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Nitric Oxide Synthesis Inhibition Attenuates Morphine-Induced Place Preference

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KIVASTIK, T., J. RUTKAUSKAITE AND A. ZHARKOVSKY. *Nitric oxide synthesis inhibition attenuates morphine-induced place preference.* PHARMACOL BIOCHEM BEHAV **53**(4) 1013–1015, 1996.—Nitric oxide (NO) has been implicated in the actions of opioids. The aim of the present study was to investigate the role of NO in the mechanisms mediating the rewarding effects of morphine. Therefore, the influence of NO synthase inhibitor L-*N*-nitroarginine (L-NOARG) on morphine-induced place preference in rats was studied. L-NOARG, when given at 20 mg/kg, IP, significantly inhibited the effect of morphine. L-NOARG by itself, when administered at 5 or 20 mg/kg, IP, appeared to have no reliable effect on place conditioning. The results suggest a possible role of NO in the opioid reward process.

Nitric oxide Morphine Place preference Rats

THE FREE RADICAL gas nitric oxide (NO) is a highly unconventional messenger molecule. Its function in the CNS is widely related to excitatory amino acids. Thus, NO is formed in response to glutamate acting upon NMDA receptor, and its release is involved in many glutamate actions in the CNS including cellular events that may underlie the processes of learning and memory (12). According to the results of recent studies, NO may be implicated in the actions of opioids; it has been demonstrated that inhibitors of NO synthase could prevent morphine tolerance (6) and attenuate the development and expression of the abstinence syndrome (5). The issue is largely unclear, however, as far as the opioid reward process and NO are concerned. Thus, the present study was addressed to investigate whether NO is involved in the mechanisms that mediate the rewarding effects of morphine. We studied the influence of NO synthase inhibitor L-*N*-nitroarginine (L-NOARG) on morphine-induced conditioned place preference (CPP) in rats.

METHODS

Animals

Male Wistar rats weighing 245–405 g were used. The rats were housed in groups of four to five with food and water available ad lib, under a 12 L : 12 D cycle (lights on at 0700 h). The experiments were carried out during the light phase of the cycle.

Drugs

Morphine sulfate (ampoules containing 20 mg/ml of morphine sulfate; Antigen Pharmaceuticals, Roscrea, Ireland) was dissolved in 0.9% NaCl solution and injected in a volume of 1 ml/kg, SC, into the neck region. The dose of morphine refers to the amount of the free base. L-NOARG (Sigma Chemical Co., St. Louis, MO) was administered IP in a volume of 2 ml/kg as 2.5% Tween 80 solution.

Place Preference Apparatus

The apparatus consisted of two square-base compartments (H 40 × 30 × 30 cm), one with white and the other with gray walls and floor. Compartments were separated by a guillotine door and covered with a transparent Plexiglas ceiling. The apparatus was placed into a dimly lit room.

Experimental Procedure

Before starting the experiment, the rats were acclimated to experimenter contact for 3 days by handling and weighing in the experiment room. The experiment consisted of three phases.

Preconditioning. During 3 days (days 1, 2, and 3), rats were given free access to both compartments of the apparatus for 15 min each day. On day 3, the time spent by rats in each compartment was recorded (the position of the rat was defined

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by the position of its front paws) and these values served as a baseline. According to the baseline values, the animals were divided into treatment groups with similar initial preference. Because most of the rats (51 from 56 animals) preferred the gray compartment (i.e., they spent over 50% of time on that side), the ones preferring the white compartment were excluded from the experiment.

Conditioning. Conditioning was conducted during 4 days (days 4, 5, 6, and 7) and included two sessions each day. The rats were conditioned for 45 min in the initially nonpreferred compartment immediately after administration of morphine, and in the preferred one after administration of saline. An interval of 4 h separated the two sessions. The order of morphine and saline presentation, paired with the given environment, was balanced across treatment groups. L-NOARG (or its vehicle) was given 15 min before morphine (or saline) administration. The following treatment groups were included: a) control [i.e., the animals receiving L-NOARG vehicle pretreatment, and saline immediately before the conditioning session (veh + sal)]; b) L-NOARG 5 mg/kg plus saline (N5 + sal); c) L-NOARG 20 mg/kg plus saline (N20 + sal); d) vehicle + morphine 3 mg/kg (veh + Mo); e) L-NOARG 5 mg/kg plus morphine 3 mg/kg (N5 + Mo); and f) L-NOARG 20 mg/kg + morphine 3 mg/kg (N20 + Mo). The dose of morphine (3 mg/kg, SC) was selected according to earlier studies (11) in which it was shown to produce reliable CPP.

Postconditioning. The postconditioning test was carried out on day 8 (24 h after the last drug administration). No injections were given before test. The rats had free choice in the apparatus for 15 min, and the time spent in each compartment was recorded by an observer unaware of the previous drug treatment.

Statistics

The data were subjected to two-factor analysis of covariance (ANCOVA) according to a 3 × 2 factorial design, in which the time spent in the drug-paired compartment during the postconditioning test served as the dependent variable, L-NOARG and morphine as categoric variables, and the baseline as covariate. Posthoc comparisons were conducted by using the contrast analysis with Bonferroni levels (i.e., the critical level 0.05 was divided by the number of the comparisons made).

RESULTS

Figure 1 shows the results. ANCOVA revealed a significant effect for the morphine factor [$F(1, 44) = 13.5$, $p = 0.001$] indicating that morphine brought about reliable CPP. In addition, a significant effect [$F(2, 44) = 3.2$, $p = 0.049$] was established for L-NOARG and a nearly significant one for the L-NOARG × Morphine interaction [$F(2, 44) = 2.9$, $p = 0.066$]. To further clarify the nature of these effects, four posthoc comparisons were conducted: the groups N5 + sal and N20 + sal were tested against the group veh + sal, whereas the groups N5 + Mo and N20 + Mo against the group veh + Mo. In most cases, no significant differences were found [$F(1, 44) = 0.02$–0.5, $p = 0.48$–0.88]. However, for the comparison of N20 + Mo vs. sal + Mo, the contrast analysis revealed $F(1, 44) = 11.6$, $p = 0.001$. This can be considered significant, as it is well below the corresponding critical p value of 0.0125 for four comparisons. Thus, L-NOARG when given at 20 mg/kg reliably attenuated the effect of morphine.

DISCUSSION

In accordance with previous studies, morphine given 3 mg/kg, SC, induced reliable CPP (11,14). This effect of morphine was significantly attenuated by NO synthase inhibitor L-NOARG given at 20 mg/kg, IP. L-NOARG itself appeared to have no reliable affect on place conditioning.

Because in our apparatus most of the rats prefer one particular (i.e., the gray) compartment, we ran the biased type of place conditioning (i.e., drug treatment was paired with the less preferred side). Such a type of procedure has been a matter for discussion, as antiaversive rather than rewarding prop-

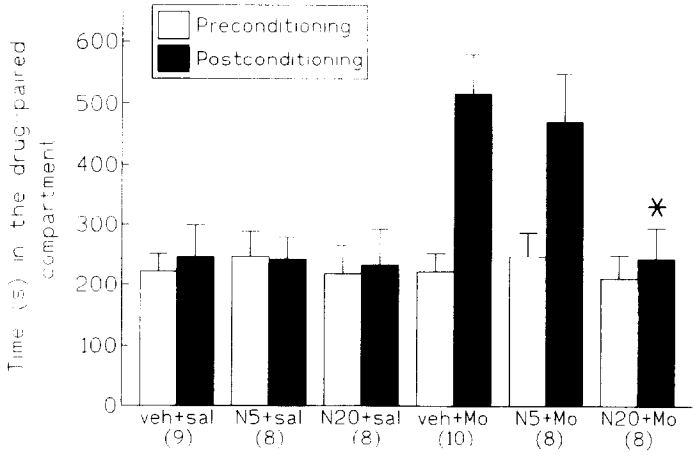

FIG. 1. Effect of NO synthase inhibitor L-NOARG on place preference induced by morphine (3 mg/kg, SC). The columns depict the mean ± ± SEM time spent in the initially nonpreferred (i.e., drug-paired) compartment during pre- and postconditioning tests (open and closed columns, respectively). The number of animals is given in brackets. sal, Saline; veh, vehicle; Mo, morphine; N5, L-NOARG 5 mg/kg, IP; N20, L-NOARG 20 mg/kg, IP. *$p < 0.05$ compared with the group veh + Mo (contrast analysis with Bonferroni adjustment).

erties of a given drug may be regarded as determinative (14). An optimal way has been proposed to counterbalance the drug treatment between the nonpreferred and preferred sides (3). With morphine, however, the experiments comparing the biased and counterbalanced procedures have provided consistent results (1,7).

The nature of L-NOARG's influence on morphine-induced CPP is a rather perplexing question, because several processes may underlie this effect. The acquisition of CPP thus involves both mnemonic and motivational components, which can be manipulated separately (15). Since NO is involved in long-term potentiation (12), L-NOARG may have impaired the acquisition of CPP as a result of its impact on mnemonic processes rather than interference with motivational properties of morphine. The role of NO in different forms of learning and memory, however, is still somewhat problematic. In the study by Bohme et al. (2), L-NOARG in the dose 25 mg/kg (i.e., similar to the one effective in the present study) given IP over 4 days was ineffective both in impairing radial-maze learning in rats and blocking LTP in ex vivo prepared hippocampal slices. The same dose almost totally inhibits brain NO synthase activity (10). This suggests that the observed inhibition of morphine-induced CPP cannot be explained solely by the impairment of mnemonic processes, and hence, our finding may have been based on the changes in the motivational properties of morphine.

Brain dopamine has been proposed to be a common neural substrate mediating the rewarding properties of different classes of abused drugs including opioids (16). NO release-inducing agents sodium nitroprusside and L-arginine have been shown to enhance the release of dopamine in striatal slices (17). However, as far as the reward process is concerned, the inhibition of NO synthesis failed to affect dopamine-dependent lateral hypothalamic brain stimulation reward (4). The role of dopamine in opioid reinforcement is, in fact, a fairly obscure question, and several studies refer to the existence of dopamine-independent components [e.g., (9)]. Hence, supposing that there is no commitment of NO in the dopamine-related reward process, it is possible that NO is involved in dopamine-independent mechanisms of opioid reward.

There are, however, alternative explanations for our finding, which cannot be excluded on the basis of the present study. First, L-NOARG may have altered the pharmacokinetics of morphine as a result of its vascular effects. Behavioural results suggest that the pharmacokinetic area under the curve of cocaine was unaffected by the inhibition of NO synthase (4); yet, it does not imply morphine. Second, L-NOARG may have interfered with the effect of morphine as a result of the inhibition of locomotor activity (13). However, at least the acute effect of the drug on motor behaviour may be ruled out, as the postconditioning test was carried out 24 h after the last L-NOARG administration. Moreover, the decreased locomotor activity has been shown to enhance the expression of morphine-induced CPP (8). Third, L-NOARG could have interfered with morphine-induced CPP as a result of some of its (possibly peripheral) aversive properties. In the present study, this effect could have been dampened by a possible floor effect, because the biased type of CPP was used. However, in our recent experiments, L-NOARG, when paired with the initially preferred compartment, failed to have any reliable place conditioning effect (unpublished results).

In conclusion, the main finding of the present study is that NO synthesis inhibition antagonizes the rewarding effects of morphine as revealed by the CPP paradigm. Our results refer to the potential involvement of NO in the opioid reward process.

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