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Short communication

Differential effects of nitric oxide synthase inhibitor, 7-nitroindazole, on discriminative stimulus and somatic effects of naloxone in morphine-dependent rats

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Abstract

Our previous report suggested that antagonists acting at NMDA receptors attenuate discriminative stimulus effects of naloxone in morphine dependent rats. Nitric oxide (NO) is a putative second messenger which mediates NMDA receptor activation. The present study evaluated behavioral effects of NO synthase inhibitor, 7-nitroindazole in morphine-dependent rats trained to discriminate 0.1 mg/kg naloxone from saline. 7-Nitroindazole did not significantly affect naloxone's discriminative stimulus effects but decreased naloxone-induced weight loss and abolished expression of several withdrawal signs — diarrhea, scream on touch, tremor and 'wet dog'-like shaking suggesting different mechanisms for subjective and somatic components of opioid withdrawal. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Naloxone; Morphine withdrawal; Nitric oxide (NO) synthase inhibitor; Drug discrimination; Y-maze; (Rat)

1. Introduction

Antagonists acting at NMDA subtype of glutamate receptors were repeatedly demonstrated to attenuate severity of opioid withdrawal syndrome (Herman et al., 1995). Subjective effects of morphine withdrawal may be attenuated by some NMDA receptor antagonists (Higgins et al., 1992; Popik and Danysz, 1997; Medvedev et al., 1998). Nitric oxide (NO) is a putative second messenger which mediates NMDA receptor activation, and accordingly NO system seems to be involved in the expression of opiate withdrawal syndrome (Adams et al., 1993; Vaupel et al., 1995; Vaupel et al., 1997).

The purpose of the present study was to evaluate behavioral effects of relatively selective inhibitor of the neuronal constitutive isoform of NO synthase, 7-nitroindazole (Moore et al., 1993) in morphine-dependent rats trained to discriminate naloxone from saline. 7-Nitroindazole was previously reported to share the ability of glutamate receptor antagonists to block development of morphine tolerance and attenuate somatic withdrawal syndrome while possessing no effect on blood pressure (Vaupel et al., 1997).

2. Materials and methods

2.1. Subjects

Ten adult experimentally naive male Wistar rats (''Rappolovo'', St. Petersburg, Russia) were used. Animals were kept individually in plastic cages with water available ad libitum. Food (standard rodent chow from ''Volossovo'', St.Petersburg, Russia) consumption was restricted to 14–18 g/day in order to maintain subjects' body weights under 350 g. All experiments were conducted during the light period of a 12/12 h day–night cycle (08:00–20:00 h).

2.2. Apparatus

The Y-maze was as described in our previous report (Medvedev et al., 1998) except for some modifications

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such as automatic guillotine doors in front of the escape and start areas and photo beams (2 cm off the floor level) to detect the rat location (i.e., runway, start or escape). The apparatus was PC-controlled through a custom-made interface.

2.3. Morphine dependence

Rats were injected subcutaneously (s.c.) with 15 mg/kg of morphine twice a day at 11:00 and 17:00. Administration of naloxone to rats that were repeatedly exposed to morphine for 4–6 weeks resulted in a dose-dependent weight loss, diarrhea, expression of forelimb tremor, ptosis, and increased frequency of 'wet dog'-like shakes and stretches (Medvedev et al., 1998).

2.4. Discrimination training

The details of the procedure were published elsewhere (Medvedev et al., 1998). Briefly, rats were trained to run from the start area to one of the escape areas to avoid or escape from the electric shocks. Each training session consisted of three trials. At the start of each trial, rat was placed into the start area and the beginning of a trial was signaled 5 s later by the opening of the guillotine door. Starting 0–60 s later (the value was randomly assigned by a computer for each trial), the maze floor was electrified everywhere except for one of the escape areas (1.5 mA, 1-s trains of positive pulses every 3 s). As soon as the rat entered the appropriate escape area, sound generator (70 dB, frequency range 1–3 kHz) was turned on for 1 s and all three guillotine doors were closed. One minute later, a new trial was initiated by removing the rat from the escape area and placing it again in the start area. Rats were returned to the home cage after the completion of the third daily trial. All training and subsequent test sessions were held daily (Monday–Sunday) between 13:00 and 14:00, i.e., 2 to 3 h after the first daily morphine injection.

All discrimination training sessions began 10 min after the s.c. injection of saline or 0.1 mg/kg of naloxone. Daily saline (S) and naloxone (N) injections were given according to two monthly alternating sequences.

Acquisition training proceeded until the escape area selection corresponded to the preceding treatment condition on all trials during 10 consecutive training days. Test days were held provided that during at least two most recent saline and two most recent naloxone training sessions the escape area selection corresponded to the preceding treatment condition on all trials. At least three training sessions intervened between consecutive tests. On test days single-trial sessions were held and the floor was not electrified at any time allowing rats to choose any of the escape areas. The test trial was terminated when rat chose one of the escape areas or after 180 s, whichever came first.

During the training and test trials, the following parameters were recorded: selection of the escape area, response latency, body weight changes within 4 h after the naloxone treatment, as well as presence of diarrhea, ''wet dog''-like shaking, stretching, forelimb tremor, ptosis, scream on touch, chewing, and salivation recorded for 15-min period immediately after the naloxone administration.

2.5. Drug tests

During each test, there were two injections given: one i.p. with 7-nitroindazole (vehicle, 10–100 mg/kg, pre-session injection time 30 min), and one s.c. with either naloxone (0.032, 0.056 or 0.1 mg/kg, pre-session injection time 10 min) or saline ($N = 6$ for each drug combination).

2.6. Data analysis

Composite index of the somatic withdrawal (withdrawal score) was calculated individually for each rat based on the expression of forelimb tremor, ptosis, diarrhea, salivation, scream on touch, chewing and shaking. Presence and absence of each sign were counted as '1' and '0', respectively. Accordingly, the individual withdrawal score could assume any integer value between '0' and '5'.

Data were analyzed using SAS-STAT software (version 6.11, SAS Institute, Cary, NC). Withdrawal signs and weight loss data were subjected to the distribution-free one- and two-factorial analysis of variance (ANOVA) with repeated measures. Naloxone-arm selection data were analyzed using probit analysis adjusted for repeated measures design. Tukey's test for gradual data and Fisher's exact test for quantal data were used wherever between-group pairwise comparisons were needed.

2.7. Drugs

The following drugs were used: morphine hydrochloride (''Endokrinnyj Zavod'', Moscow, Russia), naloxone hydrochloride (Research Biochemicals International, Natick, MA), 7-nitroindazole (Sigma, St. Louis, MO). Morphine and naloxone were dissolved in physiological saline, 7-nitroindazole — in dimethylsulfoxide. All injections delivered a solution in a volume of 1 ml/kg. Doses are based upon the forms of the drugs listed above.

3. Results

Naloxone dose-dependently generalized from the training dose (Table 1), with ED_{50} of 0.044 mg/kg (confidence limits: 0.021–0.075 mg/kg). The percentage of rats selecting naloxone-appropriate escape area was higher with the

Table 1
Effects of 7-nitroindazole in morphine-dependent rats

7-NI[a] (mg/kg)	NLX[b] (mg/kg)	% of NLX responses[c]	Latency[d] (s)	Body weight change[e] (%)	Withdrawal score[f]
Vehicle	Saline	0	12.5 ± 5.6	0.7 ± 0.5	0.0 ± 0.0
Vehicle	0.032	50	16.5 ± 6.1	−1.9 ± 0.3	2.2 ± 0.5
Vehicle	0.056	80	17.7 ± 12.1	−3.0 ± 0.3	3.8 ± 0.5
Vehicle	0.1	100	9.4 ± 3.5	−4.4 ± 0.3	5.0 ± 0.3
10	Saline	17	50.3 ± 22.2	1.4 ± 1.3	0.3 ± 0.2
10	0.032	33	64.2 ± 46.6	−1.2 ± 1.1	1.3 ± 0.3
10	0.056	50	49.7 ± 34.2	−0.4 ± 1.2	1.8 ± 0.2[g]
10	0.1	50	21.0 ± 17.2	−2.4 ± 0.9[g]	0.7 ± 0.3[g]
30	Saline	0	40.1 ± 30.8	−0.5 ± 0.9	0.0 ± 0.0
30	0.032	33	22.1 ± 13.2	−1.4 ± 0.7	1.2 ± 0.3
30	0.056	83	43.1 ± 17.9	−0.8 ± 1.3	1.3 ± 0.2[g]
30	0.1	100	18.6 ± 6.4	−2.2 ± 1.3	1.7 ± 0.4[g]
100	Saline	67	83.2 ± 14.0[g]	0.9 ± 0.7	0.7 ± 0.2
100	0.032	75	124.7 ± 18.4[g]	−0.9 ± 0.8	0.8 ± 0.4[g]
100	0.056	60	127.3 ± 16.2[g]	−1.0 ± 0.3[g]	1.2 ± 0.2[g]
100	0.1	67	87.8 ± 23.1[g]	−2.3 ± 0.6[g]	1.0 ± 0.4[g]

[a] 7-NI = 7-nitroindazole.
[b] NLX = naloxone.
[c] Percentage of the naloxone-appropriate escape area selection (% animals per group).
[d] Mean (±S.E.M.) response latency.
[e] Mean (±S.E.M.) body weight change.
[f] Mean (±S.E.M.) withdrawal score (composite of forelimb tremor, ptosis, diarrhea, salivation, scream on touch, chewing and shaking).
[g] $p < 0.05$ (Tukey's test for response latency, body weight change and withdrawal score), compared to 'Vehicle' tests.

increase in naloxone dosage ($\chi^2 = 5.5$, $P < 0.05$). Naloxone administration did not affect escape area selection latencies ($F(3,15) = 0.2$, n.s.). Magnitude of naloxone-induced weight loss change as well as the total score of withdrawal signs were dependent upon the naloxone dose ($F(3,15) = 13.0$, $P < 0.01$, $F(3,15) = 24.2$, $P < 0.01$, respectively).

7-Nitroindazole did not produce appreciable levels of naloxone-appropriate escape area selection and did not significantly affect naloxone's discriminative stimulus effects when combined with various doses of naloxone ($\chi^2 = 0.8$, $P = 0.37$). Highest doses of 7-nitroindazole produced severe behavioral toxicity and on several occasions rats could not complete test trials. Such motor impairment was also evidenced by significantly prolonged latency to select escape area (Table 1; $F(3,91) = 7.2$, $P < 0.01$). At high dose levels 7-nitroindazole appeared to partially substitute for naloxone (66% at 100 mg/kg; $n = 3$). Similar degrees of intermediate responding were observed at certain 7-nitroindazole + naloxone combinations (e.g., 50% of rats selecting naloxone escape area after pretreatment with 10 mg/kg of 7-nitroindazole and the training dose of naloxone).

Pre-treatment with 7-nitroindazole attenuated naloxone-induced weight loss (Table 1; main effect of 7-nitroindazole dose: $F(3,91) = 1.2$, $P = 0.36$; naloxone by 7-nitroindazole interaction: $F(9,91) = 2.8$, $P < 0.05$) and almost completely blocked expression of several withdrawal signs — diarrhea, scream on touch, tremor and shaking (Table 1; 7-nitroindazole dose: $F(3,91) = 14.7$, $P < 0.01$; naloxone by 7-nitroindazole: $F(9,91) = 3.6$, $P < 0.01$).

4. Discussion

The main finding of the present study is that 7-nitroindazole failed to significantly modify discriminative stimulus effect of naloxone in morphine-dependent rats. These data may be viewed as an evidence against the involvement of NO pathways in the effects of glutamate receptors mediating subjective state induced by morphine withdrawal. The present findings are in apparent contrast with the earlier reports on 7-nitroindazole-induced blockade of somatic signs of opioid withdrawal syndrome (Vaupel et al., 1997). In line with this evidence, the present study revealed inhibitory influences of 7-nitroindazole on naloxone-induced weight loss and somatic withdrawal score. It should also be noted that NO donors are capable of inducing the so called 'quasi abstinence syndrome' (e.g., increased shaking activity) and that the severity of the somatic component of morphine withdrawal can be intensified by NO synthase substrate, L-arginine methyl ester (Adams et al., 1993).

In this study, when combined with different doses of naloxone 7-nitroindazole produced only non-significant decrements in percentages of rats selecting naloxone-ap-

propriate escape areas. These effects lacked clear dose-dependence and their magnitude suggested rather non-specific interactions between 7-nitroindazole and naloxone cue. Recent report by Jewett et al. (1996) demonstrated that 7-nitroindazole generalized from phencyclidine in pigeons trained to discriminate phencyclidine from saline. Similar to phencyclidine-like NMDA receptor blockers producing intermediate levels of training drug-appropriate responding in drug discrimination studies (Koek et al., 1993), one may suggest that specific interoceptive stimulation following the administration 7-nitroindazole may overshadow the discriminative stimulus effects of naloxone and thus result in apparent reduction in stimulus control over behavior.

Overall, tests with 7-nitroindazole demonstrated the dissociation between naloxone's discriminative stimulus effects and somatic withdrawal signs since pre-treatment with 7-nitroindazole had no appreciable effects on discriminative stimulus effects of naloxone while attenuated naloxone-induced weight loss and almost completely blocked expression of several withdrawal signs — diarrhea, scream on touch, tremor and shaking. Taken together with numerous previous reports on the ability of NO synthase inhibitors to attenuate somatic and non-specific behavioral symptoms of opiate withdrawal (Vaupel et al., 1995; Vaupel et al., 1997), the present results provide further evidence for different mechanisms underlying subjective and somatic components of opioid withdrawal (Higgins and Sellers, 1994).

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