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Prior Hot Plate Exposure Enhances Morphine Analgesia in Tolerant and Drug-naive Rats

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SHERMAN, J. E., C. PROCTOR AND H. STRUB. *Prior hot plate exposure enhances morphine analgesia in tolerant and drug-naive rats.* PHARMAC. BIOCHEM. BEHAV. **17**(2) 229–232, 1982.—The associative model of morphine tolerance predicts that established tolerance should be attenuated, i.e., extinguished, by placebo injections in the former morphine injection environment. The present study examined the effect of placebo sessions, with and without accompanying nociceptive stimulation, on the extinction of analgesic tolerance. In Experiment 1, rats rendered tolerant to morphine displayed recovery of morphine's analgesic action only following placebo sessions including exposure to a painful hot plate surface (52.5°C); placebo sessions on a cool plate (23–24°C) failed to attenuate tolerance even though these placebo sessions more closely matched the stimulus conditions of tolerance acquisition. In Experiment 2, repeated hot plate exposures were similarly found to enhance morphine analgesia in drug-naive rats. These results question an extinction account of the effect of hot plate placebo sessions observed in Experiment 1. Instead, they suggest that nociceptive hot plate exposures, per se, are sufficient to enhance subsequent morphine analgesia.

Morphine analgesia Hot plate Extinction of tolerance Nociception Stress Rats

SIEGEL has recently elaborated a model of morphine tolerance that emphasizes a role for associative factors based on Pavlov's suggestion that the administration of a drug constitutes a conditioning trial [11,13]. Within this framework, environmental cues that reliably predict morphine administration serve as the conditioned stimulus (CS) and the drug serves as the unconditioned stimulus (UCS). According to the conditioning model of tolerance, learning this CS-UCS association results in the acquisition of compensatory, conditioned responses (CRs) that oppose the unconditioned responses (UCRs) elicited by the morphine UCS. Thus, the development of tolerance is explained by the conditioning of a hyperalgesic CR that progressively cancels the analgesic UCR as the CS-UCS association strengthens with repeated conditioning trials.

A unique prediction of the conditioning model is that such analgesic tolerance should be attenuated by the extinction of the hyperalgesic CR. That is, to the extent that morphine tolerance is due to the elicitation of a compensatory CR, presenting the drug-associated environmental cues unaccompanied by morphine should eventually extinguish the CR and, in turn, result in the recovery of morphine's analgesic action. Thus, following the development of tolerance, repeated placebo sessions (i.e., the administration of saline) in the presence of cues formerly associated with morphine should attenuate tolerance.

Although a number of experiments have supported this prediction [5, 11, 12, 14], exceptions have also been reported [10]. Moreover, the results of recent research pose a possible problem for the associative interpretation even of these supportive findings. Evidence now clearly suggests that exposing drug-naive rats to repeated nociceptive stimulation, or stress, can enhance subsequent responsiveness to the analgesic action of opiates ([3, 6, 7, 8]; for exception see [4]). Unfortunately, the procedures that have been used to extinguish the environment-drug association have incorporated such potentially stressful stimulation as massed saline injections [5], repeated restraint and forced limb extension [12], and explicit nociceptive thermal stimulation induced by hot plate exposure [11,14]. This argument would suggest that drug-naive rats receiving placebo "extinction" sessions similar to those employed in the morphine tolerance studies might also show an enhanced analgesic response to morphine. That is, the effect of the extinction procedures alone may have enhanced subsequent morphine analgesia independently of prior morphine experience. Thus it is possible that the enhanced analgesia following these extinction procedures might have been produced by their inherent stressfulness rather than by their uncoupling of the environment-drug association. None of these extinction studies included controls to assess this possibility.

Thus, the present study had two purposes: (1) It assessed the effect of placebo sessions with or without explicit nociceptive stimulation on the extinction of analgesic tolerance to morphine (Experiment 1) and (2) determined whether similar effects occur in drug-naive rats (Experiment

2). Nociceptive stimulation was induced by exposure to a hot-plate surface, the same stimulation accompanying successful demonstrations of extinction of tolerance [11,14].

METHOD

Subjects

Subjects were 41 (Experiment 1) and 21 (Experiment 2) male Sprague-Dawley rats obtained from Simonsen Laboratories in Gilroy, CA. The rats weighed between 310–355 g (Experiment 1) and 380–430 g (Experiment 2) on the first day of experiments. All rats were individually housed and had free access to food and water in their wire-mesh home cages. All procedures were conducted at least two weeks after the rats arrived at our laboratory and only during the light component of the 12 hr light-dark cycle. All rats were briefly handled on at least 10 separate occasions before the experiment started.

Apparatus and Drugs

Nociceptive thermal stimulation was provided with a standard hot plate apparatus. The hot plate consisted of a Haake E 51 water bath and pump that heated and circulated 52.5°C water through the channeled interior of an aluminum plate. A Plexiglas cylinder with an inner diameter of 22.1 cm, 29.2 cm high, and fitted with a removable lid, restrained the rat on the surface of the plate. The temperature of the water bath during cool plate placebo sessions was 23–24°C, approximately room temperature.

Constant white noise (62 dB) was maintained in the distinctive room but not in the home-cage environment. Ambient temperature in both environments was 23±1°C.

Morphine was administered via subcutaneous injection in the dorsal neck area. The dose was based on the following procedures: morphine sulfate was dissolved in 0.9% saline according to a concentration of 5 mg/ml; the volume injected was 1 ml/kg of the rat's weight. Saline injections were equivolume.

Procedure

During the tolerance development stage all rats in Experiment 1 were transported in individual wire mesh cages to the distinctive room in separate squads consisting of 13, 14, and 14 animals. Following a 10–24 min interval (constant for each rat), 31 of the 41 rats were injected with morphine and the remaining ten rats were injected with saline. At least three rats were injected with saline in each squad. Thirty min after injection each rats was placed on the surface of the cool plate for 45 sec. Immediately after the last rat in a squad was removed from the cool plate the squad was returned to the colony. This procedure was followed on each of three consecutive days.

Following the three tolerance development sessions the 31 morphine-experienced rats were randomly assigned to one of three groups. Morphine (M) rats assigned to the two placebo extinction groups received nine placebo sessions, one per day, in which saline was now administered in the distinctive room; thirty min after saline was injected, rats in Group M-HP (n=10) were placed on the hot plate (HP) surface for 45 sec, whereas rats in Group M-CP (n=11) were placed on the cool plate (CP) for 45 sec. The remaining morphine-experienced rats, Group M-R (n=10), simply rested (R) undisturbed in their home cages during this period to control for changes in tolerance that might occur merely with the passage of time. The morphine-naive rats, i.e., those injected with saline during their "tolerance development" stage, Group S-R (n=10), also rested undisturbed during this period.

On the day following the last placebo session, all rats were transported to the distinctive room and were injected with morphine. Thirty min later each rat was placed on the hot plate surface and latency to the first paw-lick or jump response was measured; rats were removed from the hot plate after 45 sec.

In Experiment 2, two groups of rats were treated identically to Groups M-HP and M-CP of Experiment 1 with the important exception that saline rather than morphine was injected during the "tolerance development" stage of training. Thus, Group S-HP (n=10) and Group S-CP (n=11) received three daily saline injections in the distinctive room environment, that included exposure to the cool plate. Nine daily placebo "extinction" sessions followed. Group S-HP was exposed to the hot-plate surface for 45 sec during each placebo session, whereas Group S-CP was exposed to the cool plate surface. On the test day both groups were injected with morphine for the first time and 30 min later received a hot-plate test for analgesia.

All statistical tests were conducted with the analysis of variance (ANOVA). The rejection criterion for all tests of significance was $p<0.05$. Hartley's test of homogeneity of variance on the data obtained in Experiment 1 revealed significant differences in variance, $F_{max}(4,10)=15.13$ [15]. Consequently, the data from both experiments were submitted to a square-root transformation prior to performing the ANOVA.

RESULTS

Figure 1 presents the mean hot plate response latencies for all groups in Experiments 1 (Panel A) and 2 (Panel B) following the test administration of morphine. As suggested in Panel A of the figure, a one-way ANOVA revealed significant differences among the groups, $F(3,37)=9.66$. Planned pair-wise comparisons indicated that the morphine-experienced rats of Group M-R displayed significantly shorter response latencies than the morphine-naive rats of Group S-R, $F(1,37)=17.8$. This comparison indicates that the three prior morphine administrations were sufficient to induce tolerance. Group M-CP failed to show that placebo extinction sessions on the cool plate attenuated tolerance to the analgesic action of morphine; Group M-CP did not significantly differ from Group M-R, $F(1,37)=1.11$. However, the rats of Group M-HP, which received placebo sessions that included explicit nociceptive thermal stimulation induced by hot-plate exposure, were significantly more analgesic than either of the equally morphine-experienced rats of Groups M-R and M-CP, $Fs(1,37)=4.91$ and 7.42, respectively.

The results of Experiment 2 (see Panel B) show that drug-naive rats given repeated placebo "extinction" sessions on the hot plate (Group S-HP) displayed significantly greater morphine analgesia than drug-naive rats receiving placebo sessions on the cool plate (Group S-CP), $F(1,19)=6.21$. It should be noted that comparisons of absolute response latencies across the two experiments is not appropriate because these experiments were conducted at different times and with different shipments of animals.

DISCUSSION

The results of the present study indicate that repeated

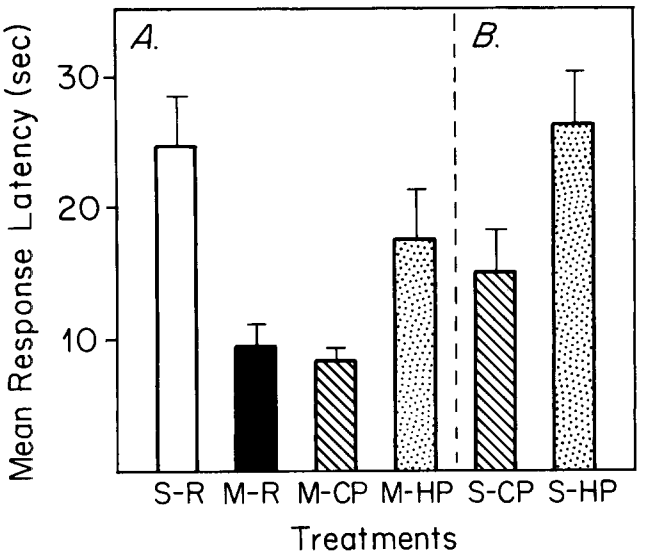

FIG. 1. Mean response latencies (±1 SEM) on the hot plate following the test administration of morphine in Experiments 1 (Panel A) and 2 (Panel B). M (morphine) and S (saline) refer to the drug given prior to the treatments: HP (hot plate), CP (cool plate) and R (rest undisturbed).

nociceptive stimulation, induced by exposure to a hot plate, enhances subsequent morphine analgesia in both tolerant (Experiment 1) and drug-naive (Experiment 2) rats. Moreover, placebo extinction sessions including exposure to a cool plate failed to attenuate tolerance (Experiment 1). These results challenge Siegel's [11,14] extinction interpretation of the effects of hot plate placebo sessions on analgesic tolerance to morphine. Three lines of evidence support this conclusion.

First, according to the conditioning model of tolerance, placebo sessions are assumed to attenuate tolerance by extinguishing the environment-morphine (CS-UCS) association. Generally, extinction is best observed when the CS presented alone in identical to the CS previously paired with the UCS [9]. Consequently, in Experiment 1 greater extinction of tolerance would be expected for Group M-CP than Group M-HP because for the former group placebo extinction sessions were identical to the tolerance development sessions, whereas for the latter group such sessions differed because of the introduction of the thermal hot plate stimulation. It should be noted that the conditioning model of tolerance does not view nociception or stress as a necessary concomitant of placebo sessions. If the acquisition of tolerance proceeds without nociceptive stimulation or stress, placebo sessions without such stimulation should successfully extinguish tolerance. Thus, the results of Experiment 1 were opposite to those expected on the basis of the conditioning model of tolerance.

Second, the results of Experiment 1 taken together with those obtained by Sherman [10], Siegel [11] and Siegel *et al.* [14] clearly show that hot-plate temperature importantly influences the degree of attenuated tolerance following a similar number of placebo sessions. When placebo sessions have been conducted with a 52.5°C or hotter temperature, attenuation of tolerance has been consistently obtained ([11,14], and Experiment 1 of the present study). When the temperature of the hot plate has been 51°C the results have been mixed; sometimes attenuation of tolerance has been observed [14], and sometimes it has not been observed [10,14]. Without exception, attenuation of tolerance has not been observed following placebo sessions conducted on a cool plate (23–24°C) ([10], and Experiment 1 of the present study). Thus, these results, all obtained with the same dose of morphine, following a highly comparable number of tolerance development sessions (3 or 4) and subsequent placebo extinction sessions (9 or 10), reveal a highly consistent pattern linking the degree of thermal nociceptive stimulation sustained on the hot plate with the recovery of morphine's analgesic action in tolerant rats. This pattern of results is not predicted by the conditioning model of tolerance.

Third, these results extend with thermal stimulation recent studies showing that repeated exposure to a variety of other nociceptive stressors enhances subsequent opiate analgesia [3, 6, 7, 8]. Importantly, in the present study under the same conditions in which hot plate placebo sessions appeared to reduce analgesic tolerance to morphine (Experiment 1), they also enhanced responsiveness to morphine in drug-naive rats (Experiment 2). Perhaps the most parsimonious explanation for these data is that repeated placebo sessions on the hot plate are sufficient to enhance subsequent morphine analgesia in both morphine-tolerant and drug-naive rats. In all prior studies of extinction of analgesic tolerance no assessment of the effect of placebo sessions, per se, were conducted. Thus, evidence for an associative interpretation of the effect of placebo sessions in morphine tolerant rats remains equivocal.

Although the present experiments call into question several studies currently supporting an associative account of morphine tolerance [5, 11, 12, 14] they do not challenge the general conclusion that associative factors importantly modulate responsiveness to opiates. The present experiments only address those studies in which exposure to nociceptive or stressful stimulation, and perhaps only hot plate stimulation, has preceded assessments of morphine analgesia.

Interestingly, the effect of hot plate exposures on subsequent morphine analgesia appears to depend on whether morphine accompanies the nociceptive stimulation. In the present study tolerant or drug-naive rats exposed to the hot plate subsequently displayed enhanced morphine analgesia. However, it has also been shown [1,2] that if morphine accompanies exposure to the hot plate, subsequent morphine analgesia is diminished, i.e., tolerance develops more quickly. Thus, the effect of hot plate exposure on morphine analgesia appears to depend on complex experiential and pharmacological interactions.

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REFERENCES

1. Adams, W. J., S. Y. Yeh, L. A. Woods and C. L. Mitchell. Drug-test interaction as a factor in the development of tolerance to morphine. *J. Pharmac. exp. Ther.* **168:** 251–257, 1969.

2. Bardo, M. T., P. J. Wellman and R. A. Hughes. The role of hot plate and general environmental stimuli in morphine analgesic tolerance. *Pharmac. Biochem. Behav.* **14:** 757–760, 1981.

3. Belenky, G. L. and J. W. Holaday. Repeated electroconvulsive shock (ECS) and morphine tolerance: Demonstration of cross-sensitivity in the rat. *Soc. Neurosci. Abstr.* **5:** 549, 1979.
4. Bodnar, R. J., D. D. Kelley, S. S. Steiner and M. Glusman. Stress-produced analgesia and morphine-produced analgesia: Lack of cross-tolerance. *Pharmac. Biochem. Behav.* **8:** 661–666, 1978.
5. Cappell, H. and C. X. Poulos. Associative factors in tolerance to morphine: dose-response determination. Paper presented in the program of Division 28, American Psychological Association, Toronto, 1978.
6. Colpaert, F. D., C. F. E. Niemegeers, P. A. J. Janssen and A. N. Maroli. The effects of prior fentanyl administration and of pain on fentanyl analgesia: Tolerance to and enhancement of narcotic analgesia. *J. Pharmac. exp. Ther.* **213:** 418–424, 1980.
7. Grau, J. W., R. L. Hyson, S. F. Maier, J. Madden IV and J. D. Barchas. Long-term stress induced analgesia and activation of the opiate system. *Science* **213:** 1409–1410, 1981.
8. Lewis, J. W., J. E. Sherman and J. C. Liebeskind. Opioid and non-opioid stress analgesia: Assessment of tolerance and cross-tolerance with morphine. *J. Neurosci.* **1:** 358–363, 1981.
9. Mackintosh, N. J. *The Psychology of Animal Learning*. London: Academic Press, 1974.
10. Sherman, J. E. The effects of conditioning and novelty on the rat's analgesic and pyretic responses to morphine. *Learn. Motivat.* **10:** 383–418, 1979.
11. Siegel, S. Evidence from rats that morphine tolerance is a learned response. *J. comp. physiol. Psychol.* **89:** 498–506, 1975.
12. Siegel, S. Morphine tolerance acquisition as an associative process. *J. exp. Psychol.: Anim. Behav. Processes* **3:** 1–13, 1977.
13. Siegel, S. The role of conditioning in drug tolerance and addiction. In: *Psychopathology in Animals*, edited by J. D. Keehn. New York: Academic Press, 1979.
14. Siegel, S., J. E. Sherman and D. Mitchell. Extinction of morphine analgesic tolerance. *Learn. Motivat.* **11:** 289–301, 1980.
15. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.