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BRIEF COMMUNICATION

Enhancement of Naloxone-Induced Analgesia by Pretreatment With Morphine

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CAPPELL, H., C. X. POULOS AND A. D. LÊ. *Enhancement of naloxone-induced analgesia by pretreatment with morphine.* PHARMACOL BIOCHEM BEHAV **34**(2) 425–427, 1989.—Recently there have been demonstrations of a form of analgesia in rats that depends on the repeated administration of an opiate antagonist for its occurrence. The mechanism of this naloxone-induced analgesia (NIA) is not clear. This experiment tested the hypothesis that the relationship between behavioral effects of previous experience with opiate agonists and antagonists would be reciprocal with respect to analgesia. Consistent with such an hypothesis, prior exposure to morphine increased sensitivity to the effect of naloxone as measured by the rate of acquisition of NIA. Although receptor functions were not measured, reciprocal changes in the regulation of opiate receptors by opiate agonists and antagonists may underlie the behavioral effects observed in this experiment.

Analgesia Morphine Naloxone Naloxone-induced analgesia (NIA) Opiate receptors

THERE have been recent independent reports of a form of analgesia that depends on the repeated administration of naloxone for its occurrence. Greely *et al.* (4) found that repeated exposure to nociceptive stimulation on the hot-plate resulted in the progressive recruitment of an analgesic response in rats implanted with naloxone or naltrexone pellets. The same phenomenon has also been seen in the case of repeated injections of naloxone (2, 4, 8). The exact basis of this naloxone-induced analgesia (NIA) is unknown. It has been hypothesized (8) that NIA occurs as the result of the activation of a collateral analgesic system in the face of blockade of endogenous opiates [cf. also (1)].

The relationship between effects of opiate agonists and antagonists would be expected to be reciprocal. On one side of the equation, there is an increase in sensitivity to effects of morphine (6,11) that is associated with continuous prior exposure to naloxone and naltrexone by means of chronic infusion or pellet implantation; this can be attributed to receptor upregulation, which has been described as an "adaptive response to treatments that block opiate agonist activity" (10). Rats become supersensitive to the analgesic (7,8) and cataleptic (7) effects of morphine following the induction of NIA using a regime of intermittent injections of naloxone.

Administration of an opiate agonist can be viewed as the reciprocal of administration of an opiate antagonist. If the relationship between agonist and antagonist actions is fully reciprocal with respect to analgesia, the other side of the equation is that increased sensitivity to naloxone would be expected in rats after chronic exposure to morphine. Specifically, morphine pretreatment should lead to a more rapid rate of acquisition of NIA. Although the data are inconsistent (10), chronic treatment with morphine has been associated with a downregulation of opiate receptors (3).

Since the manifestation of tolerance to morphine is subject to learning (9), an attempt was also made to determine whether any effect of morphine tolerance on NIA would be subject to environmental conditioning.

METHOD

Animals

The subjects were 90 male Sprague-Dawley rats (Charles River, St. Constant, Quebec) weighing 300–325 g at the beginning of the experiment. Animals were housed singly in wire-mesh

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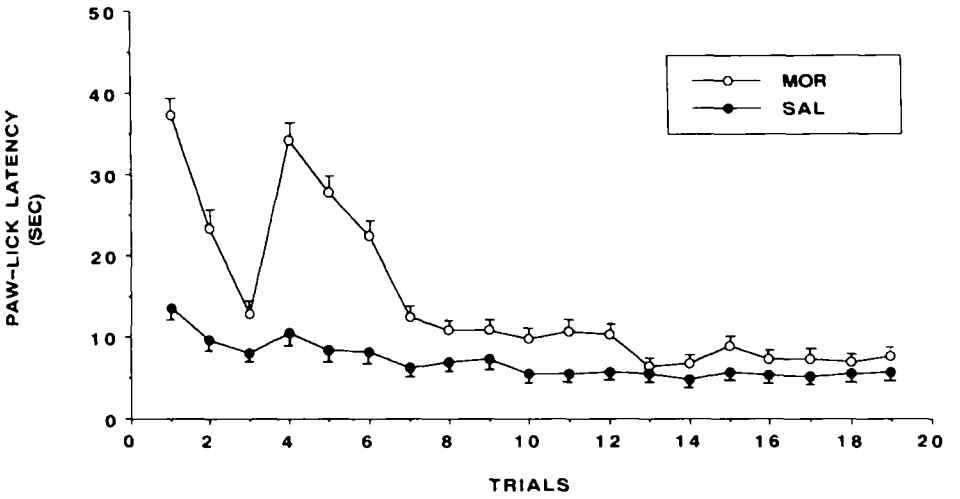

FIG. 1. Effect of chronic treatment with morphine on paw-lick latency. The dose was raised from 5 to 10 mg/kg on Trial 4. Vertical bars indicate standard error.

hanging cages. Access to water was ad lib, and the food ration was 5 Purina Lab Chow pellets (approximately 20 g) per day. The animals were maintained on a 12-hr light:12-hr dark cycle throughout the experiment.

Apparatus

Analgesia was assessed using a hot-plate apparatus consisting of a heated water bath in a plastic tub covered by an aluminum plate (Grant Instruments Model AB02). The surface temperature was maintained at 49.5 degrees C ($\pm$0.5 degrees C). This temperature was continuously monitored with a surface probe (Yellow Springs Instruments 'banjo' probe, Model 408) secured to the surface of the aluminum plate. A Plexiglas chamber (27 $\times$ 16.5 $\times$ 9 cm) with a hinged lid was mounted on the surface to confine rats to the hot-plate.

Procedure

Screening. The animals were handled daily for a week prior to the experiment. They were then screened for responsivity to the hot-plate on trials conducted in the colony room. On screening trials, rats were removed from the home cage and injected SC with physiological saline in a volume of 1 ml/kg. Thirty minutes following injection, the animal was placed on the hot-plate surface and the time elapsed until the first paw-lick was recorded. If no paw-lick occurred within 45 sec, the animal was removed and assigned a score of 45 sec. The animals were returned to their home cages immediately after these trials. There were three screening trials in all.

Establishment of tolerance to morphine. During this phase of the experiment, which began the day after the final screening trial, rats were assigned to a morphine (M, n = 60) or saline group (S, n = 30). The groups were matched in mean paw-lick latency based on the average response of the last two screening trials. To test for the effects of environmental conditioning, a discrimination training procedure was used. To anticipate, this manipulation had no effect on the results. Most of the related procedural details are omitted for the sake of brevity. (Details are available from the authors on request.) Rats in Group M were administered a total of 19 injections of morphine sulphate distributed over a 57-day period. Morphine was injected SC in a solution volume of 1 ml/kg. The dose began at 5 mg/kg and was increased to 10 mg/kg on the fourth morphine injection. Thirty minutes following injection, the rats were tested on the hot-plate and latency to the first paw-lick was recorded. If no paw-lick was observed within 45 sec, the rat was removed from the plate and assigned a score of 45 sec. The days intervening between morphine administrations were either saline days or "days off." On the former, rats were given control injections of physiological saline and tested with the hot-plate procedure. On days off, no procedures were conducted and the rats simply remained in their home cages in the colony room.

Morphine days, saline days, and days off occurred with approximately equal frequency. Experimental procedures were always scheduled to take place in the light cycle between 10:00 and 17:00 hr.

Rats in Group S were treated similarly to those in Group M with the exception that injections of saline were substituted for morphine.

Testing with naloxone. Prior to this phase of the experiment, 5 rats were eliminated on the basis that they failed to develop analgesic tolerance. Rats from Group M were tested with saline (S) or naloxone (N) according to the following design:
MOR-NAL (n = 28): Rats were tested with naloxone; MOR-SAL (n = 28): Rats were tested with saline; SAL-NAL (n = 29): rats were tested with naloxone.
There was a 4-day interval between the last day of tolerance-acquisition and the beginning of testing with naloxone. Injections of naloxone hydrochloride were administered in a dose of 5 mg/kg in a fluid volume of 1 ml/kg. The details of injection and test procedures were as described earlier, with the exception that hot-plate testing began 15 min after injection in these tests. There were a total of 7 tests with naloxone distributed over 14 days at intervals ranging from 24 hr to 5 days. Between tests the animals remained in their home cages, undisturbed except for routine maintenance.

RESULTS

The acquisition of tolerance to morphine is shown in Fig. 1. Analysis of variance (ANOVA) confirmed that morphine tolerance had been established prior to testing with naloxone ($p<0.001$).

A preliminary (ANOVA) showed that the environmental manipulation had no effect on paw-lick latencies in animals tested

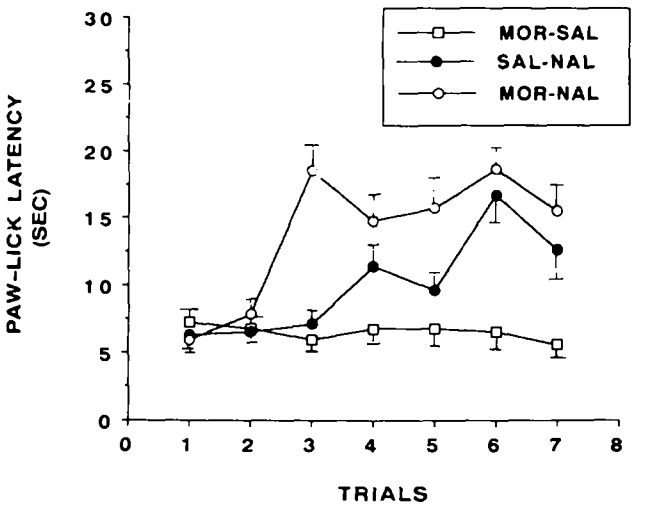

FIG. 2. Effect of repeated tests with naloxone (5 mg/kg) on paw-lick latency. The abbreviation on the left of the hyphen in the legend indicates the prior treatment, and the abbreviation on the right indicates the test injection. Vertical bars indicate standard error.

with saline or naloxone. Therefore the data obtained in both environments were combined to provide an assessment of morphine treatment per se on analgesic response. The results are shown in Fig. 2. The data were analyzed by ANOVA followed by individual comparisons using Tukey's tests. The ANOVA yielded significant effects of Treatments, $F(2,574) = 41.9$, $p<0.001$, Trials, $F(6,574) = 8.9$, $p<0.001$, and their interaction, $F(12,574) = 4.5$, $p<0.001$. Comparisons of trial means showed no between group differences on Trials 1 and 2. On Trial 3, the paw-lick latency of rats pretreated with morphine exceeded that of the other two groups ($p<0.01$), which did not differ from each other. By Trial 4 and thereafter, the effect of naloxone treatment in control rats began to take effect ($p<0.05$ or better). However, a significant effect of morphine pretreatment on NIA was still evident on Trials 4 and 5, after which the two naloxone-injected groups displayed similar paw-lick latencies.

DISCUSSION

This experiment confirmed the hypothesis that pretreatment with morphine would affect the rate of acquisition of NIA. Significant NIA became evident one trial sooner among morphine-experienced rats than among their nontolerant controls, and remained relatively greater for three trials.

There was substantial tolerance in the morphine-experienced rats; however, there was no evidence of environmental specificity of the morphine experience in the development of NIA. The reasons for this cannot be established. Although there was a negative result in this aspect of the experiment, conditions necessary for a valid test of the effect of morphine pretreatment on NIA were nonetheless met. There was no irregularity in the acquisition of pharmacological tolerance per se. The pattern of progressive recruitment of NIA over trials was as would be expected (4). Thus, the finding that experience with morphine accelerated the acquisition of NIA was clearly established.

It has been suggested (8) that NIA occurs because a collateral nonopiate system is activated when the primary endogenous pain control system is compromised by opiate receptor blockade. Such an hypothesis is consistent with our findings, which provide an interesting complement to the phenomenon of increased sensitivity to morphine as a result of prior exposure to naloxone (6,11). The downregulation of opiate receptors as a consequence of pretreatment with morphine may be the factor underlying increased sensitivity to NIA. The ability of naloxone to neutralize an opiate-mediated endogenous system should be increased if receptor downregulation has already occurred as a result of some prior experience, and the adaptive response mediated by the collateral system should come into play more quickly. Since we made no attempt to assess downregulation directly, we can only speculate about this mechanism. However, this does not mitigate the confirmation of our essential hypothesis concerning the reciprocity of the analgesic effects of morphine and naloxone at a behavioral level.

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