Pharmacology Biochemistry and Behavior, Vol. 61, No. 3, pp. 331–333, 1998

0091-3057/98 $19.00 + .00

ELSEVIER

PII S0091-3057(98)00104-X

Naloxone-Precipitated Morphine Withdrawal Induced Place Aversions: Effect of Naloxone at 24 Hours Postmorphine

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Received 15 August 1997; Revised 24 March 1998; Accepted 5 April 1998

PARKER, L. A. AND J. ANISH. *Naloxone-precipitated morphine withdrawal induced place aversions: Effect of naloxone at 24 hours postmorphine.* PHARMACOL BIOCHEM BEHAV **61**(3) 331–333, 1998.—The aversive properties of naloxone-precipitated withdrawal from acutely administered morphine were assessed using the place-conditioning paradigm. The conditioned place aversion produced by naloxone (0.5–1.0 mg/kg, SC) after two conditioning trials was enhanced by pretreatment with morphine (20 mg/kg, SC) 24 h prior to the conditioning trial. Naloxone precipitated withdrawal from acutely administered morphine produces an aversive motivational state that becomes associated with place cues. © 1998 Elsevier Science Inc.

Morphine Withdrawal Naloxone Precipitated withdrawal Acute dependence Place conditioning Place aversion Learning Rat

MORPHINE withdrawal typically is produced by either terminating chronic morphine exposure or by administering an opiate antagonist to morphine pretreated rats (7). In fact, withdrawal symptoms may be precipitated by an opioid antagonist after only a few widely spaced administrations of an opioid (1–6,8,9–12,19,20); this phenomenon has been called acute physical dependence (10). Results of both animal and human studies indicate that the symptoms of acute physical dependence are qualitatively similar to those seen following long-term opioid exposure (4,8,10). Acute dependence may be observed when naloxone is administered up to several hours after, even, a single dose of morphine (2,4,8,10), which challenges the traditional view that dependence requires chronic exposure to opiates. The withdrawal is apparent not only by behavioral symptoms of abstinence (2,4,8,20), but also by the ability of such withdrawal to serve as an aversive motivational stimulus (11).

Precipitated withdrawal produces both a place and a taste aversion. Mucha reported that that a place aversion (16) and a taste aversion (13) produced by an injection of an opiate antagonist is enhanced in rats implanted with subcutaneous morphine pellets. Recently, McDonald, Parker, and Siegel (11) reported that naloxone-precipitated morphine withdrawal induced 22 h after acutely administered morphine produced conditioned rejection reactions to a flavor with which it was paired. With a 22-h interval, the stimulus properties of morphine have dissipated at the time of the naloxone injection (2,4,7,8), but naloxone is still capable of precipitating withdrawal from acutely administered morphine as measured by its ability to produce taste aversion learning and by its ability to produce somatic withdrawal symptoms (10,19,20).

The purpose of the present experiment was to determine if acute opioid withdrawal can be observed in the place conditioning paradigm when the morphine–naloxone interval is 24 h. On each of two trials, one chamber was paired with naloxone (0.5 or 1 mg/kg, SC) 24 h after an injection of morphine (20 mg/kg, SC) and the chamber preference was assessed.

METHOD

Subjects

The subjects were 77 (42 in Experiment 1a and 35 in Experiment 1b) male Sprague–Dawley rats weighing between 200–224 g upon arrival in the laboratory. They were housed in pairs in plastic cages in a room illuminated on a 12 L:12 D cy-

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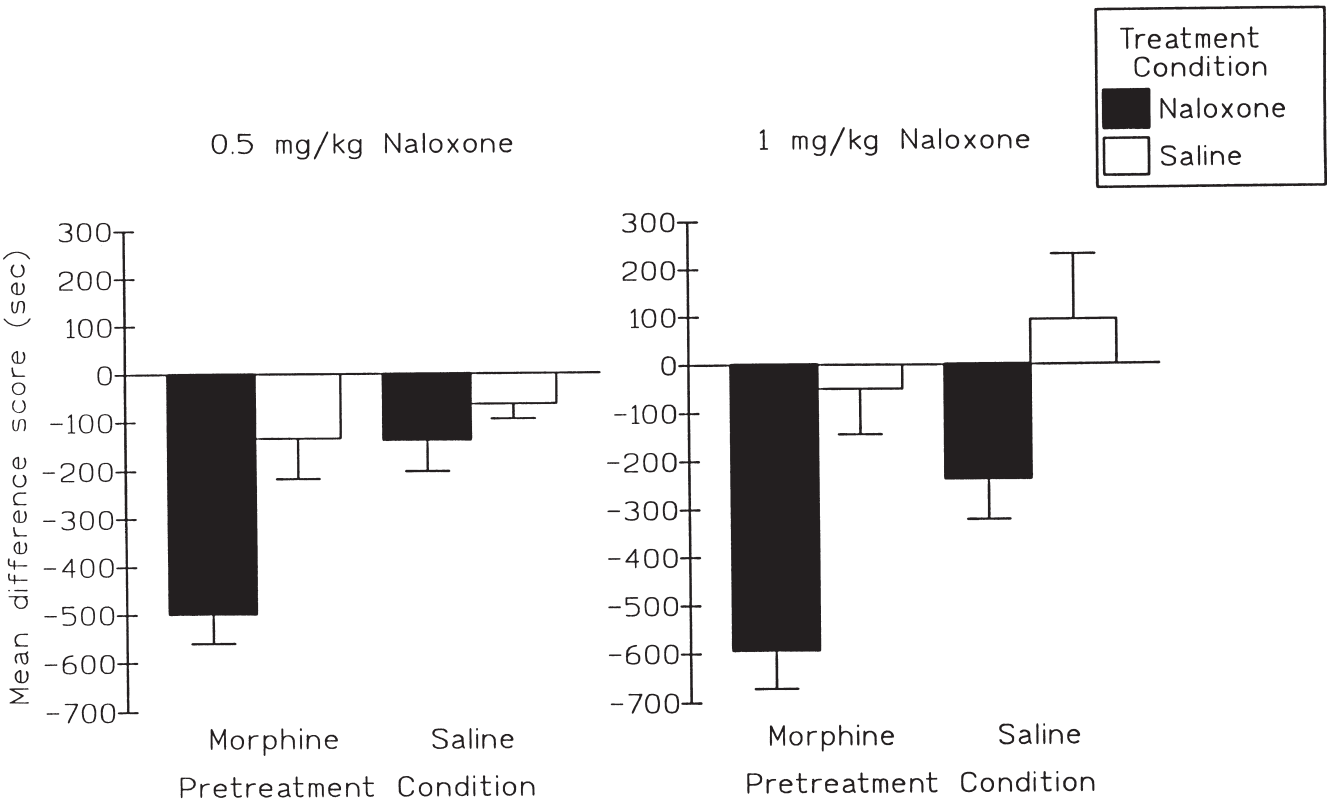

FIG. 1. Mean time (seconds) in treatment-paired chamber minus nontreatment-paired chamber during the place preference tests of Experiment 1a (0.5 mg/kg naloxone) and Experiment 1b (1 mg/kg naloxone).

cle with the lights on at 0800 h. All procedures were conducted during the light phase of the cycle. Food and water were available ad lib. Upon arrival in the laboratory, the rats were handled daily for 1 week prior to the conditioning trials.

Apparatus

The place-conditioning apparatus, previously described (17) included two chambers separated during conditioning trials by a wooden divider. The wooden walls of each chamber ($35 \times 25 \times 30$ cm) were painted flat black. The conditioning cues consisted of the textural floors in the chambers: one floor was covered with wire mesh (0.625 cm), and the other floor was covered with sandpaper strips (5 cm wide) located 5 cm apart. When assessed for their preference for each of these cues after three pairings with intraperitoneally injected saline solution, rats displayed equal preference for the two floors.

During testing, the divider between the chambers was removed allowing the rats to explore both chambers. The activity of the rats during testing was monitored by a video-tracking apparatus (Videomex-V, Columbus Instruments, Columbus, OH) from a video camera mounted to the ceiling. This provided a measure of the amount of time that the rats spent in each chamber.

Procedure

The data were collected in two replications that varied only on the basis of the dose of naloxone employed: in Experiment 1a, the naloxone dose was 0.5 mg/kg, and in Experiment 1b, the naloxone dose was 1 mg/kg. The rats received two conditioning trial cycles, with each cycle consisting of 3 days and 48 h intervening between the two cycles. On the first day of each cycle, the rats received a subcutaneous (SC) injection of saline solution (1 ml/kg) 5 min prior to being placed in one of the place-conditioning chambers for 30 min. This chamber will be called the nontreatment-paired chamber. Half of the rats were placed in the chamber with the sandpaper floor, and half of the rats were placed in the chamber with the mesh floor. On the second day of a cycle (24 h after the saline injection), the rats were injected (1 ml/kg) SC with either morphine (20 mg/kg) or saline and were returned to their home cage. On the third day of a cycle (24 h after the morphine or saline injection), the rats were injected with naloxone (1 mg/kg in Experiment 1a and 0.5 mg/kg in Experiment 1b) or saline and placed in the opposite side of the place-conditioning chamber as on the first day of the cycle. This chamber will be called the treatment-paired chamber. For each experiment, the design was a 2×2 with the factors of pretreatment drug (morphine or saline) and treatment drug (naloxone or saline) with 8–10 rats per group.

Forty-eight hours after the final conditioning trial, the rats received a place-preference test in a drug-free state. On the test trial, the rats were placed at the intersection between the two chambers with the divider removed and were allowed to explore both chambers for 15 min. The amount of time spent in each chamber was automatically recorded by the video-tracking apparatus.

RESULTS

Figure 1 presents the mean difference (seconds) in time spent in the treatment-paired chamber minus the time spent in the nontreatment-paired chamber. A $2 \times 2 \times 2$ between-factors ANOVA with the factors of pretreatment condition (morphine or saline) $\times$ treatment condition (naloxone or saline) $\times$ experiment [Experiment 1a (0.5 mg/kg naloxone), Experiment 1b (1 mg/kg naloxone)] revealed a significant pretreatment condition effect, $F(1, 69) = 14.3$, $p < 0.01$, a significant treatment condition effect, $F(1, 69) = 27.4$, $p < 0.01$, and a significant pretreatment by treatment interaction, $F(1, 69) = 4.51$, $p < 0.05$. To evaluate the interaction, subsequent Newman–Keuls pair-wise comparison tests revealed that the group pretreated with morphine and treated with naloxone displayed a significantly larger place aversion than all other groups ($p < 0.01$) In addition, the group pretreated with saline and treated with nalxone spent less time in the treatment-paired chamber than did the group pretreated and treated with saline ($p < 0.05$), suggesting that naloxone alone was also aversive.

DISCUSSION

Naloxone produced a conditioned place aversion in opiate naïve rats after only two conditioning cycles, as has previously been reported (14,15,17). However, the strength of that naloxone-induced place aversion was significantly enhanced by pretreatment with morphine 24 h prior to the conditioning trial. Consistent with our results, others have reported that behavioral withdrawal responses can be observed when an opioid antagonist is administered long after one or two doses of morphine in humans (4,8), rats (2,3,19), dogs (6,10), mice (5,20), and vermit monkeys (9). In fact, antagonist precipitated effects have been reported even when the antagonist-precipitated effects occur 48 h or more after a single morphine injection (3,5). Furthermore, in humans, at 24 h after morphine, the agonist effects, such as miosis, respiratory depression, and subjective opioid symptoms are no longer measurable (4), suggesting that morphine produces a long-term physiological or biochemical adaptational change that persists beyond its initial effects. This adaptational change appears to be hedonically aversive (18), in opposition to the hedonically rewarding initial effects of morphine (15).

Using a similar procedure, McDonald et al. (11) reported that acute naloxone-precipitated morphine withdrawal produced active rejection of a taste with which it was paired even when morphine was administered 22 h prior to the naloxone. Again, only two conditioning trials were necessary to establish the association between the taste cue and the aversive motivational properties of acute withdrawal. Therefore, acute precipitated withdrawal produced an aversive motivational state in the rats when assessed either by the taste aversion or the place aversion paradigms after only two conditioning trials.

ACKNOWLEDGEMENTS

This research was supported by a grant to L. Parker from the Natural Sciences and Engineering Research Council (NSERC-OGP 92057) of Canada and by an NSERC Undergraduate Student Award to A. Joshi. We would like to thank Sarah Mercer, Joan Cyr, and Marion Corrick for their assistance in collecting some of the data.

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