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BLOCKING OF ENHANCED SENSITIVITY TO BEHAVIORAL EFFECTS OF NALOXONE INDUCED BY NARCOTIC AGONISTS IN RATS

RAKA JAIN* AND RAJAT RAY

Department of Psychiatry,
National Drug Dependence Treatment Centre,
All India Institute of Medical Sciences,
New Delhi – 110 029

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Abstract : The present experiment evaluated whether prior treatment with naloxone could block the sensitization to opiate antagonist induced by single dose administration of pure agonist (morphine) or mixed agonist (buprenorphine). Food deprived male Wistar rats were trained to respond for food on a multiple-trial, fixed-interval 3 min schedule. Reinforcement was contingent upon a response within a 10-s limited hold period following a fixed-interval of 3 min. A trial consisted of three fixed interval of 3 min separated by a 10 min timeout period during which responses were not reinforced. The rate decreasing effects of the opioid antagonist naloxone was determined by cumulative dosing. Pretreatment with morphine (0.3 mg/kg, SC) and buprenorphine (0.03 mg/kg, SC) resulted in an increase sensitivity to the rate decreasing effect of naloxone compared to saline pretreatment. Administration of naloxone (0.3 mg/kg) 10 min prior to pretreatment doses of buprenorphine (0.03 mg/kg; 1.0 mg/kg) and morphine (0.3 mg/kg) increased sensitization to naloxone. However, greater sensitization was observed at low dose of buprenorphine. The increased sensitivity was partially blocked at high dose of buprenorphine (1.0 mg/kg) by naloxone pretreatment. These results suggest that the doses of naloxone used to block opioid induced sensitization might be different from those required in animals with normal sensitivity to opioid antagonists. Further agonist-induced sensitization to behavioral effects of opioid antagonist appears to be opioid receptor specific.

Key words : naloxone morphine buprenorphine operant behavior sensitization

INTRODUCTION

The pure opioid antagonists have relatively few effects on non-dependent subjects, but can produce behavioural effects on their own at much higher dose even on drug free subjects (1, 2). Following chronic exposure to morphine, the administration of opioid antagonists elicits effects related to the precipitation of opioid withdrawal. Earlier studies have, however, shown naloxone precipitated signs of opioid

*Corresponding Author : Tel. No. : 91-11-26593595, Fax : 91-11-26588663, Email : rakajain2001@yahoo.com

withdrawal which can be observed after a single exposure to morphine (3–6). Such a phenomenon is termed as 'acute sensitization'. These are enhanced following a second morphine exposure after 24 hours (7). The magnitude of physical dependence has been assessed by the severity of gross behavioural and physiological changes following antagonist administration as well as the degree of sensitivity to antagonists (8, 9). Thus, sensitization to the opioid antagonists is commonly used as an index to measure physical dependence on opioid (10).

Studies have shown that severity or withdrawal also depends upon the dose of naloxone (4), the dose of opiate agonist (3) and the interval between opiate (agonist) pretreatment and antagonist administration (5, 11, 12). Antagonist sensitization (enhanced sensitivity) can be observed with repeated high dose administration (cumulative dosing) even in the absence of agonist pre-treatment (13). Suppression of food-reinforced operant response behaviour is also a sensitive index for detecting opioid withdrawal changes. For example, operant behavior is attenuated at lower doses of antagonists than those required to produce other signs of withdrawal, such as weight loss (14, 15). Rates of food-reinforced response are suppressed by naloxone administration, thus, rate-decreasing effects have been used to study sensitization to antagonists (16). Sensitization to the effects of opioid antagonists appears to be mediated largely through μ receptor (17, 18). Pretreatment with δ or κ receptor agonists produces far less sensitization than those mediated by administration of mu-receptor agonist like morphine (10). In addition, morphine induced sensitization is greatest for those antagonists having the highest affinity for μ opioid receptor (19). However, such an evidence following administration of non-specific agonist like buprenorphine is unclear.

The aim of the present study was to evaluate whether prior treatment with naloxone could block the sensitization to opiate antagonist induced by single administration of pure agonist (morphine) or mixed agonist (buprenorphine). Studies on acute sensitization following cumulative dosing of naloxone now provide valuable insight to understand the mechanism of development of acute physical dependence.

METHODS

Subjects

Eight male adult albino Wistar strain, drug naïve rats (Bred at Experimental Animal Facility, All India Institute of Medical Sciences, New Delhi, India), weighing 120–150 g were used in the study. The rats were housed individually in plastic cages in a temperature-controlled room with 12 : 12 h light : dark cycle at Experimental Animal Facility of the All India Institute of Medical Sciences, New Delhi. Rats were food deprived to approximately 80% of their free feeding weight by restricted feeding of rat chow (Purina Mills, St. Louis, Mo.). Water was available, continuously in the home cages throughout the experiment. The mean group body weight was 140 g at the

beginning of the experiments and 165 g at the end.

Apparatus

Experiment was conducted in two standard operant chambers (24 × 30 × 33 cm; Coulbourn Instruments, Lehigh Valley, Pa.) each housed in a ventilated, sound-attenuating cubicle. Chambers were equipped with a single lever, food receptacle and house light located on one wall. Session events and data collection was controlled via computer program run on a desktop microcomputer (IBM compatible PC).

Behavioral training and testing

Training of rats were based on procedures used by White – Gbadedo and Holtzman (19). Rats were trained to press the liver for food reinforcement (45 mg pellets: Bioserve Inc; French – town, N.J.) on a fixed interval 3 min schedule with a 10-s limited hold. The schedule delivered food reinforcement following the first response after a 3 min time period had elapsed provided the response was made within 10-s, otherwise that opportunity for food was lost and the next interval began. Once animals consistently responded throughout a 1-hr session, the multiple trial procedure was introduced. Response periods of three fixed intervals (with 10–s limited hold) was preceded by time out periods that were gradually increased to 5–min. During the time-out, the house – light was off and responses had no programme consequences. Commencement of the response period was signaled by the illumination of the house – light and the automatic delivery of a food pellet. Light cues were used for conditioning the rats. Rats were trained 6 days per week for two to four trials, each trial consisting of both a time out and a response period. Drug tests began when animals had acquired stable performance. After training each rat was pretreated with saline or with drug dose combinations as under :

1) Saline
2) 0.3 mg/kg naloxone + Saline
3) 0.3 mg/kg naloxone + 0.3 mg/kg morphine
4) 0.3 mg/kg naloxone + 0.3 mg/kg buprenorphine
5) 0.3 mg/kg naloxone + 1.0 mg/kg buprenorphine
6) Saline + 0.3 mg/kg morphine
7) Saline + 0.3 mg/kg buprenorphine

All doses refer to free base. Each rat serves as its own control. The saline and drug dose combinations were tested in a Latin Square design. In the current study naloxone was used along with other combination drugs like morphine and buprenorphine in variable dosing. Naloxone was given 10 min before morphine or buprenorphine administration prior to testing. Subsequently, cumulative dosing (0.01, 0.03, 0.1, 0.3, 1.0, 3.0 mg/kg) procedure was used 4 hrs. later to generate a naloxone dose – effect curve in the rest session. Cumulative dosing continued until response rates decreased to less than 10% of control for two consecutive trials or after four trials were over. Cumulative dosing procedure enables determination of an entire dose response function in a single test session. Immediately just before the start to

cumulative dosing of naloxone each rat received n-saline and placed in the experimental chamber. After each test drug dose combination each rat was put up back for training till the stable response was achieved for three consecutive days. During training each rat received saline injection. If this criteria was not met, the drug test was postponed until the next week. Each rat was tested with drug administration not more frequently than once a week.

Drugs

Pharmaceutical preparations of naloxone hydrochloride (David Bull Laboratories, Australia), morphine hydrochloride (Civil Drug Laboratories, Delhi) and buprenorphine (Rusan Pharma Ltd. Mumbai) were used in the study. All drug doses were administered in a volume of 1 ml/kg body weight with doses expressed as the free base.

Data analysis

Control data were obtained weekly by averaging response rates over trials conducted on non-injections days preceding test days. A two way non-parametric (Friedman) ANOVA with post-hoc analysis was performed for the significance of within the groups and Wilcoxon's Rank Sum test to see the difference between the groups. P values 0.05 has been considered as statistical significance level. Data was analyzed using the Biomedical data processing (BMDP) statistical package (Version 7.0).

RESULTS

Response rates averaged 31.96 responses per minutes for the eight rats used in the study. Descriptive Statistics of the eight rats for naloxone following various pretreatment

TABLE I: Descriptive statistics of rate-reducing effects of the eight rats for naloxone following various pretreatment drugs.

Pretreatment drugs	*Mean±SD*	*Mean±SD of Naloxone doses (mg/kg)*					
	Saline	0.01	0.03	0.1	0.3	1.0	3.0
Saline	33.70 (24.78)	43.32 (26.64)	40.32 (28.93)	44.13 (19.49)	43.82 (19.49)	32.34 (19.77)	24.168* (16.00)
0.3 NX+ Saline	30.28 (22.23)	29.11 (21.29)	33.49 (23.23)	28.21 (22.49))	24.69 (20.18)	22.65* (18.32)	15.76* (14.81)
0.3 NX+ 0.3 Mor	14.97 (16.18)	18.11 (18.63)	13.45 (15.59)	14.15 (13.83)	11.98 (14.33)	9.69* (9.82)	3.72* (4.63)
3.0 NX+ 0.03 Bup	1.38 (2.80)	2.75 (5.36)	6.48 (9.07)	4.22 (6.29)	3.39 (6.99)	1.97 (3.54)	1.31* (2.57)
0.3 NX+ 1.0 Bup	7.49 (11.71)	8.96 (16.10)	7.95 (14.01)	12.35 (23.62)	10.24 (15.08)	10.02 (15.04)	6.01* (10.75)
Saline+ 0.3 Mor	34.07 (26.75)	24.68 (21.60)	24.98 (21.48)	22.63 (22.55)	17.74* (16.63)	11.92* (18.52)	12.55* (20.61)
Saline+ 0.03 Bup	17.05 (18.80)	20.60 (25.60)	25.66 (26.05)	20.22 (24.28)	18.40 (13.81)	15.60 (14.72)	10.07* (9.81)

*P<0.05

drugs is shown in Table I. Fig. 1A, shows the comparison of sensitivity to the rate reducing effect of naloxone following pretreatment with drugs (saline, 0.3 mg/kg naloxone + saline, 0.3 mg/kg naloxone + 0.3 mg/kg morphine and saline + 0.3 mg/kg morphine). Dose – effect curves of naloxone showed no significant difference between pretreatment doses of saline and saline + 0.3 mg/kg naloxone. Pretreatment with morphine (0.3 mg/kg) resulted in increased sensitivity to the rate reducing effect of naloxone as compared to saline and was found to be significant at all doses of naloxone dose response curves (0.01, P=0.038; 0.03, P=0.05; 0.1, P=0.015; 0.3, P=0.00; 1.0, P=0.015; 3.0, P=0.005). Sensitization to naloxone was increased by administration of naloxone (0.3 mg/kg) 10 min prior to treatment doses of morphine (0.3 mg/kg).

The rate – reducing effect of naloxone following pretreatment with saline, 0.3 mg/kg naloxone + saline, 0.3 mg/kg naloxone + 0.3 mg/kg buprenorphine, 0.3 mg/kg naloxone + 1.0 mg/kg buprenorphine, saline + 0.3 mg/kg buprenorphine) is shown in Fg. 1B. Pretreatment of low dose (0.3 mg/kg) of buprenorphine caused a significant dose-related decrease in response rates of naloxone doses (0.01, P=0.001; 0.03, P=0.005; 0.1, P=0.001; 0.3, P=0.00; 1.0, P=0.001; 3.0, P=0.001) and (0.01, P=0.001; 0.03, P=0.015; 0.1, P=0.007; 0.3, P=0.005; 1.0, P=0.002; 3.0, P=0.021) as compared to saline as well as to 0.3 mg/kg saline – naloxone pretreatment respectively. Similarly, pretreatment with high dose (1.0 mg/kg) of buprenorphine caused a significant dose-related decrease in response rates of naloxone doses (0.01, P=0.005; 0.03, P=0.010; 0.1, P=0.028; 0.3, P=0.005; 1.0, P=0.021; 3.0, P=0.015) as compared to saline whereas significant difference of naloxone dose effect was only seen at two doses (0.01, P=0.028; 0.03, P=0.010; 0.1, P=0.105; 0.3, P=0.065; 1.0, P=0.083; 3.0, P=0.065) when compared with saline – 0.3 mg/kg naloxone pretreatment. Thus, these results indicate that sensitization to naloxone further resulted in enhanced sensitivity to the rate reducing effect of naloxone by administration

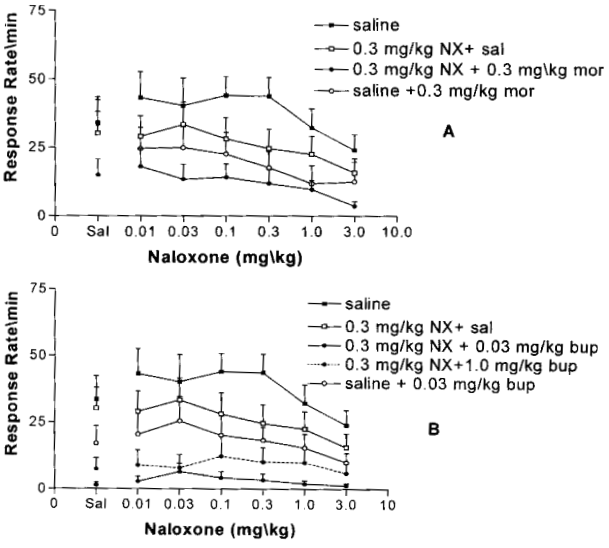

Fig. 1 : Comparison of sensitivity to the rate reducing effect of naloxone following pretreatment with **A** saline (closed squares) and the combination of 0.3 mg/kg naloxone + saline (open squares), 3.0 mg/kg naloxone + 0.3 mg/kg morphine (closed circle), saline + 0.3 mg/kg morphine (open circles); **B** saline (closed squares) and the combination of 0.3 mg/kg naloxone + saline (open squares), 0.3 mg/kg naloxone + 0.03 mg/kg buprenorphine (closed circle), 0.3 mg/kg naloxone + 1.0 mg/kg buprenorphine (closed circles with dotted lines) saline + 0.03 mg/kg buprenorphine (open circles). All pretreatment doses were administered 4 hrs before cumulative dosing with naloxone. Naloxone was administered 10 min before morphine or buprenorphine administration prior to testing. Each point is a mean ± SEM.

of naloxone (0.3 mg/kg) 10 min prior to treatment doses of 0.03 mg/kg buprenorphine and 1.0 mg/kg buprenorphine. Buprenorphine showed a biphasic effect. Greater sensitization was observed at low dose of buprenorphine (0.03 mg/kg) as compared to higher dose (1.0 mg/kg). Pretreatment with 0.3 mg/kg naloxone did not block the sensitization to naloxone induced by low (0.03 mg/kg) dose of buprenorphine as indicated by the downward displacement in the naloxone dose response curves (Fig. 1B). However, pretreatment with 0.3 mg/kg naloxone partially blocked the sensitization to naloxone (doses 0.1, 0.3, 1.0 and 3.0 mg/kg) induced by high dose of buprenorphine (1.0 mg/kg).

DISCUSSION

In the present study pretreatment with morphine increased sensitivity to the rate-decreasing effect of naloxone in rats responding for food compared with saline pretreatment in rats. This effect was consistent with the earlier reports (20, 10, 21). Administration of 0.3 mg/kg SC dose of naloxone 10 min prior to the treatment dose of morphine (3.0 mg/kg SC) failed to block morphine – induced sensitization to opioid antagonist as indicated in Fig. 1A. The current results do not appear to reflect reports made by others at doses 3.0 mg/kg SC of morphine and 0.3 mg/kg SC of naloxone under the same experimental conditions (19). The lack of blockade by antagonist pretreatment could be that rats used in the present study had no previous history of opioid antagonists treatment and the species of rats was different as used in earlier reports (19). Sensitization to the effects of opioid antagonist was seen 4 h after acute pretreatment of opioid agonist (morphine). The sensitization to opioid antagonists induced by morphine pretreatment is dose and time dependent. In the current study for the sake of consistency and comparability to earlier studies, opioid antagonist was used 4 h after the acute pretreatment of opioid agonist (10). This pretreatment interval results in the maximum sensitization to naltrexone by a single dose of peripherally administered morphine (20). There are also reports of increased sensitivity to naltrexone testing (10, 13, 22) in both primates and rats. Therefore animals in the present study might be less sensitive to opioid antagonists as compared to previous reports (22). Moreover, blockade effects of morphine was studied only at a single dose of naloxone (0.3 mg/kg, SC). The blockade effect with higher dose of naloxone pretreatment is warranted which was not used in the present study. Similarly, sensitization to the rate decreasing effects of opioid antagonists induced by acute pretreatment with buprenorphine a mixed opioid agonist-antagonist, at low (0.03 mg/kg, SC) and high dose (1.0 mg/kg, SC) was observed in the present study (Fig. 1B). These findings are in accordance with previous reports of the acute effects of buprenorphine on food-maintained responding, however, the dose required to effectively suppress food-maintained responding differs among species (23). This is also suggestive of its agonistic property like morphine with regard to rate decreasing effects of schedule-controlled responding. Pretreatment with morphine induced sensitization to rate reducing effect to naloxone was substantially less than with pretreatment with buprenorphine at low dose. These findings

accord well with other studies that indicate buprenorphine is also much more potent analgesic than morphine and its duration of action exceeds that of on a number of other behavioral measures (24, 25). Further, a significant degree of sensitization occurred more at low dose of buprenorphine than at high dose. This may be on account of its partial agonist characteristic at low doses and an antagonist characteristic at high doses (24). Furthermore, buprenorphine has greater binding affinity at μ opioid receptor and has high intrinsic activity, which relates to its agonist property. Administration of 3.0 mg/kg SC naloxone 10 min prior treatment of low dose of buprenorphine 0.03 mg/kg did not block the sensitization to naloxone as indicated by the absence of overlapping in the naloxone dose-response curve of 0.3 mg/kg naloxone + saline. These findings suggest that dose of naloxone was inadequate to block sensitization to rate-decreasing effects of antogonist and it appears that higher dose of naloxone may be required to observe this effect. However, pretreatment with 0.3 mg/kg naloxone, partially blocked the sensitization to naloxone induced by high dose of buprenorphine (1.0 mg/kg), as no significant differences was seen in naloxone dose effect curves. Buprenorphine has been shown to have antagonist activity at κ opioid receptor under a wide range of behavior conditions (26–29). It also does not block the effects of μ agonists on responding maintained by food, but it does antagonize the effects of an antagonist at the κ opioid receptor and complement data collected in other behavioral assays that suggest that buprenorphine has kappa antagonist properties (30). In the current experiment partial blockade was observed at higher doses which may be on account of its κ antagonist property but noting could be said conclusively from the current study.

The most likely conclusion that can be drawn from the present results is that acute sensitization to opioid antagonist induced by opioid pretreatment is an opioid specific. The degree of sensitization differed among opioid agonists, further supporting the hypothesis that the phenomenon reflects initial changes in opioid systems that underlie physical dependence. The dose of naloxone used to block opioid induced sensitization might be different from those required in animals with normal sensitivity to opioid antagonists.

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