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BRIEF COMMUNICATION

Naloxone-Induced Hypoalgesia: Effects of Noradrenergic Antagonists and Agonist

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FOO, H. AND R. F. WESTBROOK. *Naloxone-induced hypoalgesia: Effects of noradrenergic antagonists and agonist.* PHARMACOL BIOCHEM BEHAV **39**(3) 795–797, 1991.—The present experiments confirmed that rats injected with naloxone and exposed to a heated floor acquired a hypoalgesic response, as indexed by the latencies to lick their paws. The expression of these latencies were unaffected by yohimbine, clonidine, propranolol, or by relatively moderate doses of prazosin, suggesting that the conditioned hypoalgesic response induced by pairings of naloxone and a heated floor is not mediated by the release and turnover of noradrenaline.

Naloxone Hypoalgesia Noradrenaline Yohimbine Clonidine Prazosin Propranolol

REPEATEDLY injected with the opioid antagonist, naloxone, and tested in a hot-plate or tail-flick apparatus, rats acquire a hypoalgesic response, as indexed by their latencies to lick their paws or flick their tails (5–9). The acquisition of long latencies to paw lick is a function both of the concentration of the drug (range 0.7–5.0 mg/kg) at the time of exposure to the heated floor, and of the temperature of that floor (9). These increases in latencies are mediated by conditioning processes because they are a) contingent upon pairing of the drug and the heat stressor (5, 6, 9), b) specific to the place where these pairings occurred (9), and c) can be detected there in the absence of the drug (6, 8, 9).

There is evidence that this conditioned hypoalgesia involves neural, rather than hormonal, mechanisms because acquisition of the long latencies to paw lick was not impaired by either hypophysectomy or adrenalectomy (5). Further, this neurally mediated conditioned hypoalgesic response is nonopioid in nature because the long paw lick latencies are a) detected in the presence of the drug (5–9), b) do not show cross-tolerance with morphine (8,9), and c) do not decrease across repeated pairings of the drug and exposure to the heated floor (5–7, 9).

Evidence from pharmacological studies suggests that neurally mediated, nonopioid form of conditioned hypoalgesia is mediated by noradrenergic mechanisms. Specifically, acquisition of the conditioned hypoalgesia induced by pairings of a distinctive environment with brief continuous footshock was cross tolerant with clonidine hypoalgesia, whereas expression of the conditioned hypoalgesia was reversed by yohimbine but not by phentolamine, suggesting that the effects were due to the release of NE and its subsequent action upon alpha adrenoceptors (1–4). Consequently, the present experiments examined whether noradrenergic mechanisms are involved in the expression of the conditioned hypoalgesia induced by pairings of naloxone and a heat stressor. If mediated by the release and turnover of noradrenaline, then the long paw-lick latencies detected in the place where these pairings had occurred should be a) reversed by yohimbine; b) enhanced by clonidine and c) prazosin; but d) unaffected by propranolol, a nonselective beta adrenergic blocker. These experiments were conducted separately but are presented together for convenience of exposition.

METHOD

Animals

The animals used in these studies were male, Wistar rats with an average weight of 347 g (range 292–400 g). They were obtained from the University of New South Wales Animal Breeding and Housing Unit. Throughout the course of the experiment, they were housed in plastic laboratory boxes (60 × 40 × 20 × cm), with a maximum of 5 rats per box, and with continuous access to food and water.

Apparatus

The hot-plate apparatus consisted of a 24 × 48 cm (diameter × height) Plexiglas chamber with a copper floor (1 mm thick) that stood 12 cm above the base of the chamber. The portion below the copper floor was drilled with 3 cm diameter holes to permit the circulation of water below the floor. This chamber

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stood in a water bath whose temperature was maintained at 51.5 ± 0.5°C by a Haake D1 circulator. This resulted in a floor temperature of 50.5°C as measured by a digital thermometer (Anritsu). The laboratory also contained wooden boxes (30 × 30 × 30 cm) with air holes drilled to the sides. These served as chambers where rats were kept in isolation from each other when brought to the laboratory.

Drugs

The drugs used were naloxone-HCl (E.I. duPont de Nemours & Company, USA), yohimbine-HCl (Sigma, USA), clonidine-HCl (Sigma, USA), prazosin-HCl (Pfizer, Australia) and propranolol-HCl (I.C.I., Australia). Prazosin was dissolved in a ratio of 1 to 50, glycerol to saline. The vehicle for all the other drugs was 0.9% w/v saline. All drug solutions were prepared immediately before injections, and were administered at a volume of 1.0 ml/kg. Naloxone was injected subcutaneously in the dorsal neck area at a dose of 2.5 mg/kg. The noradrenergic drugs were injected intraperitoneally and at the following dosages: yohimbine (0.625, 1.25, 2.5, 5.0 mg/kg), clonidine (0.003125, 0.00625, 0.0125, 0.025 mg/kg), prazosin (0.01, 0.05, 0.1, 1.0 mg/kg) and propranolol (1.25, 2.5, 5, 10 mg/kg).

Procedure

In all experiments, the rats were handled and weighed each day for three days. Rats trained with naloxone-stressor pairings were allocated to five weight-matched groups (n = 10 in Experiments 1 and 2; n = 8 in Experiments 3 and 4). On each of the three training days, subjects were injected with naloxone and placed

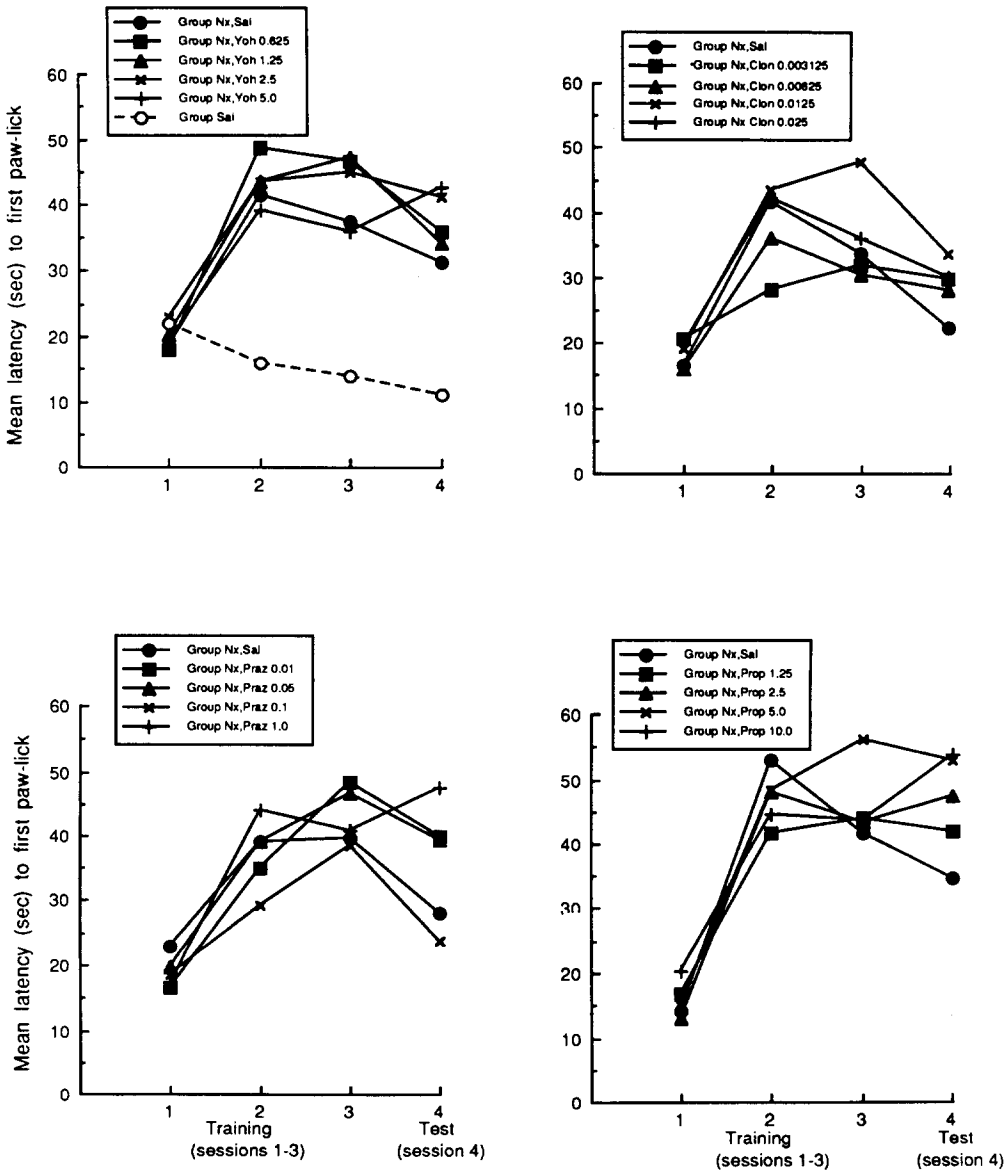

FIG. 1. The mean latencies to the first paw lick across the three training sessions and on test. Rats were trained with pairings of the heat stressor and saline (Sal) or naloxone (Nx). Naloxone-trained subjects were then tested on the heated floor either with the vehicle or with various doses of a noradrenergic drug.

in the wooden boxes for 30 min. They were then exposed to the heated floor for 60 s and the latencies to lick their front and back paws were recorded with pushbuttons connected to a microprocessor. A latency of 60 s was recorded if the animal failed to lick its paws within this time. On day 4, rats were injected with either the vehicle or the appropriate dose of a noradrenergic drug, and placed in the wooden boxes for 30 min. They were then tested for 60 s on their latencies to paw lick in response to the heated floor. In order to show that rats were rendered hypoalgesic by naloxone-stressor pairings, an additional group of rats (n = 10) received separate exposures to the drug and the stressor. On each of these days, rats in Group Sal were injected with saline and isolated in the chambers for 30 min. They were then exposed to the heated floor for 60 s and their paw-lick latencies were recorded in the manner described previously. Three hours later, they were injected with naloxone in their home cages. The procedures used were approved by the University of New South Wales Committee on the Use of Animals in Research and Teaching, and do not cause tissue damage.

Statistical Analysis

Because a preliminary analysis failed to reveal any substantial differences in the latencies with which subjects licked their front and back paws, the latency to the first paw lick was used as the dependent variable. Paw-lick latencies on the final training session were used as a covariate in the statistical analysis of the test results. This analysis consisted of a set of planned, nonorthogonal contrasts written to compare the differences between rats tested with a given drug dose and those tested with saline. Because multiple comparisons were conducted within each experiment, a Bonferroni Inequality procedure was used to determine the critical F values. These values were 6.8 (1,44 *df*, a = 0.05) for Experiments 1 and 2, and 7.0 (1,34 *df*, a = 0.05) for Experiments 3 and 4.

RESULTS

Figure 1 shows the mean latencies to the first paw lick across training sessions and on test. Subjects were exposed to the stressor with either naloxone (Groups Nx) or saline (Group Sal). On test, the naloxone-trained subjects received either the vehicle (Groups Nx, Sal) or a dose of a noradrenergic drug in advance of exposure to the heated floor.

There was evidence for conditioned hypoalgesia because rats tested with saline where they have come to expect exposure to the heated floor with naloxone took longer to lick their paws than those expecting saline (Groups Nx, Sal vs. Group Sal, $ps<0.05$). This conditioned hypoalgesia was neither attenuated by yohimbine (Groups Nx, Sal vs. Nx, Yoh 0.625, $F=0.77$, $p=0.77$; Groups Nx, Sal vs. Nx, Yoh 1.26, $F=1.84$, $p=0.18$; Groups Nx, Sal vs. Nx, Yoh 2.50, $F=0.32$, $p=0.32$; Groups Nx, Sal vs. Nx, Yoh 5.0, $F=4.75$, $p=0.035$) nor potentiated by clonidine, at any of the doses used (Groups Nx, Sal vs. Nx, Clon 0.003125, $F=1.31$, $p=0.53$; Groups Nx, Sal vs. Nx, Clon 0.00625, $F=0.47$, $p=0.80$; Groups Nx, Sal vs. Nx, Clon 0.0125, $F=0.06$, $p=0.50$; Groups Nx, Sal vs. Nx, Clon 0.025, $F=0.40$, $p=0.26$). The three lower doses of prazosin also failed to produce an enhancement (Groups Nx, Sal vs. Nx, Praz 0.01, $F=0.01$, $p=0.93$; Groups Nx, Sal vs. Nx, Praz 0.05, $F=0.02$, $p=0.89$; Groups Nx, Sal vs. Nx, Praz 0.1, $F=4.73$, $p=0.04$). However, the largest dose of prazosin, 1 mg/kg, produced a statistically significant increase in paw-lick latencies (Groups Nx, Sal vs. Nx, Praz 1.0, $F=7.90$, $p=0.008$). Finally, none of the doses of propranalol affected the conditioned hypoalgesia (Groups Nx, Sal vs. Nx, Prop 1.25, $F=0.64$, $p=0.43$; Groups Nx, Sal vs. Nx, Prop 2.50, $F=0.27$, $p=0.61$; Groups Nx, Sal vs. Nx, Prop 5.0, $F=0.25$, $p=0.62$; Groups Nx, Sal vs. Nx, Prop 10.0, $F=3.90$, $p=0.056$).

DISCUSSION

The present results have shown that the conditioned hypoalgesia which accrues from exposures to the context associated with naloxone-heat stressor pairings is not mediated by the actions of noradrenaline on alpha-2 receptors. Neither the alpha-2 antagonist, yohimbine, nor the agonist, clonidine, had any effect upon this conditioned hypoalgesia. In contrast, there was evidence of a role for alpha-1 adrenoceptors because the conditioned hypoalgesia was increased by prazosin. However, the dose at which the enhancement was observed, 1 mg/kg, is considered a very high one and the rats appeared to have been less mobile than those given the lower dosages. Consequently, the observed effect may be due to a motoric impairment rather than to prazosin's inhibition of nociception. Finally, there was no evidence for the involvement of beta-adrenoceptors in mediating the conditioned hypoalgesia because their blockade by propranolol failed to produce any detectable changes on the long paw-lick latencies.

In summary, the present experiments showed that the conditioned hypoalgesia provoked by exposure to the place associated with naloxone-stressor pairings is not mediated by noradrenergic mechanisms. Consequently, these results suggest that the hypoalgesia so induced is mediated by other neural, nonopioid mechanisms, such as those which rely upon the transmission of serotonin or GABA.

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