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Acquisition and Expression of Conditioned Hypoalgesia in Morphine-Naive and Morphine-Tolerant Rats

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FOO, H. *Acquisition and expression of conditioned hypoalgesia in morphine-naive and morhphine-tolerant rats.* PHARMACOL BIOCHEM BEHAV **62**(3) 433–437, 1999. — The present study used a within-subject design to examine acquisition and expression of conditioned hypoalgesia in 50 male Wistar rats. Morphine-naive rats preexposed to a heat stressor with saline were hypoalgesic when subsequently tested for latencies to tail flick or paw lick. However, morphine-tolerant rats preexposed to the heat stressor with saline failed to display hypoalgesia when tested for latencies to tail flick, but showed hypoalgesia when tested for latencies to paw lick. Taken together, these findings suggest that expression of conditioned hypoalgesic responses in morphine-tolerant rats may depend on the nociceptive test used. Both morphine-naive and morphine-tolerant rats preexposed to the heat stressor with morphine failed to display hypoalgesia on either the tail-flick or the hot-plate test, demonstrating that morphine's ability to block acquisition of conditioned hypoalgesia is independent of the test used to assess nociceptive sensitivity. © 1999 Elsevier Science Inc.

Morphine Stressor Hypoalgesia Tail flick Hot plate

PREEXPOSURE to a heat stressor, such as, the heated floor of a hot-plate apparatus, has been shown to provoke hypoalgesic responses in rats (11,17,20,26). Specifically, rats preexposed to a 54°C floor of a hot-plate apparatus show longer paw-lick latencies, when tested on a 52°C floor, than those preexposed to a nonheated (23°C) floor. There is evidence that the long latencies to paw lick thus accrued are mediated by associative learning processes, because they are extinguished when exposures to the heated floor are interpolated by exposures to a nonheated one (2,9,11,12,26).

The conditioned hypoalgesia resulting from preexposure to the heat stressor is not a selective reduction of nociceptive sensitivity to acute pain induced by the thermal stimulus of the hot-plate test. This is because decreases in nociceptive sensitivity have also been detected with other pain assays. Rats tested in a context (CS) associated with exposure to the heated floor have been shown to attend less to a formalin-injected paw (7–10,12), and to take longer to flick their tails in response to the application of radiant heat (6), than those preexposed to a nonheated floor.

Although conditioned hypoalgesic responses elicited by the place associated with exposure to the heat stressor are detected using different pain assays, the antinociceptive mechanisms activated depend on the pain test employed. The conditioned hypoalgesic responses observed with the hot-plate test appear to be subserved by non-mu mechanisms, because the long paw-lick latencies are reversed by naloxone and unaffected by pretreatment with morphine (5,17,18,25,26). In contrast, mu opioid mechanisms mediate conditioned hypoalgesic responses detected with either the formalin or the tail-flick radiant heat test, because the decreases in paw attendant behaviors (7–10,12), or the long tail-flick latencies (6), are reversed by naloxone and are prevented by a history of exposures to morphine. These findings suggest that the form of conditioned hypoalgesia evoked relies critically upon the test used for nociceptive assessment [cf. (15)].

Although expression of conditioned hypoalgesic responses on the hot-plate assay is mediated by non-mu mechanisms, two findings suggest that acquisition of such responses is based on mu opioid mechanisms. First, naloxone potentiates acquisition of conditioned hypoalgesia, because rats preexposed to the heated floor with naloxone display longer paw-lick latencies than those given separate exposures to naloxone and the heated floor (5,7–9,11,17,25). Secondly, morphine ap-

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pears to impair acquisition of such responses, because rats preexposed to the heated floor with morphine do not acquire the long paw-lick latencies that are otherwise observed (10,11,26). Morphine's ability to block acquisition of the long paw-lick latencies cannot be attributed to its hypoalgesic properties, because morphine-tolerant rats preexposed to the heat stressor with morphine also fail to show the long paw-lick latencies (10,11,26). However, the lack of hypoalgesia among morphine-naive and morphine-tolerant rats preexposed to the heated floor with morphine has been documented only with the hot-plate test, and may not generalize to other pain assays.

Consequently, the present experiment uses a within-subject design to further examine acquisition and expression of conditioned hypoalgesic responses in morphine-naive and morphine-tolerant rats with the tail-flick radiant heat and hot-plate tests. Each subject was tested with the tail-flick test first followed by the hot-plate test approximately 24 h later. This order of testing was used to minimize carryover effects between tests because prior tail-flick testing does not induce hypoalgesia (6), whereas prior hot-plate testing induces hypoalgesia (6,25,26). The first aim of this study is to confirm that morphine-naive rats preexposed to a heat stressor with saline display hypoalgesia when subsequently tested for tail-flick or paw-lick latencies in a place associated with preexposure to the heat stressor (6). The second aim is to confirm that morphine-tolerant rats given such exposures display hypoalgesia when tested with the tail-flick test but not when tested with the hot-plate test. In a previous investigation (6), I found that expression of conditioned hypoalgesia in morphine-experienced rats appears to depend on the pain test used, but did not confirm that the morphine-treated rats had acquired tolerance to the hypoalgesic effects of the opioid. The final aim is to examine whether morphine-naive and morphine-tolerant rats exposed to the heat stressor with morphine would fail to show conditioned hypoalgesic responses across both tail-flick and hot-plate tests.

METHOD

Subjects

Subjects were 50 experimentally naive, male Wistar rats, with an average weight of 260 g (range 240–290 g) at the start of the experiment. They were obtained from the colony maintained by the Laboratory Animal Services, University of Adelaide. The rats were housed in plastic boxes (60 × 40 × 20 cm) throughout the course of the experiment, and were given free access to food and water. There were five rats to each box. The boxes were kept in a colony room maintained on a 12 L: 12 D cycle, with experimentation conducted during the light cycle.

Apparatus

The hot-plate apparatus consisted of a Plexiglas cylinder (23 cm inner diameter × 48 cm high) with a brass floor (1 mm thick) fixed 12 cm above the base of the cylinder. The portion of the cylinder below the brass floor was perforated with 2-cm diameter holes to permit circulation of water under the floor. The cylinder stood in a water bath whose temperature could be maintained at a particular value (±0.5°C) by a Grant VFK Open Bath Circulator. The tail-flick apparatus consisted of a 500-W projection bulb housed within a fan-forced, air-cooled stainless steel box (25 × 16 × 12 cm high) [cf. (3,24)]. A 2-mm aperture was located on the surface of the box directly above the bulb and between 3-mm high aluminum side rails. A photocell, connected to a digital timer, was mounted 12 cm above the aperture. Lateral deflection of the rat's tail activates the photocell, terminating the light source and the digital timer. Tail-flick latencies (TFLs) were measured to the nearest ms. A cutoff latency of 15 s was used. The heat source was adjusted to produce baseline TFLs of approximately 3 s in naive rats. The restraining procedure used for tail-flick testing consisted of wrapping the rat's torso and limbs with towelling material to prevent body movement, with the entire tail left exposed for testing. The laboratory also contained wooden boxes (30 cm long × 28 cm wide × 30 cm high), which served as chambers where rats were kept in isolation when brought to the laboratory from the adjacent colony room.

Drug

Morphine HCl (Faulding) dissolved in 0.9% physiological saline was used. The morphine dosages used were 5, 10, 15, and 20 mg/kg. Both morphine and saline were injected subcutaneously in the dorsal neck area at a volume of 1 ml/kg.

Procedure

Morphine/saline pretreatment (days 1–12). After handling, the rats were allocated to six weight-matched groups. The rats in three of these groups were injected with morphine (groups Mor,Sal-amb + Mor,Sal-hot + Mor,Mor-hot, n = 5, 10, 10, respectively), whereas those in the other three groups received saline (groups Sal,Sal-amb + Sal,Sal-hot+Sal,Mor-hot, n = 5, 10, 10, respectively). These injections took place in the colony room. The morphine dosage commenced at 5 mg/kg and was increased by 5 mg/kg every 3 days. By the end of the pretreatment period, the drug treated rats had received three injections of each of the four doses of morphine (i.e., 5, 10, 15, and 20 mg/kg), whereas the saline-treated rats received equivalent volumes of physiological saline. The injection regimen was discontinued for 5 days to allow the rats to recover from any acute withdrawal.

Familiarization. On each of days 15–17, the last 3 days of recovery from the morphine/saline injections, the rats were familiarized to the apparatus, and to the injection and restraint procedures. Familiarization was conducted to remove any hypoalgesia induced by novelty, the injection procedure, and restraint before the start of conditioning and testing (6). On each of these days, the rats were brought to the laboratory, given an injection of saline, and placed into the wooden boxes for 20 min. They were then exposed for 3 min to the floor of the hot-plate apparatus. The temperature of the water surrounding this floor was maintained at 23°C (amb). After each exposure, the floor was cleaned and wiped with 0.5% acetic acid solution to mask any stress odors. On these days, the rats were also familiarized for 12 min to tail-flick restraint. There was at least a 1.5 h interval between exposure to the 23°C floor and restraint.

Conditioning. On day 18, the rats were first familiarized to tail-flick restraint for 12 min. This was conducted before exposures to the drugs and the hot-plate apparatus to prevent any association between restraint and drug. Approximately 1.5 h later, the rats were brought to the laboratory, injected with saline (Sal) or morphine (Mor), and placed into the wooden chambers for 20 min. They were then exposed for 30 s to the floor of the hot-plate apparatus. The water surrounding this floor was maintained at 23°C (amb) or 54°C (hot). Thus, rats in groups Sal,Sal-amb and Mor,Sal-amb were given saline and exposed to the 23°C floor, rats in groups Sal,Sal-hot and Mor,Sal-hot received saline and were exposed to the 54°C

TABLE 1

MEAN AND STANDARD ERROR (SE) OF THE LATENCIES TO FIRST PAW-LICK (PLLS) FOR RATS IN EACH OF THE FOUR GROUPS EXPOSED TO THE HEATED FLOOR ON THE CONDITIONING DAY (DAY 18)

Groups	Mean PLLs (sec)	SE
Sal, Mor-hot	7.52	0.91
Mor, Mor-hot	5.71	0.86
Sal, Sal-hot	3.96	0.44
Mor, Sal-hot	4.03	0.24

floor, and rats in groups Sal,Mor-hot and Mor,Mor-hot were injected with 5 mg/kg morphine and exposed to the heated floor. Approximately 1.5 h after exposure to the 23°C or the 54°C floor, rats that had received saline were given a home cage injection of morphine in order to control for any nonspecific effects of the drug, whereas those that had received morphine were given a control injection of saline in their home cages. The latencies to first paw lick in response to placement on the heated floor were recorded with push buttons connected to a microprocessor.

Tail-flick tests. On day 19, the rats were brought to the laboratory, injected with saline, and placed in the wooden boxes for 20 min. They were then tested for TFLs immediately and at 2, 4, 6, and 8 min after removal from the wooden chambers.

Hot-plate test. On day 20, the rats were brought to the laboratory, injected with saline, and placed in the wooden boxes for 20 min. They were then tested for paw-lick latencies (PLLs) on the heated floor. The temperature of the water surrounding this floor was maintained at 52°C. PLLs were recorded in the manner described previously.

RESULTS

Conditioning (Hot Plate)

There was evidence that morphine induced hypoalgesia in drug-naive rats tested with morphine (Table 1). The contrast that tested for differences between group Sal,Mor-hot and the other three groups was significant, $F(1, 31) = 11.07$, $p = 0.003$. Pretreatment with morphine had rendered morphine-experienced rats tolerant to the hypoalgesic effects of the drug, because there were no reliable differences between group Mor,Mor-hot vs. groups Sal,Sal-hot + Mor,Sal-hot, $F = 3.08$, $p = 0.089$. However, this pretreatment did not alter nociceptive sensitivity in those tested with saline, because there were no significant differences between group Sal,Sal-hot and group Mor,Sal-hot, $F < 1.0$.

The latencies to tail flick and paw lick on the test sessions were analyzed using two sets of post hoc, orthogonal contrasts. With α set at 0.025 in each of these analyses, the critical *F*s calculated according to Rodger's (19) technique are 9.35 for the tail-flick tests ($df = 5, 44$) and 9.45 for the hot-plate test ($df = 5, 38$). Owing to a computer error on the hot-plate test day, data from three subjects from group Sal,Mor-hot and three from froup Mor,Mor-hot were not recorded. Thus, the analysis of the results from these two groups are based on $n = 7$.

Tail-flick tests. As can be seen from the left panel of Fig. 1, saline pretreated rats given separate exposures to morphine and the heated floor were hypoalgesic when subsequently tested for latencies to tail flick. This observation was confirmed by statistically significant differences between group Sal,Sal-hot and the other groups, $F = 53.02$, $p = 0.000$. In contrast, there was no evidence of hypoalgesia in morphine-tolerant rats that had received unpaired exposures to morphine and that floor, because there were no reliable differences between

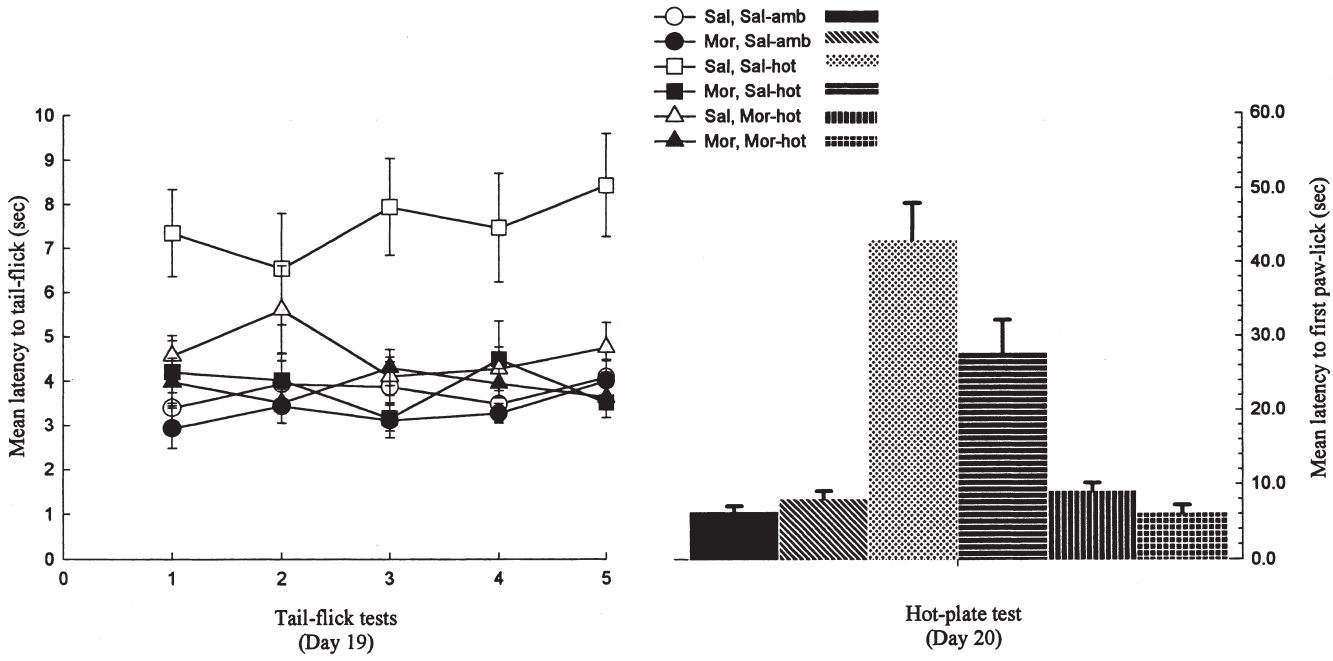

FIG. 1. The left panel shows the mean and ±SEM of the latencies to tail flick across the five consecutive test trials for rats in each of the six groups. The rats were pretreated with saline (Sal) or morphine (Mor) before conditioning with saline (Sal-) or morphine (Mor-) on the 54°C (hot) or 23°C (amb) floor. They were then tested with saline for latencies to tail flick immediately and at 2, 4, 6, and 8 min after removal from the wooden chambers. The right panel shows the mean latencies to first paw lick of rats in each of the groups when they were tested with saline on the heated floor the next day.

group Mor,Sal-hot and groups Sal,Mor-hot+Mor,Mor-hot + Sal,Sal-amb + Mor,Sal-amb, $F < 1.0$. There was also no evidence for hypoalgesia in morphine-naive or morphine-tolerant rats given paired exposures to morphine and the heated floor. The contrast that tested for differences between groups Sal,Mor-hot + Mor,Mor-hot vs. groups Sal,Sal-amb + Mor,Sal-amb was not significant, $F = 1.75$. Pretreatment with morphine did not affect nociceptive sensitivity/reactivity, because the contrasts that tested for differences between group Sal,Mor-hot vs. group Mor,Mor-hot and between group Sal,Sal-amb vs. group Mor,Sal-amb were not significant, Fs = 0.21 and 1.59, respectively.

Hot-Plate Test (Right Panel)

There was evidence that morphine-naive rats given separate exposures to morphine and the heated floor acquired hypoalgesia, because there were statistically significant differences between group Sal,Sal-hot and the other groups, $F = 62.65$, $p = 0.000$. Similarly, morphine-tolerant rats that had been exposed to the heated floor separately from morphine were also hypoalgesic. The contrast that tested for differences between group Mor,Sal-hot vs. groups Sal,Mor-hot+Mor,Mor-hot+Sal,Sal-amb + Mor,Sal-amb was significant, $F = 24.05$, $p = 0.000$. However, rats that had been given paired exposure to morphine and the heated floor failed to acquire hypoalgesia, as evidenced by the lack of statistically significant differences between groups Sal,Mor-hot + Mor,Mor-hot vs. groups Sal, Sal-amb + Mor,Sal-amb, $F < 1.0$. The contrasts that tested for differences between group Sal,Mor-hot vs. group Mor,Mor-hot and between group Sal,Sal-amb vs. group Mor,Sal-amb were not significant, $F < 1.0$.

DISCUSSION

The present results have confirmed that morphine-naive rats given separate exposures to a heat stressor and morphine acquire hypoalgesia, as evidenced by long latencies to tail flick (6) and paw lick (26). In contrast, morphine-tolerant rats given such exposures failed to display hypoalgesia when tested for latencies to tail flick, but then showed hypoalgesia when tested for latencies to paw lick. Taken together, these findings provide further evidence that expression of conditioned hypoalgesic responses by morphine-tolerant rats may depend on the pain assay used (6). However, morphine's blockade of acquisition of conditioned hypoalgesia was independent of the test measure. Morphine-naive and morphine-tolerant rats preexposed on the heated floor with the drug failed to show hypoalgesic responses on both the tail-flick and hot-plate (26) tests.

One explanation for the differential expression of conditioned hypoalgesic responses by morphine-tolerant rats might begin by considering the mechanisms mediating conditioned hypoalgesic responses elicited by a place associated with exposure to a heat stressor. Several findings suggest that the form of conditioned hypoalgesic responses elicited may depend on the pain test employed. Specifically, conditioned hypoalgesic responses observed with the hot-plate test appear to be based on non-mu mechanisms, because the long PLLs are neither reversed by naloxone nor cross-tolerant with morphine (5,17,18,25,26). In contrast, conditioned hypoalgesic responses detected with the tail-flick test are mediated by mu mechanisms, because the long TFLs are reversed by naloxone and are not acquired by morphine-experienced rats (6). The present study showed that morphine-tolerant rats failed to display conditioned hypoalgesia with the tail-flick test, but were then able to display conditioned hypoalgesia with the hot-plate test. This differential expression of conditioned hypoalgesia can be interpreted in terms of a cross-tolerance effect. That is, conditioned hypoalgesic responses among morphine-tolerant rats were not detected when such responses are mu opioid mediated (i.e., tail-flick test), but are observed when they are based on non-mu mechanisms (i.e., hot-plate test).

In conclusion, the present results have documented a critical role of the pain test used in determining whether morphine-tolerant rats express conditioned hypoalgesic responses. The importance of this factor may explain some of the inconsistencies in the literature regarding the evidence for contextually controlled morphine hypoalgesic tolerance and conditioned hyperalgesic responses [cf. (1,4,13,14,16,21–23)] and implicate the use of multiple pain measures in such studies [cf. (13)].

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