ELSEVIER

Pharmacology, Biochemistry and Behavior 70 (2001) 193–197

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

The dopamine antagonist, alpha-flupenthixol, interferes with naloxone-induced place aversion learning, but not with acute opiate dependence in rats

Andrea N. Santi, Linda A. Parker*

Department of Psychology, Wilfrid Laurier University, Waterloo, Ontario, Canada N2L 3C5

Received 27 October 2000; received in revised form 15 May 2001; accepted 7 June 2001

Abstract

Pretreatment with the dopamine antagonist alpha-flupenthixol (alpha-flu) interfered with the establishment of a naloxone-induced place aversion in two experiments. In Experiment 1, the potential of pretreatment with alpha-flu to interfere with the establishment of a naloxone-induced place aversion was evaluated in rats administered morphine (Group MN) or saline (Group SN) 24 h prior to the naloxone conditioning trial. Naloxone-precipitated withdrawal from morphine administered 24 h prior to the trial produced a stronger place aversion than produced by naloxone alone. The neuroleptic, alpha-flu, attenuated the naloxone-induced place aversion, but did not selectively interfere with the place aversion produced by acute opiate dependence. Experiment 2 replicated demonstration of interference with naloxone-place aversion learning by neuroleptic pretreatment with the inclusion of saline controls. These results suggest that dopamine modulates either the aversive motivational properties of naloxone or learning, even in opiate naïve rats. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Dopamine; Antagonist; Alpha-flupenthixol; Neuroleptic; Place aversion; Opiate withdrawal; Conditioning; Acute dependence

1. Introduction

Opiate antagonists, such as naloxone, produce a conditioned place aversion in both morphine dependent (Hand et al., 1988) and non-dependent (Mucha et al., 1982) rats. Dopamine has been reported to modulate the motivational properties of opiate antagonists (Acquas et al., 1989; Bechara et al., 1992, 1995; Shippenberg and Herz, 1988); however, the specific role played by dopamine is the subject of some controversy. The D1 dopamine receptor antagonist, SCH23390, reportedly blocked naloxone-induced place aversions in nondependent rats (Acquas et al., 1989; Shippenberg and Herz, 1988). On the other hand, Bechara et al. (1992, 1995) report that the nonspecific neuroleptic alpha-flupenthixol (alpha-flu) interfered with conditioned place aversions produced by naloxone and spontaneous opiate withdrawal in opiate-dependent rats, but not in opiate-naïve rats.

Opiate withdrawal (an indicator of opiate dependence) is typically produced by either terminating chronic morphine exposure or by administering an opiate antagonist, such as naloxone, to chronically morphine-pretreated humans or rats (Jaffee and Martin, 1990). However, antagonist-precipitated opiate withdrawal may also be observed when naloxone is administered up to several hours after, even, a single dose of morphine, which is called acute opiate dependence (Eisenberg, 1982; Gellert and Sparber, 1977; Heishman et al., 1990; June et al., 1995). The withdrawal is apparent not only by behavioral symptoms of abstinence, but also by the ability of such withdrawal to serve as an aversive motivational stimulus (McDonald and Parker, 2000; Mucha, 1991; Parker and Joshi, 1998). In fact, Parker and Joshi (1998) report that when morphine is administered 24 h prior to each of two conditioning trials with naloxone, rats display a stronger place aversion than that produced by naloxone alone. Since the aversive motivational properties are evident well after the agonist effects of morphine have dissipated, acute morphine administration appears to produce long-lasting changes at the opiate receptor.

* Corresponding author. Tel.: +1-519-884-0710, +1-519-884-3900; fax: +1-519-746-7605.

E-mail address: lparker@wlu.ca (L.A. Parker).

0091-3057/01/$ – see front matter
PII: S0091-3057(01)00594-9

2. Experiment 1

Although Bechara et al. (1992, 1995) suggest that neuroleptics should not interfere with morphine withdrawal in nondependent animals, they did not specifically evaluate the effect of neuroleptic pretreatment on acute morphine dependence. In the present investigation, rats were be administered either alpha-flu or saline 2.5 h prior to naloxone conditioning (according to the procedure of Bechara et al., 1995); half of the rats were pretreated with morphine 24 h earlier to induce acute dependence, the other half were pretreated with saline. The ability of alpha-flu to block naloxone-precipitated withdrawal was assessed following two exposures to morphine and then again after four exposures to morphine.

2.1. Method

2.1.1. Subjects

Subjects were 48 male Sprague–Dawley rats obtained from Charles River Labs, St. Constant, Quebec, weighing approximately 200–225 g upon arrival in the laboratory. They were housed in pairs in polyethylene cages and maintained on a 12:12 h light/dark schedule. Purina Rat Chow and water were provided ad lib throughout the experiment. The rats were handled daily for 1 week prior to Conditioning trial 1.

2.1.2. Drugs

Morphine was given at a dose of 20 mg/kg, alph-flu was given at a dose of 0.8 mg/kg, and naloxone was given at a dose of 1.0 mg/kg. Morphine and naloxone were administered subcutaneously (sc) and alph-flu was administered intraperitoneally (ip). All drugs were mixed with saline and administered at a volume of 1 ml/kg. Saline injections were, also, given in a volume of 1 ml/kg.

2.1.3. Apparatus

The place conditioning apparatus was separated into two equal-sized chambers by a removable wooden divider. Each chamber ($35 \times 25 \times 30$ cm) was painted flat black. The texture of the floor differed between chambers, which provided the conditioning cues. One chamber had wire mesh covering the floor and the other chamber had sandpaper strips covering the floor. During testing, the divider was removed and the rats were allowed access to both chambers. A camera that was mounted to the ceiling recorded the movement of the rat between chambers, and a video-tracking apparatus (Videomex-V, Columbus Instruments, Columbus, OH) determined the time spent in each chamber.

2.1.4. Procedure

There were four groups ($n = 12$/group) that differed on the basis of pretreatment (alpha-flu or saline) and conditioning treatment (morphine–naloxone [MN] or saline–naloxone [SN]). Over the course of the experiment, the rats received a total of four pairings of naloxone with a chamber; they were tested following two pairings and four pairings.

The first test occurred following two cycles of conditioning, with each cycle consisting of 3 days. On the first day of each cycle, rats received an injection of alpha-flu ($n = 24$) or saline ($n = 24$) 2.5 h prior to a second injection of saline. Saline was administered 5 min prior to placement in one of the chambers for 30 min. Half of the rats were placed in the chamber with the sandpaper floor and half were placed in the chamber with the wire mesh floor. On the second day of each cycle the rats were injected with morphine or saline and then replaced in their cage. On the third day of each cycle the rats received an injection of either alpha-flu or saline 2.5 h prior to a second injection of naloxone. The naloxone was injected 5 min prior to placement in the opposite chamber from the first day of the cycle for a duration of 30 min. Twenty-four hours after the completion of the second cycle, the first test was conducted. During testing, each rat was placed in the chamber for 15 min, with the divider between the floors removed, and the amount of time that the rat spent on each floor was measured.

The second test was conducted following another two cycles of conditioning, which began 48 h after the first test The second two cycles of conditioning trials were conducted in a manner identical to that of the first two cycles. On the day following the second cycle, the rats received a second 15-min place preference test. The video-tracking apparatus recorded the time spent in each chamber during each test.

2.2. Results

The mean amount of time (s) spent on the naloxone-paired floor minus the saline-paired floor for Group MN (closed bars) and Group SN (open bars) during the first and second test trial is presented in Fig. 1. As is evident in the figure, Group MN displayed a stronger place aversion than Group SN, reflecting the presence of acute opioid dependence. The difference scores were analyzed as a $2 \times 2 \times 2$ mixed factors analysis of variance (ANOVA), which revealed significant main effects of conditioning treatment [$F(1,44) = 30.19$, $P < .01$] and pretreatment [$F(1,44) = 9.65$, $P < .01$]. Groups MN displayed stronger place aversions than Groups SN and groups pretreated with alpha-flu displayed weaker place aversions than groups pretreated with saline. The analysis did not reveal a significant conditioning treatment by pretreatment interaction [$F(1,44) = 0.17$; $P = .68$], which suggests that alpha-flu did not differentially affect the strength of the naloxone aversion on the basis of prior opiate treatment.

The analysis also revealed significant interactions of conditioning treatment by test [$F(1,44) = 5.39$, $P < .025$]. Analysis of simple main effects revealed that Group MN displayed a stronger place aversion than Group SN on both Test 1 [$F(1,46) = 12.8$; $P < .001$] and on Test 2

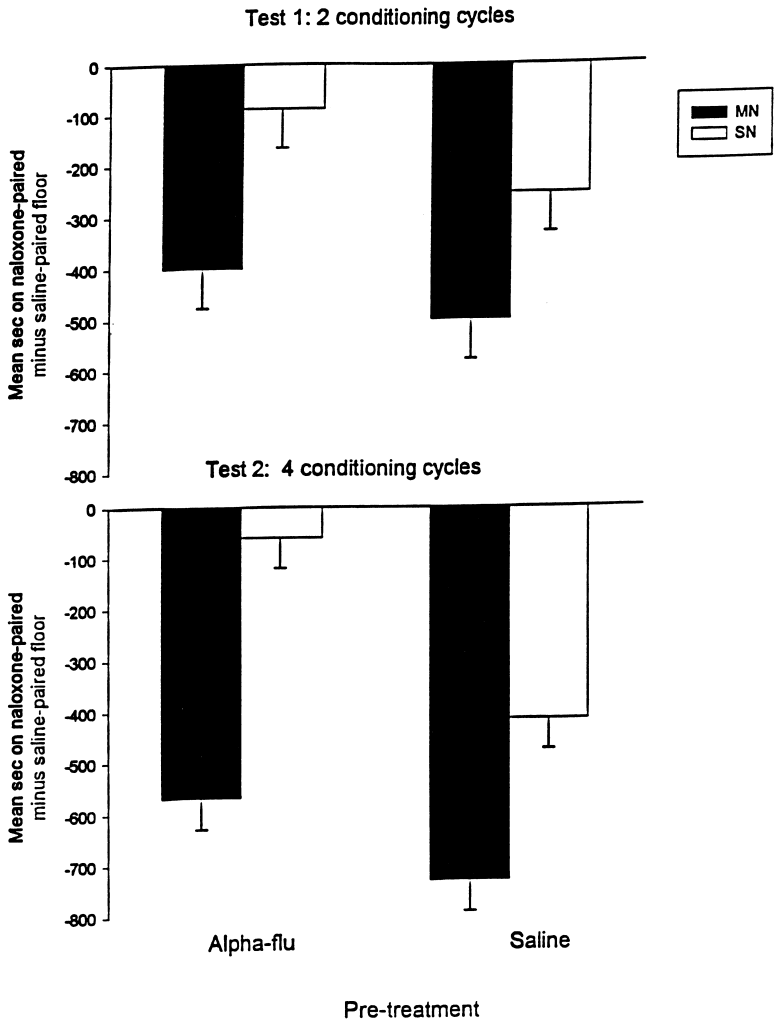

Fig. 1. Mean time (s) morphine- and saline-treated rats visited naloxone-paired floor minus saline-paired floor given a pretreatment of alpha-flu or saline during place conditioning after two and four conditioning trials.

[$F(1,46) = 33.5$; $P < .001$]. The analysis also revealed a significant pretreatment by test interaction [$F(1,44) = 4.87$; $P < .05$]. Analysis of simple main effects revealed that the group pretreated with alpha-flu displayed a weaker place aversion than the group pretreated with saline on Test 2 [$F(1,46) = 9.14$; $P < .01$], but not on Test 1 [$F(1,46) = 2.37$; $P = .13$]. The three-way interaction was not significant [$F(1,44) = 1.28$; $P = .26$].

2.3. Discussion

Naloxone-precipitated withdrawal from morphine administered 24 h prior to the trial produced a stronger place aversion than that produced by naloxone alone. The neuroleptic, alpha-flu, attenuated the naloxone-induced place aversion, but did not selectively interfere with the place aversion produced by acute opiate dependence. This implies that the dopamine system may be involved in the mediation of the aversive effect of naloxone in rats that are both morphine naïve and those acutely exposed to morphine.

3. Experiment 2

The results of Experiment 1 suggested that the dopamine antagonist alpha-flu interfered with the establishment of a naloxone-induced place aversion regardless of whether the rats had previously been pretreated with morphine (acute dependence) or saline. However, the absence of animals conditioned with saline precluded evaluation of the possibility that alpha-flu completely blocked the establishment of a naloxone-induced place aversion. In Experiment 2, the potential of alpha-flu to interfere with naloxone-induced place conditioning was evaluated with the inclusion of saline control groups.

3.1. Method

3.1.1. Subjects

The subjects were 48 male Sprague–Dawley rats weighing between 262 and 296 g on the first conditioning trial. As in Experiment 1, they were handled daily

for a week prior to the first trial. The rats were maintained identically as in Experiment 1 and the same apparatus was used. The doses of naloxone (1 mg/kg sc) and alpha-flu (0.8 mg/kg ip) were identical to those of Experiment 1.

3.1.2. Procedure

There were four groups ($n = 12$/group) that differed on the basis of pretreatment drug (alpha-flu or saline) and conditioning drug (naloxone or saline). The rats received a total of four pairings of the conditioning drug with a chamber. On the first day of each conditioning cycle, the rats were injected with alpha-flu ($n = 24$) or saline ($n = 24$) 2.5 h prior to a second injection of saline that was administered 5 min prior to placement in one of the chambers for 30 min. Half of the rats were placed in the chamber with the sandpaper floor and half were placed in the chamber with the wire mesh floor. On the second day of each cycle, the rats received an injection of either alpha-flu or saline 2.5 h prior to an injection of either naloxone or saline. Five minutes later the rats were placed in the opposite chamber from the first day of the cycle for 30 min. Forty-eight hours after the completion of the fourth cycle, the rats were tested. During testing, each rat was placed in the chamber for 15 min, with the divider between the floors removed, and the amount of time that the rat spent on each floor was measured. The video-tracking apparatus recorded the time spent in each chamber during the test.

3.2. Results and discussion

The mean amount of time (s) that each pretreatment group spent on the treatment-paired floor minus the non-treatment-paired floor for the groups conditioned with naloxone (dark bars) and the groups conditioned with saline (open bars) is presented in Fig. 2. As is evident in the figure, alpha-flu interfered with a naloxone-induced place aversion. The difference scores were analyzed as a 2×2 analysis of variance (ANOVA), which revealed significant main effects of conditioning treatment [$F(1,44) = 10.73$; $P < .01$, pretreatment, $F(1,44) = 4.13$; $P < .05$] and a significant conditioning treatment by pretreatment interaction [$F(1,44) = 5.45$; $P < .025$]. A simple main effects analysis revealed that among the rats pretreated with saline, naloxone produced a stronger place avoidance than saline[$F(1,22) = 11.44$; $P < .01$]; however, among the rats pretreated with alpha-flu, naloxone did not produce a greater place aversion than saline [$F(1,22) = 0.71$; $P = .41$]. Furthermore, among the naloxone-conditioned rats, saline pretreated rats displayed a greater place aversion than alpha-flu-pretreated rats [$F(1,22) = 6.21$; $P < .025$]; while alpha-flu had no effect on the rats pretreated with saline [$F(1,22) = 0.10$; $P = .76$].

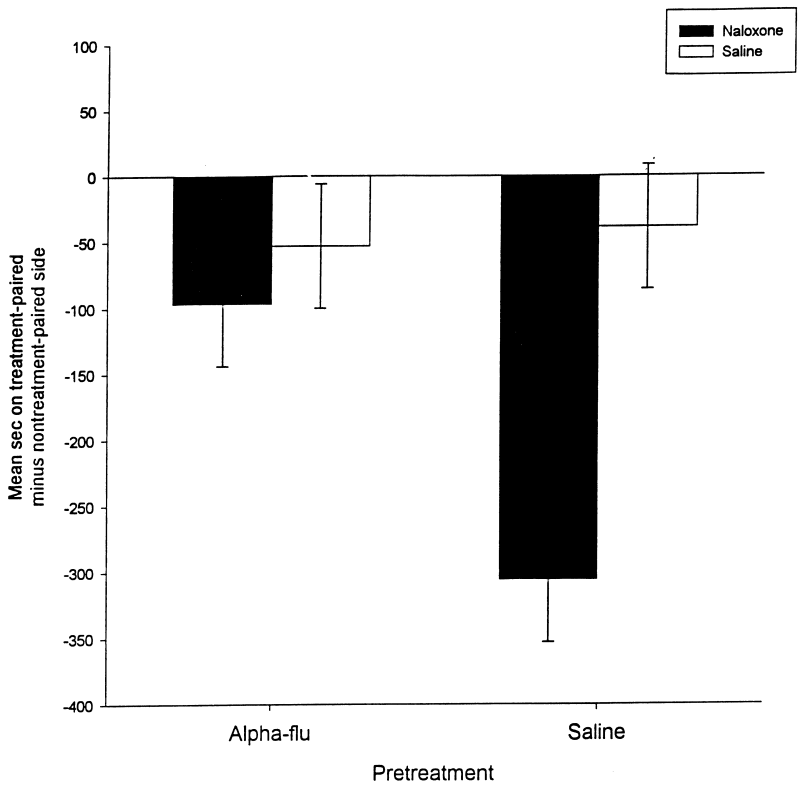

Fig. 2. Mean time (s) in treatment-paired floor minus saline-paired floor following pretreatment with either alpha-flu or saline after four conditioning trials.

The neuroleptic, alpha-flu, completely blocked a four-trial naloxone-induced place aversion.

4. General discussion

In Experiment 1, naloxone-precipitated withdrawal from morphine administered 24 h prior to the trial produced a stronger place aversion than did naloxone alone. The neuroleptic, alpha-flu, attenuated the naloxone-induced place aversion, but did not selectively interfere with the place aversion produced by acute opiate dependence. Experiment 2 replicated this effect with the inclusion of saline-conditioned rats. This implies that the dopamine system is involved in the mediation of the aversive effect of naloxone in rats that are both morphine naïve and those acutely exposed to morphine. Furthermore, in contrast with Bechara et al. (1992, 1995), in rats with no prior morphine experience alpha-flu completely blocked the naloxone place aversion. Our results are consistent with previous research (Acquas et al., 1989; Shippenberg and Herz, 1988) suggesting that the dopamine system is involved in the aversive motivational properties of naloxone. However, the design of the present experiments cannot rule out the possibility that alpha-flu nonspecifically interfered with learning (see Beninger and Miller, 1998).

Acknowledgments

This research was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC-OGP 92057) to L.A.P. and by an NSERC Undergraduate Training Award to A.N.S. We thank Marion Corrick for excellent animal care and Robert Sorge for aid in collecting the data. All procedures were approved by the Wilfrid Laurier Animal Care Committee according to the guidelines of the Canadian Council on Animal Care.

References

Acquas E, Carboni E, Leone P, Di Chiara G. SCH 23390 blocks drug-conditioned place-preference and place-aversion: anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? Psychopharmacology 1989;99:151–5.

Bechara A, Harrington F, Nader K, van der Kooy D. Neurobiology of motivation: double dissociation of two motivational mechanisms mediating opiate reward in drug-naive versus drug-dependent animals. Behav Neurosci 1992;106:798–807.

Bechara A, Nader K, van der Kooy D. Neurobiology of withdrawal motivation: evidence for two separate aversive effects produced in morphine-naive versus morphine-dependent rats by both naloxone and spontaneous withdrawal. Behav Neurosci 1995;109:91–105.

Beninger RJ, Miller R. Dopamine-D1-like receptors and reward-related incentive learning. Neurosci Biobehav Rev 1998;22:335–45.

Eisenberg RM. Further studies on the acute dependence produced by morphine in opiate naïve rats. Life Sci 1982;31:1531–40.

Gellert VF, Sparber SB. A comparison of the effects of naloxone on body weight loss and suppression of fixed-ratio operant behavior in morphine dependent rats. J Pharmacol Exp Ther 1977;201:44–54.

Hand TH, Koob GF, Stinus L, LeMoal M. Aversive properties of opiate receptor blockade are centrally mediated and are potentiated by previous exposure to opiates. Brain Res 1988;474:364–8.

Heishman SJ, Stitzer ML, Bigelow GE, Liebson IA. Acute opioid physical dependence in humans: effects of naloxone at 6 and 24 hours postmorphine. Pharmacol, Biochem Behav 1990;36:393–9.

Jaffee JH, Martin WR. Opioid analgesics and antagonists. In: Gilman AG, Rall TLW, Nies AS, Taylor P, editors. Goodman's and Gillman's: the pharmacological basis of therapeutics. New York: Pergamon Press, 1990. pp. 485–521.

June HL, Stitzer ML, Cone EC. Acute physical dependence: time course and relation to human plasma morphine concentrations. Clin Pharmacol Ther 1995;57:270–80.

McDonald RV, Parker LA. Reinstatement of both a conditioned place preference and a conditioned place aversion with drug primes. Pharmacol, Biochem Behav 2000;66:559–61.

Mucha RF. What is learned during opiate withdrawal conditioning? Evidence for a cue avoidance model. Psychopharmacology 1991;104:391–6.

Mucha RF, van der Kooy D, O'Shaughnessy M, Bucenieks P. Drug reinforcement studied by the use of place conditioning in rat. Brain Res 1982;243:91–105.

Parker LA, Joshi A. Naloxone-precipitated morphine withdrawal induced place aversions: effect of naloxone at 24 hours postmorphine. Pharmacol, Biochem Behav 1998;61:331–3.

Shippenberg TS, Herz A. Motivational effects of opioids: influence of D-1 versus D-2 receptor antagonists. Eur J Pharmacol 1988;151:233–42.