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BRIEF COMMUNICATION

Naloxone-Induced Hypoalgesia: Evidence From the Formalin Test

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FOO, H. AND R. F. WESTBROOK. *Naloxone-induced hypoalgesia: Evidence from the formalin test.* PHARMACOL BIOCHEM BEHAV 45(2) 501-505, 1993.—Two experiments examined the hypoalgesic effects that accrue from pairing exposure to a heat stressor with the opioid antagonist naloxone. Experiment 1 confirmed that rats given separate exposures to a heated floor and to naloxone are hypoalgesic when then tested on that floor. Further, the results confirmed that pairing the initial exposure to the heat stressor with naloxone resulted in a more profound hypoalgesic response. Experiment 2 provided new evidence for the hypoalgesic effects of separate exposures to the heat stressor and to naloxone, and for naloxone's enhancement of acquisition of the hypoalgesia, in rats tested for responsiveness to formalin. These results, therefore, demonstrate generalisability of the hypoalgesic effects across assays for acute and chronic pain.

Naloxone Stressor Hypoalgesia Hot-plate Formalin

RECENT evidence has shown that exposures to various stressors with an opioid antagonist can come to induce hypoalgesia (2,3,7-12,14-19). For example, when repeatedly exposed to a heated floor with naloxone rats paw-lick with the same latencies as saline-treated ones on the initial exposure but paw-lick with longer latencies on subsequent exposures. There is also evidence that this naloxone-induced hypoalgesic response is mediated by associative learning processes. First, the increases in paw-lick latencies are a consequence of pairing naloxone with exposure to the heat stressor because such latencies are not detected in rats given separate exposures to the drug and heat stressor (7-11,14,15,17) or in those given combined exposures to the drug and apparatus (9,15). Second, the long latencies to paw-lick are contextually controlled, detected when rats are tested in the place associated with naloxone-stressor pairings but not when they are tested in the place associated with saline-stressor ones (17). Third, these latencies are not a result of state-dependent learning because the long latencies are detected when rats are tested with saline where they had received naloxone-stressor pairings (7,9-11,15,17-19).

The hypoalgesia induced by naloxone-heat stressor pairing appears to be due to the drug's ability to increase the aversive-hypoalgesic properties of the stressor. Rats exposed to a heated floor are not only hypoalgesic when subsequently tested on that floor but also more afraid of that place. This increase in fear is evidenced by the longer latencies with which rats preexposed to the heat stressor take to step-down from a platform onto the nonheated floor of the hot-plate apparatus in comparison to those preexposed to a nonheated floor (18). Moreover, the long latencies to paw-lick and step-down are enhanced by pairing the initial exposure to the heated floor with naloxone (17,18). Finally, the long latencies to paw-lick resulting from exposure to naloxone-heat stressor pairings are not specific to the use of a heated floor as the stressor because rats exposed to pairings of a cold floor with naloxone are hypoalgesic when then tested with saline on a heated one (9).

Thus far, naloxone-induced hypoalgesia has been evidenced by the latencies with which rats come to lick their paws on a heated floor (2,3,7-12,14-19) or flick their tails in response to the application of heat (15). It is possible, therefore, that the hypoalgesia is selective to a reduction of nociceptive sensitivity to noxious thermal stimulation. The pain elicited by the application of heat to the rat's paws in the hot-plate test, or to its tail in the tail-flick test, is acute and localized and has been described as "sharp pricking" (1) or "phasic" (4). This type of pain differs from that associated with tissue damage, such as the one produced by an injection of dilute formalin into the rat's paw (5). The pain following tissue injury is chronic and diffused and has been described as "dull continuous" or "tonic" (4).

Consequently, the present study used a chronic pain measure, the formalin test, to assess nociceptive sensitivity of rats

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given separate exposures to a heat stressor and to naloxone or a combined exposure to that stressor and the drug. However, to provide evidence for the hypoalgesic effects of these pairings with an acute pain measure Experiment 1 aims to demonstrate a) hypoalgesia in rats trained and tested on a 52°C floor and b) naloxone's ability to enhance acquisition of this conditioned hypoalgesic response. Experiment 2 examined a) whether hypoalgesia is observed in rats trained on a 52°C floor and tested with formalin and b) whether naloxone-induced hypoalgesia is detected in rats trained on a heated floor with naloxone and tested with formalin. In each of these experiments, four groups of rats were used in a 2 × 2 design. On training, rats in group Nal-hot were exposed to a pairing of naloxone and the heated floor, while those in group Sal-hot received separate exposures to the drug and that floor. To control for the effects of naloxone on novelty-induced hypoalgesia (9), two other groups were included. The rats in group Nal-amb were given a pairing of naloxone and the nonheated floor of the hot-plate apparatus, while those in group Sal-amb received separate exposures to the drug and that floor. Rats were then tested for nociception on the heated floor (Experiment 1) or to formalin (Experiment 2). These experiments were conducted separately but are presented together for convenience of exposition.

METHOD

Subjects

There were 20 experimentally naive, male Wistar rats in each of the experiments. They weighed an average of 400 g (range 330–450 g) in Experiment 1 and 365 g (range 258–440 g) in Experiment 2. They were obtained from the Specific Pathogen Free Unit (Little Bay, Sydney) and were housed in plastic boxes (65 × 40 × 22 cm) across the course of the experiment. Rats were housed five to a box and had continuous access to food and water. These boxes were kept in a colony room maintained on a normal 12 L : 12 D cycle.

Apparatus

The hot-plate apparatus consisted of a Plexiglas chamber (24 × 48 cm, diameter × height) with a copper floor (1 mm thick) fixed 12 cm above the base of the chamber. The portion of the chamber below the copper floor was perforated with 3-cm diameter holes to permit the circulation of water under the floor. The chamber stood in a water bath whose temperature was maintained by a Haake D1 Open Bath Circulator. The laboratory also contained wooden boxes (30 × 27 × 30 cm) with transparent Plexiglas doors that served as chambers where rats were kept in isolation when brought to the laboratory from the adjacent colony room.

Drug

Naloxone HCl (E. I. duPont de Nemours & Co., Wilmington, DE) was used. The drug was dissolved in 0.9% w/v saline solution and was given at a dosage of 2.5 mg/kg. Both naloxone and physiological saline were injected SC into the dorsal area of the neck at a volume of 1.0 ml/kg.

Procedure

Training. On day 1, rats in two of the groups were injected with either naloxone (group Nal-hot) or saline (group Sal-hot), placed in the wooden chambers for 30 min, and then exposed for 60 s to the heated floor of the hot-plate apparatus. The temperature of the water surrounding this floor was maintained at 51.5 ± 0.5°C. Rats in the remaining two groups were given either naloxone (group Nal-amb) or saline (group Sal-amb) and placed in the wooden chambers for 30 min. They were then exposed for 60 s to the nonheated floor of the hot-plate apparatus, whose temperature was maintained at 23°C. Three hours after exposure to the heated or nonheated floor, rats in groups Sal-hot and Sal-amb were given naloxone to control for any nonspecific effects of the drug, while those in groups Nal-hot and Nal-amb were given a control injection of saline. These injections took place in the colony room. The latencies with which rats licked their paws on the heated floor were recorded with push-buttons connected to a microprocessor.

Test. On day 2, rats were tested for their latencies to paw-lick on the heated floor or for their responsiveness to lift/lick a formalin-injected paw. In Experiment 1, rats were taken to the laboratory, injected with saline, and placed in the wooden chambers for 30 min. They were then tested for 60 s on the heated floor. The latencies to the first paw-lick were recorded in the manner described previously. In Experiment 2, the rat was taken to the laboratory and anesthetised with halothane. Formalin (0.05 ml, 3%) was then injected SC into the dorsal surface of the right forepaw. After recovery from the anesthetic (approximately 12 min), the rat was injected with saline, placed in the wooden chamber for 30 min, and then exposed to the nonheated floor for 5 min. The occurrences of lifting or licking the injected paw in the wooden chambers and on the nonheated floor were recorded on a 2-s time sampling schedule.

RESULTS

There were no differences in the latencies with which rats in groups Nal-hot and Sal-hot licked their paws on the initial exposure to the heated floor ($p < 0.05$, mean latencies = 13.7 and 10.1 s, respectively). The mean latencies with which rats in each of the groups in Experiment 1 licked their paws in response to the heated floor, on test, are shown in Fig. 1. These results were analysed with a set of planned, orthogonal contrasts (12). With $\alpha = 0.05$, df = 1 and 16, the critical F is 4.49. It is clear that rats preexposed to the heated floor took longer to lick their paws than those preexposed to the nonheated one. Thus, evidence for hypoalgesia resulting from preexposure to the saline- or the naloxone–heat stressor pairing were the reliable differences in paw-lick latencies between rats in groups Sal-hot and Nal-hot vs. those in groups Sal-amb and Nal-amb, $F = 32.45$. There was also evidence that acquisition of this hypoalgesia was enhanced by pairing the initial exposure to the heated floor with naloxone. The contrast that tested for differences in paw-lick latencies between rats in group Sal-hot vs. those in group Nal-hot was significant, $F = 25.79$. This enhancement was not due to naloxone maintaining or enhancing the hypoalgesic properties of a novel environment because there were no significant differences in paw-lick latencies between rats in group Sal-amb vs. those in group Nal-amb ($F = 0.20$).

There were no differences in the latencies with which rats in groups Nal-hot and Sal-hot licked their paws on exposure to the heated floor ($p < 0.05$, mean latencies = 12.5 and 11.9 s, respectively). However, the results of interest are the subsequent responses of rats in each of the four groups in Experiment 2 to lift/lick a formalin-injected paw. The left panel of Fig. 2 shows the mean number of paw-lifts and paw-licks for rats in each of these groups across the 30-min expo-

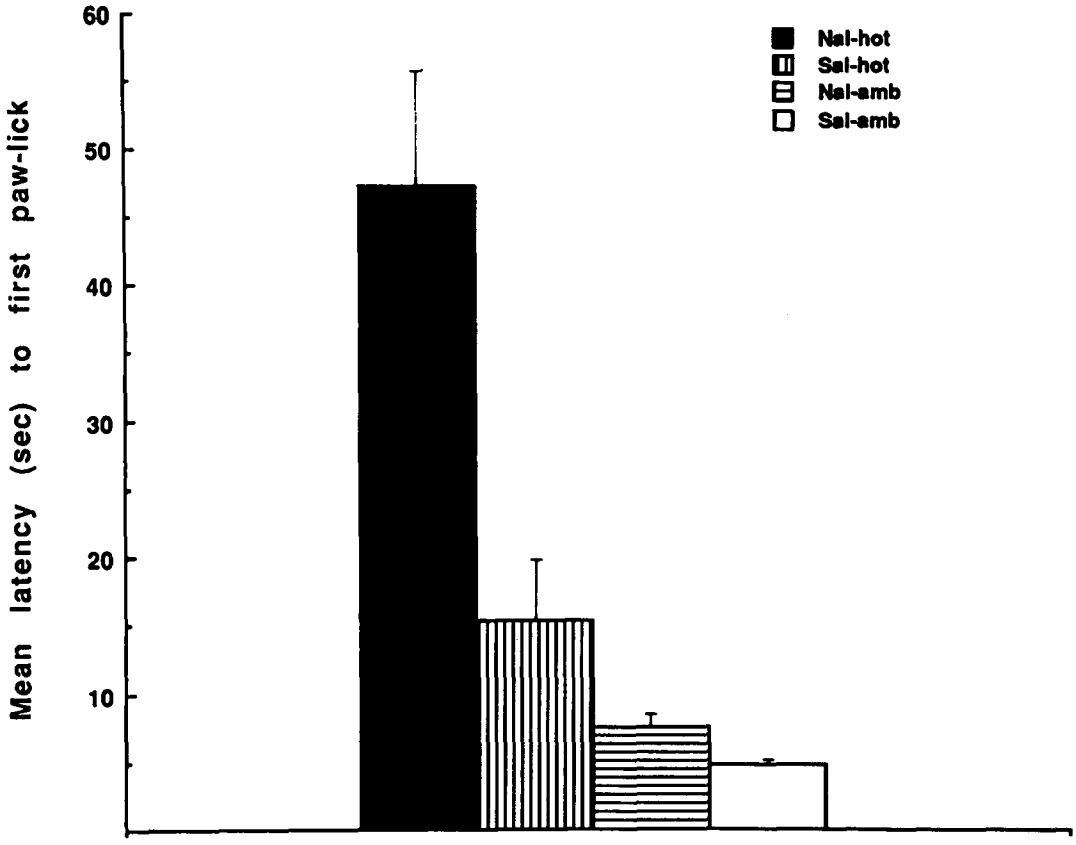

FIG. 1. Mean (and SEM) latencies to the first paw-lick for rats in each of the four groups in Experiment 1 in response to testing on the heated floor. On conditioning, rats in two of these groups were given combined exposures to naloxone and the heated (group Nal-hot) or the nonheated (group Nal-amb) floor. Rats in the remaining two groups were given separate exposures to naloxone and the heated (group Sal-hot) or the nonheated (group Sal-amb) floor. On test, all rats were injected with saline and then exposed to the heated floor.

sure to the wooden chambers. These results were analysed with the set of planned, orthogonal contrasts used in Experiment 1 (1 and 16 df; $\alpha = 0.05$; $F_{critical} = 4.49$). It is clear that rats preexposed to the heated floor responded less to a formalin-injected paw than those preexposed to a nonheated one. Thus, there were statistically significant differences in the number of paw-lifts and paw-licks between rats in groups Sal-hot and Nal-hot vs. those in groups Sal-amb and Nal-amb, $F = 10.92$. Acquisition of this hypoalgesia was enhanced by pairing exposure to the heated floor with naloxone because there were reliable differences in the frequency to lift/lick the formalin-injected paw between rats in group Sal-hot vs. those in group Nal-hot, $F = 4.60$. Further, there was no evidence that naloxone had maintained the hypoalgesic properties of the novel environment because there were no reliable differences in the number of paw-lifts and paw-licks between rats in group Sal-amb vs. those in Nal-amb ($F = 0.55$).

The right panel of Fig. 2 depicts the mean number of paw-lifts and paw-licks for rats in each of the groups in Experiment 2 across the 5-min exposure to the nonheated floor. An analysis of these results also revealed a hypoalgesic effect of preexposure to the heated floor (groups Sal-hot and Nal-hot vs. groups Sal-amb and Nal-amb, $F = 8.46$). However, there was no evidence for enhancement in acquisition of hypoalgesia by pairing naloxone with preexposure to the heat stressor (group Sal-hot vs. group Nal-hot, $F = 0.54$). In contrast, there was evidence for a hypoalgesic effect of pairing naloxone with exposure to the nonheated one (group Sal-amb vs. group Nal-amb, $F = 5.21$).

DISCUSSION

The results of Experiment 1 have confirmed two previous findings. First, rats given separate exposures to a heated floor and to naloxone are hypoalgesic when subsequently tested on that floor (17–19) and, second, naloxone given in combination with exposure to the heat stressor enhanced acquisition of the hypoalgesia (2,3,7–12,14–19). Experiment 2 provided evidence that these effects are not selective to the inhibition of phasic nociceptive stimulation because rats given separate exposures to the heated floor and to naloxone were hypoalgesic when then tested for responsiveness to formalin. Further, there was evidence for an enhancement in acquisition of hypoalgesia by pairing exposure to the heated floor with naloxone, that is, rats exposed to the heated floor with the drug were more hypoalgesic, when subsequently tested for responsiveness to formalin, than those given separate exposures to the heat stressor and naloxone.

The present results have shown that the hypoalgesic response resulting from separate exposures to the heat stressor

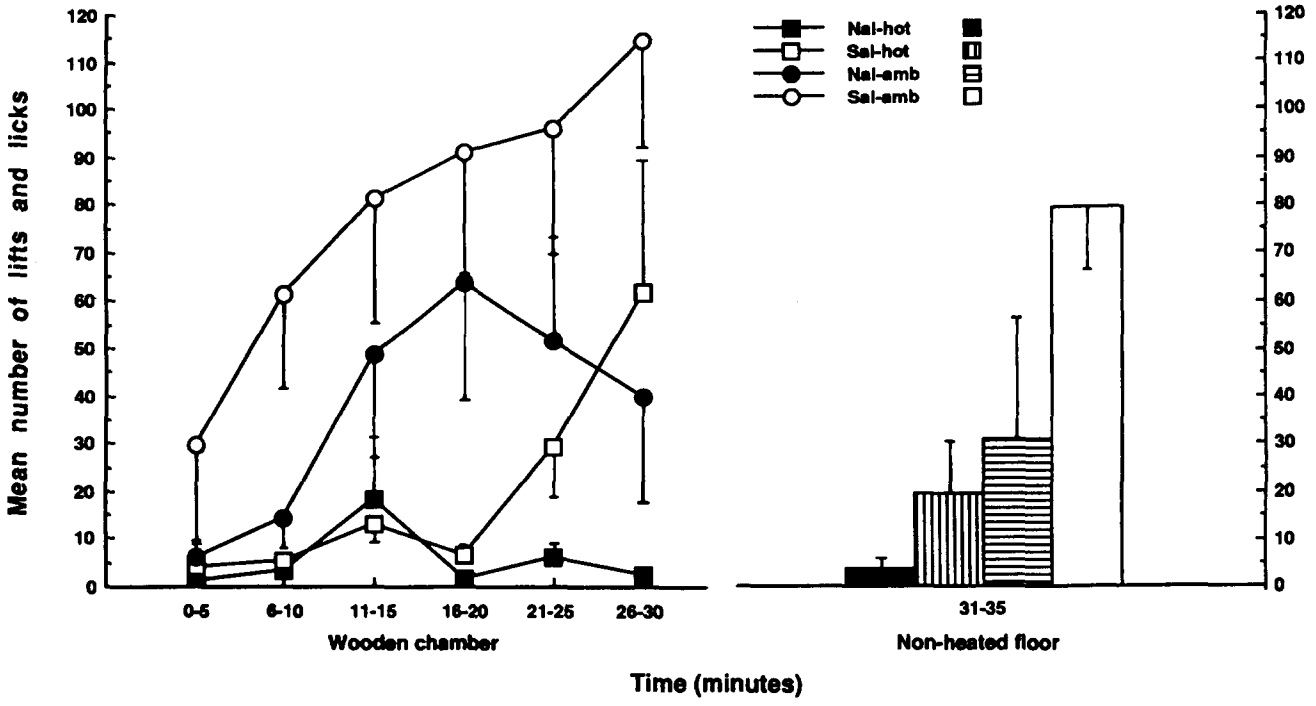

FIG. 2. The mean (and SEM) number of lifts and licks in response to formalin for each of the four groups in Experiment 2 across the 30-min exposure to the wooden chambers (left panel) and the 5-min exposure to the nonheated floor (right panel). On conditioning, rats in two of these groups were given combined exposures to naloxone and the heated (group Nal-hot) or the nonheated (group Nal-amb) floor. Rats in the remaining two groups were given separate exposures to naloxone and the heated (group Sal-hot) or the nonheated (group Sal-amb) floor. On test, all rats were injected with saline and then assessed for responsiveness to formalin for 30 min in the wooden chambers, followed by 5 min on the nonheated floor.

and to naloxone is not specific to some property of testing on the heated floor or to exposure to the hot-plate apparatus per se. Specifically, Experiment 2 provided evidence for hypoalgesia in rats tested for responsiveness to formalin in the hot-plate apparatus that was the source of the noxious, thermal stimulation, as well as in the wooden boxes that were associated with exposure to that stressor. Further, the results demonstrated that acquisition of conditioned hypoalgesia was enhanced in rats exposed to the heat stressor with naloxone. However, evidence for this enhancement was selective to the wooden boxes that had been paired with exposure to the heated floor rather than to the hot-plate apparatus itself. An examination of the data suggests that the lack of differences may be due partly to a floor effect in the paw-lift/lick responses of rats in group Nal-hot. Presumably, then, a more sensitive test might have detected enhancement of the hypoalgesia.

A further discrepancy in the present findings was in naloxone's effects upon rats given the drug in combination with exposure to the nonheated floor. It may be recalled that rats given a combined exposure to naloxone and to the nonheated floor (group Nal-amb) were hypoalgesic when tested for responsiveness to formalin on the nonheated floor (Experiment 2) but not when tested for sensitivity to the heated floor (Experiment 1). However, there is evidence to suggest that naloxone, when given in combination with repeated exposures to the nonheated floor, comes to maintain the hypoalgesic properties of the novel apparatus (9). A number of factors may account for the present failure to replicate this finding, for example, the relatively small sample size used.

There is evidence to suggest that naloxone comes to produce enhancement in acquisition of the conditioned hypoalgesia by acting upon central mechanisms (16) and, more specifically, upon supraspinal receptors (11). Naloxone's blockade of such receptors could mediate increase in acquisition of the conditioned hypoalgesia in at least two conceivable ways. For example, it is possible to suppose that naloxone's blockade of opioid receptors alters the sensitivity of endogenous pain control mechanisms so as to produce an unconditioned hyperalgesia. This hyperalgesic response could serve to provoke a compensatory hypoalgesia whose activation becomes conditionally activated by cues that have been paired with the hyperalgesic response (3,14,15). However, several investigators have reported acquisition of conditioned hypoalgesia resulting from preexposure to naloxone–heat stressor pairing in the absence of an unconditioned hyperalgesic response (7–11,15,17–19). Alternatively, naloxone's blockade of opioid receptors could be viewed in terms of its impact upon a motivational system so as to augment the level of fear conditioned by the heat stressor (6). For example, there is evidence that rats preexposed to the heated floor take longer to step-down onto the nonheated floor of the hot-plate apparatus than those preexposed to the nonheated floor (18). Moreover, rats preexposed to the heated floor with naloxone take even longer to step-down than those preexposed to the heated floor with saline, suggesting that naloxone increases aversive conditioning to the heat stressor (18). Further, if it is assumed that the motivational system that mediates fear has inhibitory connections with the one that modulates pain (6) the increase in aversive

conditioning would result in a concomitant increase in hypoalgesia.

In conclusion, the present experiments have generalised the conditioned hypoalgesic effect of separate exposures to a heat stressor and to naloxone, and an enhancement of its acquisition by pairing exposure to that stressor with naloxone, to the inhibition of chronic pain. These results may have some implications for the therapeutic use of naloxone in pain management because the formalin test has been employed to model continuous pathologic pain.

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