between σ receptors and calcium-dependent protein tissue transglutaminase (TG-2). Our previous study suggests that (±)-PPCC may act as a $\sigma_{1/2}$ agonist (Prezzavento et al., 2007). Nevertheless, since the subcellular mechanisms by which σ ligands exert their effects have not been explained in detail, and the concept of σ agonist or antagonist remains unclear (Skuza and Rogoz, 2006), our experiments further clarify the pharmacological profile of (±)-PPCC.

The data obtained with (±)-PPCC support the hypothesis that the σ system is involved in the modulation of opioid analgesia (Pasternak et al., 2006; Marrazzo et al., 2006). Furthermore, the σ_1 selective agonist (+)-pentazocine antagonizes systemic (Chien and Pasternak, 1993), spinal and supraspinal (Mei and Pasternak, 2002) morphine analgesia, while haloperidol, the non-selective and putative σ_1 antagonist, increases it in mice and rats, which implies that this antiopioid system is tonically active (Chien and Pasternak, 1995). Moreover, haloperidol enhances KOP and delta opioid peptide (DOP) analgesia (Chien and Pasternak, 1994) more dramatically than morphine, which indicates that the σ system is active against the opioid analgesic system, but is more active against KOP analgesia. This opioid antagonism has also been confirmed by the antisense approach, in which down-regulation of the σ_1 receptor increases the analgesic activity of KOP agonists (−)-U-50,488H and naloxone benzoylhydrazone (King et al., 1997). Gear and co-workers (2006) have recently reported that haloperidol enhances nalbuphine analgesia which shows that a σ ligand can block the antianalgesic effect of a KOP agonist–antagonist.

The σ ligand (+)-MR200, which is structurally related to haloperidol, increased the analgesic effect induced by s.c. (−)-U-50,488H and reversed the effect of (+)-pentazocine on the KOP agonist, which demonstrated σ_1 antagonist activity (Ronsisvalle et al., 2001). It is interesting to note that (±)-PPCC, which is structurally related to (+)-MR200, with a 4-phenylpiperidin-4-ol amino moiety instead of 4-(4-chlorophenyl)piperidin-4-ol, and with the *p*-methyl substituent on the phenyl present on the cyclopropane ring, displays an inversion of pharmacological activity on the σ_1 receptor. Both compounds have a similar $\sigma_{1/2}$ binding affinity, (Prezzavento et al., 2007; Ronsisvalle et al., 2001), but in our study, (±)-PPCC showed the same effect as the σ_1 selective agonist (+)-pentazocine, and even though PPCC was tested as a racemate, it is almost certain that the agonist σ_1 activity was present in at least one of the two enantiomers. This inversion of activity on the σ_1 receptor is very interesting: our hypothesis is that one of the two hydrophobic sites proposed by Ablordeppey and co-workers (2002) may be implicated other than for σ affinity but also for σ activity. The stereochemistry study, the role of the substituent present on the aromatic ring in (+)-MR200 and its opposite enantiomer, the separation of the single enantiomers of (±)-PPCC and the synthesis of structurally related compounds, may make an important contribution to clarifying the requirement for the activity of these compounds on the σ receptor.

Conclusion

The present study indicates that (±)-PPCC alone has no analgesic action. However, when co-administered with (−)-U-50,488, (±)-PPCC effectively blocked the analgesia induced by the opioid. The present data suggest that (±)-PPCC is a new σ_1 agonist that may provide useful insight into the functional antagonism of the σ_1 receptor on the modulation of opioid analgesia.

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