Pharmacology Biochemistry & Behavior, Vol. 28, pp. 267–273. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Chronic Naltrexone Supersensitizes the Reinforcing and Locomotor-Activating Effects of Morphine

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Received 3 November 1986

BARDO, M. T. AND J. L. NEISEWANDER. *Chronic naltrexone supersensitizes the reinforcing and locomotor-activating effects of morphine.* PHARMACOL BIOCHEM BEHAV **28**(2) 267–273, 1987.—Rats were implanted for 10 days with a slow-release naltrexone pellet and then the pellet was removed. Sham-control animals were treated similarly, except no pellet was implanted. One day after pellet removal or sham treatment, animals were assessed for morphine-induced conditioned place preference (CPP) or locomotor activity. CPP was evident in sham animals following two conditioning trials using 5 mg/kg subcutaneous morphine (Experiment 1) and following one conditioning trial using 8 mg/kg intravenous morphine (Experiment 2). Animals conditioned while implanted with a naltrexone pellet showed no morphine-induced CPP. More important, one day after pellet removal, naltrexone-pretreated animals given one conditioning trial with 5 mg/kg intravenous morphine displayed a greater preference for morphine-associated cues relative to sham animals given morphine (Experiment 3 and 4). This single IV morphine dose was insufficient to produce CPP in sham animals, suggesting that naltrexone-induced supersensitization may only be evident at a morphine dose below the reinforcing threshold in control animals. Further, chronic naltrexone potentiated the locomotor-activating effect of 2 mg/kg subcutaneous morphine but not of either 1 or 5 mg/kg morphine (Experiment 5). Behavioral supersensitization assessed by morphine-induced locomotor activation was transient, as it was evident one day, but not either three or 10 days following pellet removal (Experiment 6). These results confirm the functional significance of opiate receptor up-regulation following chronic opioid blockade.

Naltrexone Morphine Conditioned place preference Locomotor activity Opiate receptor Receptor up-regulation

CHRONIC exposure to opiate antagonist drugs produces a compensatory increase in the number of opiate receptors in the central nervous system. This up-regulation of opiate receptors has been demonstrated *in vitro* with neuroblastoma-glioma cell cultures [2,7] and *in vivo* using receptor assays involving either autoradiography [37] or tissue homogenate fractions [15,23]. *In vivo* exposure to naltrexone by implantation of a slow-release pellet induces maximal up-regulation within eight days, with the greatest increase observed in the limbic system and hypothalamus [43]. Following removal of the naltrexone pellet, opiate receptor levels in whole brain return to normal within 12 days [39], although only two to four days are required in hypothalamus, striatum and prefrontal cortex [5]. This transient up-regulation in opiate receptors occurs at μ and δ binding sites [12,43], but not at either κ or σ binding sites [38].

Concomitant with the up-regulation in opiate receptors, various functional changes are evident following chronic opiate antagonist treatment. Electrophysiologic evidence indicates that chronic opiate antagonism decreases the spontaneous firing rate of neurons within the brainstem locus coeruleus and supersensitizes these neurons to the inhibitory effect of morphine [3]. Chronic opiate antagonism also supersensitizes the guinea pig ileum twitch response to the inhibitory effect of opiates [35] and potentiates morphine-induced hyperactivity in mice [12]. In rats, chronic opiate antagonism potentiates the antinociceptive efficacy of morphine as measured by the hot-plate, tail-flick, shock-vocalization and jump-flinch tests [5, 29, 36, 38, 42]. However, not all behavioral responses are supersensitized to the effects of opiates. For example, chronic opiate antagonism produces no change in the hypoactive effect of high-dose morphine administration in either infant or adult rats [4,42], nor is morphine-induced conditioned taste aversion altered in adult rats [5]. Behaviors which do not involve primarily μ or δ receptors may not be subject to supersensitization.

It is unclear presently whether morphine-induced reinforcement is supersensitized following chronic opiate antagonism. In humans, chronic naltrexone treatment is effective in blocking the reinforcing efficacy of opiates and helps prevent re-addiction in some opiate-dependent patients [13, 20, 24, 32]. To the extent that opiate reinforcement involves μ or δ receptors, up-regulation of these receptors by chronic opiate antagonism may increase the reinforcing effect of opiates. If this is the case, then an opiate addict who relapses following chronic naltrexone treatment may be supersensitized to opiate reward. Consistent with this possibility, heroin-induced reinforcement assessed by electrical self-stimulation of the lateral hypothalamus is enhanced in rats pre-exposed to naltrexone [34].

In the present study, we examined the effect of chronic naltrexone treatment on morphine-induced conditioned place preference (CPP) in rats. CPP is an animal model of drug reinforcement which has been used extensively to

assess the reinforcing effect of μ-type opiates in rats [10, 17, 22, 25, 27, 28, 33]. Unlike the self-administration procedure [40], CPP can be assessed following a single intravenous morphine injection [6,28]. We therefore used this procedure to assess the reinforcing value of morphine given either one or two days after termination of chronic naltrexone treatment. In addition to CPP, we assessed the effect of chronic naltrexone treatment on the hyperactive response produced by low-dose morphine.

METHOD

Animals

The animals were male Sprague-Dawley albino rats (Harlan Industries, Indianapolis, IN), initial weight 250–425 g, housed individually with food and water available ad lib.

Naltrexone Pellets

Animals were divided randomly into one of three main treatment groups. Two groups were anesthetized by ether inhalation and were implanted subdermally with a pellet of naltrexone free base (30 mg, National Institute on Drug Abuse, Rockville, MD). The pharmacokinetics of the slow-release naltrexone pellet have been characterized previously [41]. Ten days after pellet implantation, one group of animals was anesthetized again and the pellet was removed. The second group was treated similarly, except the pellet was left intact. The third treatment group was a sham surgery control group. These animals were anesthetized like the previous two groups and a subdermal incision was performed, but no pellet was implanted. Ten days later, this sham surgery was repeated.

Apparatus

CPP was conducted in a chamber that had three different compartments separated by guillotine doors. The two end compartments measured 22×26×30 cm, while the middle compartment measured 22×14×30 cm. One end compartment had white walls, a wire-mesh floor, and pine wood chips under the floor. The other end compartment had black walls, a metal-grid floor, and cedar shavings under the floor. The middle compartment had gray walls and a solid wood floor which was also gray.

Locomotor activity was assessed in a plywood chamber measuring 30×30×42 cm. The chamber walls were painted white and the floor was wire-mesh with cedar bedding underneath. Mounted 4 cm above the floor were two photocells and light sources which divided the chamber into four equal quadrants. Each time a photobeam was interrupted, a counter connected to a photocell relay registered one count. The number of photobeam interruptions were recorded in 20-min blocks.

Conditioned Place Preference

In Experiment 1, animals in each of the three treatment groups (i.e., pellets removed, pellets intact and sham) were assessed for morphine-induced CPP on the day following pellet removal or sham surgery. Each animal was placed in the black compartment for 30 min with the guillotine door closed. Immediately afterward, each animal received either morphine sulfate (5 mg/kg, SC) or saline in a volume of 1 ml/kg and then was placed into the white compartment for 30 min post-injection. This procedure was repeated on the next day. On the third day, each animal was placed into the middle gray compartment with the guillotine doors open and allowed free-choice access to all compartments for 15 min. The duration spent in each compartment and the number of entries into each compartment were recorded by an observer who was unaware of each animal's individual treatment.

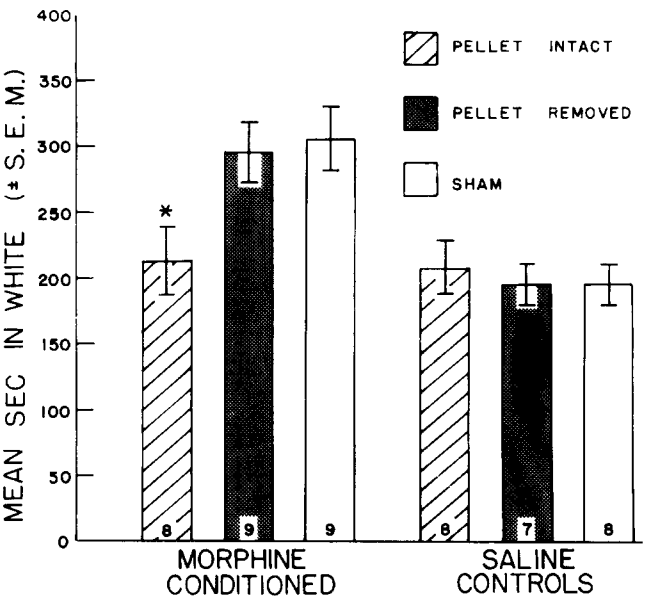

FIG. 1. Mean duration spent in white compartment for animals in Experiment 1 given two-trial CPP with morphine (5 mg/kg, SC) or saline. One group was conditioned and tested with an intact naltrexone pellet; one group was conditioned beginning one day after pellet removal; and one group was given sham treatment. Number of animals per group represented at base of each bargraph. Asterisk (*) represents significant difference from pellet-removed and sham groups, $p<0.05$.

In Experiments 2–4, single-trial CPP was assessed using intravenous morphine sulfate (5 or 8 mg/kg). Two days before pellet removal or sham surgery, each animal was implanted with a catheter (PE 50 tubing) into the right jugular vein under chloral hydrate anesthesia (330 mg/kg, IP). The catheters were flushed with 0.5 ml saline on the two days following surgery. Conditioning began one day after pellet removal or sham surgery. Each animal was placed into the white compartment for 30 min and, on an alternate day, was placed into the black compartment for 30 min. The order in which animals were placed into the white and black compartments was counterbalanced across treatment groups. Immediately after placement into white, half of the pellet-removed and half of the sham animals received morphine (8 mg/kg in Experiment 2 or 5 mg/kg in Experiments 3 and 4). The morphine infusion (1 ml/kg) was followed by 0.5 ml saline in order to ensure that the drug was flushed entirely from the catheter. This infusion procedure took about 60 sec. The remaining animals received saline immediately after placement into white. On the day following conditioning, animals were tested for CPP as described previously.

Locomotor Activity

In Experiment 5, animals were assessed for morphine-induced changes in locomotor activity. On the day following pellet removal or sham surgery, each animal was placed into the activity apparatus for 30 min for acclimation. Following

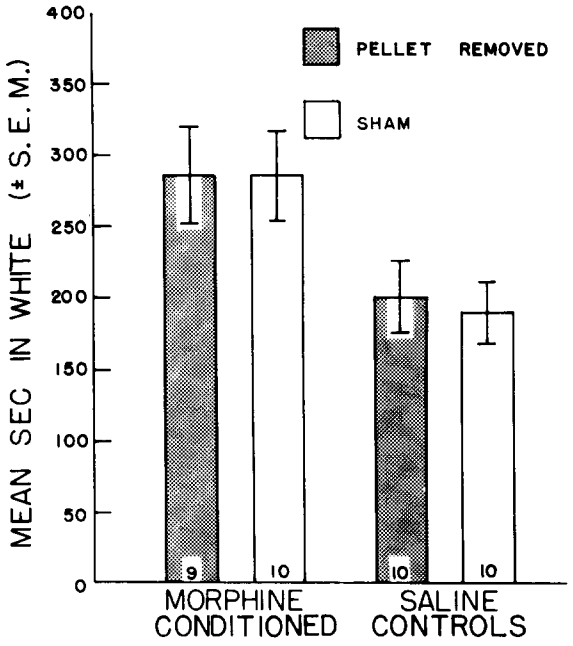

FIG. 2. Mean duration spent in white compartment for pellet-removed and sham animals in Experiment 2 given one-trial CPP with morphine (8 mg/kg, IV) or saline. Number of animals per group represented at base of each bargraph.

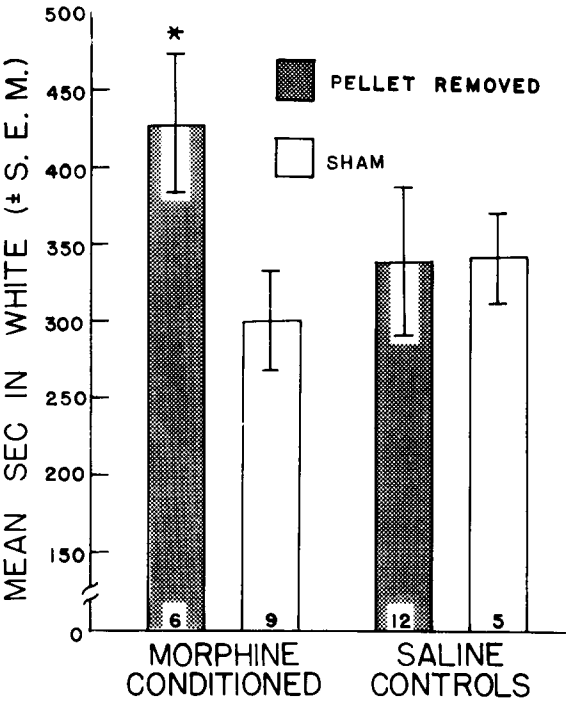

FIG. 3. Mean duration spent in white compartment for pellet-removed and sham animals in Experiment 3 given one-trial CPP with morphine (5 mg/kg, IV) or saline. Number of animals per group represented at base of each bargraph. Asterisk (*) represents significant difference from sham group, $p<0.05$.

this, pellet-removed and sham animals received either 0, 1, 2 or 5 mg/kg morphine subcutaneously in a volume of 1 ml/kg. Immediately following injection, each animal was placed back into the activity apparatus and the number of photobeam interruptions were counted in 20-min blocks for two hours. In Experiment 6, morphine-induced activity was assessed at either one, three, or 10 days following pellet removal or sham surgery. Animals were placed in the activity apparatus for 30 min for acclimation. They then received 2 mg/kg morphine and were tested in the activity apparatus for 3 hr post-injection.

Statistics

The data were analyzed using analyses of variance, and pairwise comparisons were made using tests of simple main effects or a-priori t-tests. Multiple comparisons between means were made using the Newman-Keuls test of significance.

RESULTS

Conditioned Place Preference

The results from the two-trial CPP test using 5 mg/kg subcutaneous morphine are presented in Fig. 1. As expected, CPP was evident as an increased duration spent in the white compartment for morphine-conditioned groups relative to saline-controls, $F(1,43)=11.35$, $p<0.01$. Pairwise comparisons between the morphine-conditioned groups revealed that pellet-removed and sham animals did not differ significantly, indicating that supersensitization to the reinforcing effect of morphine was not obtained. However, pellet-intact animals spent significantly less time in white than both pellet-removed and sham animals, $t(15)\geq 1.79$, $p<0.05$, demonstrating that naltrexone blocked acquisition of morphine-induced CPP.

In Experiment 2, single-trial CPP was obtained using 8 mg/kg intravenous morphine (see Fig. 2). A 2×2 factorial analysis of variance indicated that morphine-conditioned groups spent significantly more time in white than saline-controls, $F(1,35)=8.86$, $p<0.01$. A pairwise comparison between the two morphine-conditioned groups revealed that pellet-removed and sham animals did not differ significantly, indicating that supersensitization to the reinforcing effect of morphine was not obtained.

While single-trial CPP was obtained in both naltrexone- and sham-pretreated groups using 8 mg/kg intravenous morphine, the results from Experiments 3 and 4 indicated that single-trial CPP was obtained only in naltrexone-pretreated groups using 5 mg/kg intravenous morphine. Using this lower dose of morphine, two independent replications (Experiment 3 and 4) were conducted. In the first replication, a pairwise comparison between morphine-conditioned groups revealed that pellet-removed animals spent significantly more time in white than sham animals, $t(14)=2.61$, $p<0.05$; see Fig. 3. In contrast, a comparison between saline-control groups revealed no significant difference between pellet-removed and sham animals. In the second replication, similar results were obtained, except that the comparison between morphine-conditioned groups only approached statistical significance ($p<0.10$; data not shown). Nonetheless, combining the four morphine-conditioned groups from the two replications revealed that pellet-removed animals spent significantly more time in the morphine-associated white compartment than sham animals, $F(1,35)=5.68$, $p<0.05$, suggesting that the reinforcing efficacy of morphine was enhanced.

Further, the results from Experiment 4 indicated that naltrexone pretreatment decreased the number of entires into

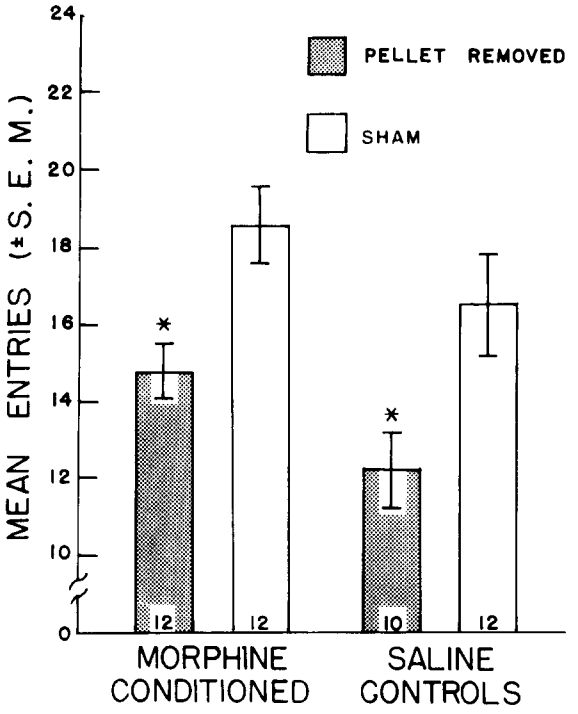

FIG. 4. Mean number of entries into both white and black compartments for pellet-removed and sham animals in Experiment 4 given one-trial CPP with morphine (5 mg/kg, IV) or saline. Number of animals per group represented at base of each bargraph. Asterisk (*) represents significant difference from sham group, $p<0.05$.

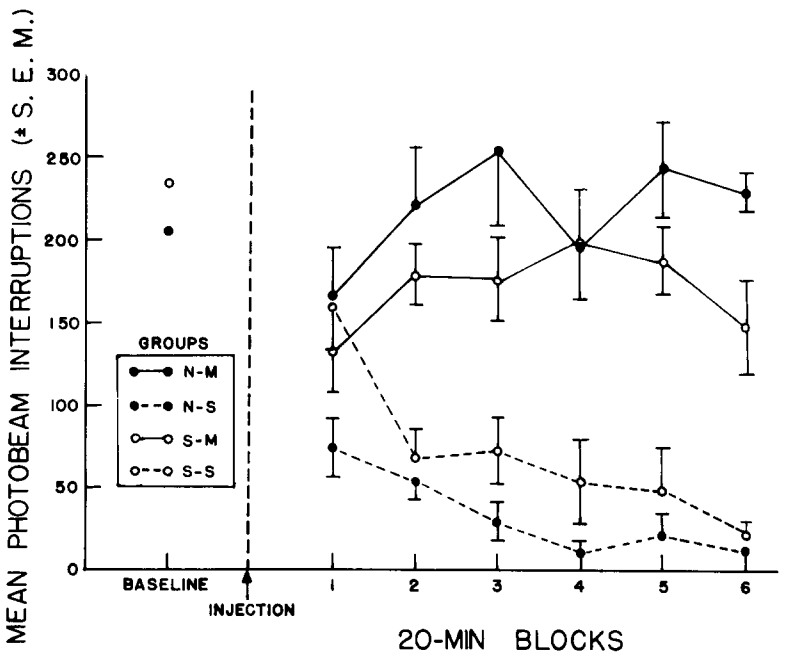

FIG. 5. Mean number of photobeam interruptions for naltrexone (N) pellet-removed and sham (S) animals in Experiment 5 tested following either 2 mg/kg morphine (M) or saline (S). Each baseline mean based on 16 animals and post-injection mean based on eight animals.

both the white and black compartments (see Fig. 4). A 2×2 analysis of variance indicated that pellet-removed animals made significantly fewer compartment entries than sham animals, $F(1,38)=6.34$, $p<0.01$. The mean number of white entries for pellet-removed and sham groups were 6.5 and 8.0 respectively, and the mean number of black entries for pellet-removed and sham groups were 7.1 and 9.6 respectively. Thus, the decrease in entries observed in naltrexone-pretreated animals did not reflect a change specific to either the white or black compartment, but rather reflected a general decrease in locomotor activity in the entire apparatus.

Locomotor Activity

The results from Experiment 5 revealed that animals tested one day after pellet removal were significantly more active than sham animals following 2 mg/kg morphine, $t(1,15)=2.88$, $p<0.01$, demonstrating supersensitization to the hyperactive effect of morphine. However, supersensitization was not evident with either 1 or 5 mg/kg morphine, as pellet-removed and sham animals did not differ significantly following these doses (data not shown). Analysis of the data across test blocks revealed that the hyperactive effect of 2 mg/kg morphine was not evident across all post-injection test blocks (see Fig. 5). A 2×2×6 repeated measures analysis of variance of these data revealed a significant drug × block interaction, $F(5,125)=9.99$, $p<0.01$. A Newman-Keuls test ($p<0.05$) indicated that, regardless of pellet treatment, morphine-injected animals (groups N-M and S-M) were significantly more active than saline-injected animals (groups N-S and S-S) on blocks 2–6, but not on block 1. Further, the analysis of variance revealed a significant pellet × drug interaction effect, $F(1,25)=5.79$, $p<0.05$. Collapsed across blocks, a Newman-Keuls test ($p<0.05$) revealed that group N-M was significantly more active than group S-M, demonstrating supersensitization to morphine-induced hyperactivity. However, groups N-S and S-S did not differ significantly. There was also no significant difference between pellet-removed and sham animals on the baseline test prior to the injection of morphine or saline. Thus, in contrast to the entry data from the CPP test shown in Fig. 4, locomotor activity did not differ between naltrexone-pretreated and sham animals in the absence of morphine.

In Experiment 6, all animals received 2 mg/kg morphine either one, three, or 10 days following pellet removal or sham treatment. As shown in the previous experiment, when administered morphine, pellet-removed animals were significantly more active than sham animals one day following treatment, $t(19)=1.92$, $p<0.05$, thus demonstrating supersensitization to the hyperactive effect of morphine. In contrast, pellet-removed and sham animals did not differ significantly following morphine when tested either three or 10 days after treatment. There was also no significant difference in activity between pellet-removed and sham animals during the 30-min pre-injection baseline activity measure assessed either one, three, or 10 days after treatment (data not shown)

DISCUSSION

Chronic naltrexone exposure produces behavioral supersensitivity to morphine-induced reinforcement and hyperactivity. Implantation of a naltrexone pellet blocked completely acquisition of two-trial CPP using 5 mg/kg subcutaneous morphine. More important, removal of the naltrexone pellet enhanced one-trial CPP using 5 mg/kg intravenous morphine. This effect was clearly evident in one experiment and was marginally evident in a replication experiment, indicating a small effect using the present procedure. In addition, chronic naltrexone potentiated the locomotor-activating effect of 2 mg/kg morphine, but not the effect of either 1 or 5 mg/kg morphine. This dose-related supersensitization was transient, as it was evident one day, but not either three or 10 days after pellet removal. In the absence of morphine, chronic naltrexone treatment also reduced locomotor activity measured in the CPP apparatus and in the photobeam activity monitor, although this latter effect was not statistically reliable. The mechanism underlying the naltrexone-induced attenuation in locomotor activity is unclear presently.

One problem with the CPP procedure is that it is not very sensitive to morphine dosage [11, 28, 31]. For example, the magnitude of CPP following four conditioning trials is similar for morphine doses ranging from 0.08 to 10 mg/kg [28]. This suggests that although chronic naltrexone may increase the reinforcing potency of morphine, it may not induce a CPP effect greater than that observed in sham animals. Consistent with this, we found that when CPP was obtained in sham animals using either two-trial subcutaneous morphine (5 mg/kg) or one-trial intravenous morphine (8 mg/kg), naltrexone-pretreated animals displayed CPP which was not greater than sham animals. In contrast, when CPP was *not* obtained in sham animals using one-trial intravenous morphine (5 mg/kg), naltrexone-pretreated animals did display CPP. These results suggest that naltrexone-induced supersensitivity to the reinforcing effect of morphine may be evident only at doses below the threshold at which CPP is obtained in control animals.

Recent evidence indicates that a modified CPP procedure may be sensitive to drug dosage [8]. With this modified procedure, several drug doses are contrasted to a single reference dose. For example, Barr *et al.* [8] administered 1 mg/kg morphine in one conditioning compartment and administered either 0.1, 0.3, 3.0 or 5.0 mg/kg morphine in an alternative conditioning compartment. Within this range, dose-dependent CPP was obtained against the reference dose of 1 mg/kg morphine. Such a modified procedure may be useful in assessing the reinforcing potency of morphine following chronic naltrexone.

Nonetheless, the present results are consistent with a previous report indicating that the reinforcing efficacy of heroin is potentiated following chronic naltrexone [34]. In that report, the facilitory effect of heroin on electrical self-stimulation of the lateral hypothalamus in rats was enhanced following 20 daily injections of naltrexone (10 mg/kg, SC). To the extent that CPP and self-stimulation represent a similar reinforcement process, these results indicate that up-regulation of opiate receptors following chronic naltrexone enhances opiate reward. Further, since chronic naltrexone up-regulates μ and δ sites, but not κ or σ sites [38], these results suggest that opiate reward may be mediated by μ or δ receptors. This contrasts with other findings indicating that opiate-induced conditioned taste aversion involves a mechanism independent of μ and δ receptors [5, 9, 26]. Thus, the rewarding and aversive consequences of opiates appear to involve different receptor populations.

Chronic naltrexone also potentiated morphine-induced hyperactivity in the present study. The neuropharmacologic basis for this finding is unclear presently. However, the nigrostriatal and mesolimbic dopamine pathways may have been supersensitized to the locomotor-activating effect of

morphine. Direct injection of opiates into these dopaminergic pathways results in hyperactivity [18,19]. Further, neuropharmacologic and electrophysiologic studies have confirmed that these pathways respond to morphine by increasing neuronal firing rate [14,16] and by increasing dopamine synthesis and turnover [1]. If chronic naltrexone treatment increased the number of opiate receptors on these dopaminergic pathways, then firing rate and dopamine release evident in response to morphine challenge may have been enhanced in these pathways. However, this possible explanation is surely over-simplified, as the hyperactive effect of opiates also involves a non-dopaminergic substrate [19, 21, 30].

Regardless of the neural substrate involved, we found that the effect of naltrexone on morphine-induced hyperactivity was transient. This finding is consistent with neurochemical data indicating that naltrexone-induced up-regulation of opiate receptors is also transient [39]. In the striatum, up-regulation is evident for only two days following cessation of opiate antagonist treatment [5]. To the extent that the nigrostriatal pathway is involved in morphine-induced hyperactivity, one would expect that the duration of receptor up-regulation and behavioral supersensitization would be parallel. Consistent with this, potentiation of morphine-induced hyperactivity was evident one day, but not three days, after naltrexone treatment. These results corroborate the functional significance of opiate receptor up-regulation following chronic opioid blockade.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance of Rosalyn Ennis and David Mayleben in helping collect some of the data. This research was supported by USPHS grant DA03460.

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