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Short communication

The cyclo-oxygenase inhibitor nimesulide induces conditioned place preference in rats

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Abstract

Two cyclo-oxygenase inhibitors, indomethacin and nimesulide, have been shown to potentiate morphine-induced stimulation of meso-accumbens dopamine neurons. In this study, an unbiased conditioned place preference procedure was used to evaluate whether nimesulide produces motivational effect after systemic administration in rats. These results show that nimesulide, at doses 0.1, 0.5 and 1 mg/kg, even lower than those usually applied for inflammatory conditions, induces conditioned place preference in rats, suggesting that it might possess rewarding properties in humans.

Keywords: Cyclo-oxygenase inhibitor; Nimesulide; Conditioned place preference; Abuse liability

1. Introduction

Cyclo-oxygenase is the rate-limiting enzyme involved in the synthesis of prostanoids from arachidonic acid. Prostanoids have been demonstrated to be important lipid mediators, not only in periphery but also in the brain (Breder et al., 1995), where they appear to modulate synaptic transmission (Williams, 1997).

Recent studies demonstrated that cyclo-oxygenase pathway might modulate the neurotransmission of γ-aminobutyric acid (GABA) and dopamine in the central nervous system (Ono et al., 1992; Vaughan et al., 1997; Ross et al., 1999; Melis et al., 2000). In particular, cyclo-oxygenase blockade has been shown to potentiate opioid inhibition of GABA-ergic synaptic transmission (Vaughan et al., 1997), whereas cyclo-oxygenase inhibitors, such as acetylsalicylic acid, indomethacin and piroxicam, inhibited catalepsy induced by the dopamine receptor inhibitors haloperidol and raclopride, even though none of them produced catalepsy when used alone (Ono et al., 1992; Ross et al.,1999). In line with these findings, we have recently reported that the cyclo-oxygenase inhibitors indomethacin and nimesulide potentiate the stimulant effect of morphine on dopaminergic neurons projecting to the nucleus accumbens, even though they do not produce any effect when administered alone (Melis et al., 2000).

The ability to stimulate the firing rate of meso-accumbens dopaminergic neurons, to induce self-administration, to lower brain self-stimulation reward threshold as well as to induce conditioned place preference, is considered as an index of rewarding properties of drugs (Wise, 1987). Since behavioural evidence supports the involvement of the same neuronal pathways in reward and analgesia (Franklin, 1989), we decided to investigate whether nimesulide, a widely used new generation cyclo-oxygenase inhibitor, might possess the ability to induce conditioned place preference in rats.

2. Materials and methods

Male Sprague–Dawley rats (Charles River, Italy) weighing 150–175 g at the beginning of the experiments, were housed four per cage with free access to food and water, and maintained on a 12-h reversed light–dark cycle (light on 8:00 p.m.). Experiments were performed in accordance with the EC regulations for animal use in research (CEE no. 86/609).

Nimesulide (Sigma, Italy), dissolved in two drops of Tween 80, and morphine HCl (Sigma, Italy) were diluted

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in saline solution at a volume of 0.5 ml/kg and administered intraperitoneally (i.p.) 5 min before starting the 30-min conditioning session.

The experimental apparatus and procedure were as previously described (Fattore et al., 2000). Briefly, the apparatus consisted of eight rectangular plastic shuttle boxes ($30 \times 60 \times 30$ cm^3), each divided by a guillotine door into two distinct compartments of equal size, containing different visual and tactile cues. Visual cues were present in the walls, which were either brown or black and white striped; tactile cues were present in the floor, being either grid or chequered. Particular care was taken to ensure that all these cues — producing four possible combinations — were represented in the compartments in a counterbalanced order. The experimental room was sound attenuated and dimly lit. The unbiased procedure consisted of three consecutive phases: preconditioning (phase I), conditioning (phase II) and postconditioning or test (phase III). Before starting the experiment, each animal was randomly assigned to one of five different treatment groups ($n = 8$/group).

During phase I, 3 days were dedicated to accustoming the animals to conditioning boxes. The guillotine doors were raised, each rat was placed in one of the compartments, defined as the start box, ensuring representation of all different cues in equal number in the start compartments, and allowed to explore both sides for 15 min/day. Time spent by each animal in the two compartments was recorded on the third day (pretest) to exclude strong spontaneous preference expressed by rats. Thus, animals showing an unconditioned preference to one specific cue combination were excluded from the subsequent (conditioning) phase of the experiment. An animal was considered to be in a particular compartment only when all four paws were in that area.

Conditioning training (phase II) lasted 10 days and consisted of five alternated presentations of nimesulide (0.1, 0.5, or 1 mg/kg) and saline. Morphine (5 mg/kg i.p.) was used to provide a positive control for method, whereas the vehicle (Tween 80), which has been previously shown to not induce any behavioural effect in this conditioned place preference paradigm (data not shown), was not included in the experiments. On odd-numbered training days, nimesulide-treated rats were confined to their previous start side for 30 min, whereas on even-numbered training days each animal was given the vehicle and confined in the opposite non-start side for the same period of time. Control group received daily saline in both compartments. On the test day (phase III) the guillotine doors were raised, animals did not receive any treatment and were placed in the start compartment. Time spent by each drug-free animal in both sides during a 15-min period was again recorded. The difference in time spent in the drug-paired compartment between post and preconditioning test, expressed as difference scores (Δt), was considered the critical measurement for evaluation of conditioned preference induced by the drug: a positive difference was considered an index of reward.

A separate set of animals was pretreated with nimesulide as described for conditioned place preference experiments and individually placed into the motility cages (Omnitech Digiscan Animal Activity Monitor, Columbus, OH, USA). Each cage had two sets of 16 photocells located at right angles to each other, projecting horizontal infrared beams 2.5 cm apart and 2 cm above the cage floor. Motor activity was defined as the horizontal activity counts and scored for 60 min. All data were collected every 10 min and analysed using one-way analysis of variance (ANOVA) followed by post-hoc Newman–Keuls' test for between-group comparisons.

3. Results

The mean times (s) ± S.E.M. spent by rats in the drug-paired (start) compartment before conditioning, i.e. baseline values, were 503 ± 21 for saline, 458 ± 18 for morphine, and 486 ± 32, 426 ± 44 and 451 ± 26 for nimesulide 0.1, 0.5 and 1 mg/kg, respectively. Compared to the preconditioning phase, on the test day all morphine-treated and the majority of nimesulide-treated rats spent significantly more time in drug-paired compartment. Indeed, the mean times (s) ± S.E.M. spent by rats in the drug-paired compartment on test day were 634 ± 8 for morphine and 512.5 ± 12, 554 ± 19 and 544 ± 22 for nimesulide 0.1, 0.5 and 1 mg/kg, respectively. On the contrary, control (saline) group exhibited no significant shift in preference, reporting values of 493 ± 11.

Fig. 1 shows that statistical analysis revealed a significant difference with respect to control group for both morphine and the two higher doses of nimesulide tested,

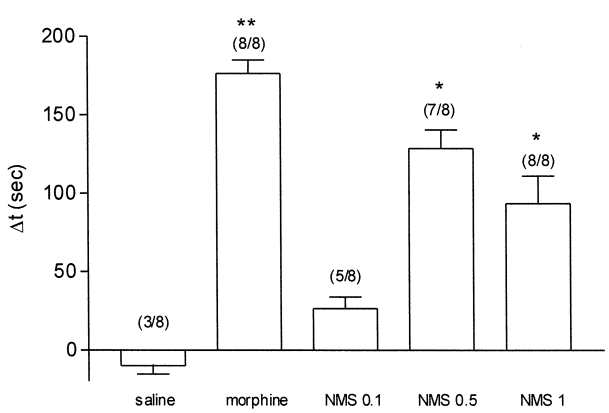

Fig. 1. Conditioned place preference induced by nimesulide in rats. Each bar represents the mean ± S.E.M. ($n = 8$ rats/groups) of difference in time spent in the drug-paired environment between post- and preconditioning test sessions. Doses are expressed in mg/kg. Numbers in parentheses: number of animals which showed preference for drug-paired side/total number of animals in the group. * $P < 0.01$ versus both saline and vehicle group (Dunnett's t-test).

Table 1
Spontaneous locomotor activity in nimesulide pretreated rat

Time (min)	0	0.1	0.5	1
0–30	6544 ± 506	7003 ± 215.5	6988 ± 351	6679 ± 498
0–60	9355 ± 431.5	11231 ± 519	10098 ± 299	9989 ± 322.5

Doses are expressed in mg/kg. Values represent mean ± S.E.M. ($n = 6$ rats/group) of cumulative locomotor activity counts over 30 and 60 min of observation.

whereas no motivational response was observed after conditioning with a lower dose (0.1 mg/kg) of the drug. It should be noted that behaviourally active doses of nimesulide (0.5 and 1 mg/kg) induced conditioned place preference in almost all (20/24) treated animals.

Moreover, at the same doses, nimesulide did not significantly affect motor activity in any direction in rats, as shown in Table 1.

4. Discussion

The present results demonstrate that nimesulide, one of the newer nonsteroidal anti-inflammatory drugs which acts mainly through the selective inhibition of cyclo-oxygenase-2, induces conditioned place preference in rats. Indeed, i.p. administration of 0.5 and 1 mg/kg of nimesulide induced a significant shift in preference for the environment previously paired with the drug. Animals pretreated with the lower dose (0.1 mg/kg) did not significantly differ from saline-treated counterparts, even though they did show a tendency in preferring the environment paired with the drug.

Morphine, introduced as a positive control of the method at the dose of 5 mg/kg, (Mackey and Van der Kooy, 1985) proved to be a stronger reinforcer than nimesulide. However, the cyclo-oxygenase inhibitor induced conditioned place preference in 83.33% of treated animals at doses lower than those clinically used for inflammatory conditions (1–6 mg/kg). Interestingly, we observed that nimesulide-induced conditioned place preference was not further enhanced when the drug was tested at higher doses (up to 6 mg/kg) and following 10 + 10 conditioning trials (data not shown).

Finally, the finding that nimesulide pretreatment does not affect spontaneous locomotor activity in rats rules out any confounding variables deriving from locomotor activating or sedative action.

Since conditioned place preference is considered both a reliable and highly predictive animal model of drug-induced reward and abuse potential of drugs (Tzschentke, 1998), the present results raise the possibility that this drug might also exert a positive reinforcing effect in man, which might explain its wide popularity (Elseviers and De Broe, 1998). However, since our finding is the first evidence suggesting that a cyclo-oxygenase inhibitor could possess rewarding properties in rats, further confirmation of our data using other cyclo-oxygenase inhibitors and alternative animal model of addiction is required. More generally, our study supports the hypothesis that the inhibition of cyclo-oxygenase cascade might participate in the modulation of the reward pathways, probably through an involvement of dopaminergic or GABA-ergic systems. Nevertheless, additional investigations are necessary to determine the exact mechanism of such interaction.

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