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Lack of Effect of Naloxone on Autoanalgesia[1]

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CHANCE, W. T. AND J. A. ROSECRANS. *Lack of effect of naloxone on autoanalgesia.* PHARMAC. BIOCHEM. BEHAV. 11(6) 643–646, 1979.—Autoanalgesia (behaviorally-induced antinociception) may be elicited by acute stress or clasically conditioned fear. Antinociception within both of these paradigms is reportedly associated with increased CNS opioid peptide activity. Large doses of naloxone (20 mg/kg) failed to modify antinociception elicited by acute footshock or conditioned fear in rats. Naloxone (4 mg/kg) was also ineffective against antinociception following footshock in mice. These data suggest that if an endorphin does mediate autoanalgesia, the affinity of its receptor for naloxone is very low. Alternatively, parallel opioid and non-opioid systems may be activated by autoanalgesic procedures, with antagonism of the opioid component being insufficient to reduce the antinociception.

Naloxone Autoanalgesia Stress Pain Conditioned fear Antinociception

RECENT experiments have demonstrated that antinociception, as assessed by the tail-flick procedure, can be reliably elicited by a variety of behavioral procedures. Thus, hyperemotionality-producing brain lesions [7, 8, 22], classically conditioned fear [8,22] or acute footshock [17] are paralleled by immediate analgesia equal to that produced by a moderate dose of morphine. The centrifugal nature of behaviorally-induced antinociception (autoanalgesia) has been suggested by its obviation upon sectioning of the spinal cord at the thoracic level [9,17]. Further experimentation has indicated that autoanalgesia is related to increased CNS opioid peptide (endorphin) activity. Thus, classically conditioning fear to the antinociceptive test procedure resulted in decreased binding of ^{3}H-etorphine [9,10] and ^{3}H-NLeu-enkephalin [12] to rat brain homogenate. In addition, a significant inverse relationship was observed between antinociception and ^{3}H-NLeu-enkephalin binding [9,12]. These results suggest that more endogenous ligand has been released and bound in the more analgesic animals, as indicated by less binding of exogenously-administered peptide. Similarly, binding of ^{3}H-naloxone has been reduced by a chronic schedule of footshock [1,19].

A particular problem for interpreting autoanalgesia as due to increased endorphin activity has been the lack of effectiveness of opiate antagonists in reducing the antinociception. Thus, naloxone did not block antinociception elicited by acute shock [17], naltrexone was also ineffective against conditioned fear-induced antinociception [9] and chronic footshock-induced analgesia was only partially reversed by naloxone [1]. Naloxone has been reported to block chronic footshock-induced analgesia in mice [13]. This analgesic test, however, was the abdominal constriction test induced by the injection of formic acid or prostaglandin El and probably does not reflect similar nociceptive processes as the radiant-heat test. Therefore in the present experiment, the effect of naloxone, at doses higher than necessary to antagonize morphine analgesia, were investigated in both rats and mice within autoanalgesic paradigms.

METHOD

Subjects

Thirty-five adult, male, Sprague-Dawley rats (Flow Laboratories, Dublin, VA USA) and 46 adult (30 g), male, ICR mice (Flow Laboratories) served as subjects in these experiments. The rats were individually-housed, and the mice were group-housed under ad lib conditions on a 12 hr light/dark cycle.

Apparatus

Antinociception was assessed using a modification of the radiant-heat tail-flick procedure [14] consisting of a 100 W lamp mounted in a reflector and focused on a photocell. The lamp and photocell were connected to a timer so that activation of the photocell, by the subject reflexively withdrawing its tail, interrupted the circuit to give a reaction time to the nearest hundredth of a sec. The intensity of the lamp could also be controlled and in these experiments was adjusted to elicit reaction times of 3–4 sec in nondrugged control rats, while at the same intensity mice responded in 2–3 sec. To avoid tail damage a 10 sec response latency cut-off criterion was maintained. Nonscrambled shock was delivered by a Lafayetter shocker (A-615C) to a 21×21 cm grid platform for rats, while a smaller (11×8 cm) grid platform was used for shocking the mice. These grids were 3 mm in diameter and were spaced 15 mm apart in the larger platform and 4 mm apart in the smaller platform. Both platforms were elevated (8 cm) and were used to support a subject during the tail-flick tests.

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Acquisition of Autoanalgesia and Tests of Antagonism

Autoanalgesia was elicited as previously reported [8] by the classical conditioning of fear to the environmental stimuli associated with the tail-flick procedure. In the present experiments, the effects of naloxone HCl (Endo Laboratories, Garden City, NJ USA) upon autoanalgesia elicited by acute footshock and conditioned fear were investigated. The rats were randomly assigned to four groups. Groups NE (n=8) and NC (n=9) were injected with 20 mg/kg (IP) naloxone, while groups SC (n=8) and SE (n=10) were injected with an equal volume of normal saline 15 min prior to the determination of baseline tail-flick latencies. Ten sec after the basal test, each rat in groups NE and SE received footshock (0.9 mA; 15 sec), with the tail-flick latencies again being determined 10 sec after the termination of the shock. Tail-flick latencies were also determined for rats in groups SC and NC at these times but no shock was administered. To assess acquisition of autoanalgesia, on each of the following 4 days the schedule of footshock continued to be administered with antinociception being measured prior to the shock. On these subsequent trials, the naloxone treatment and tail-flick test acutely-following the shock were no longer administered. Since on days 2–5 each tail-flick test preceded the shock by 10 sec, the effects of the prior (24 hr) shock on antinociception were being assessed. To assess the effect of naloxone on conditioned fear-induced antinociception, naloxone (20 mg/kg; IP) was again administered to groups NC and NE 15 min prior to the antinociceptive test of Day 6. Groups SC and SE were also injected with an equal volume of saline, with tail-flick latencies being determined 15 min later.

In mice investigation of the acquisition and antagonism of autoanalgesia preceded in an analogous fashion. Although the effect of naloxone (4 mg/kg; IP) pretreatment (15 min) upon analgesia acutely-elicited by footshock was investigated, no test of naloxone-induced antagonism of conditioned fear-induced analgesia was conducted.

RESULTS

The mean (± SEM) tail-flick latencies of rats prior to and following footshock for both saline and naloxone pretreatments are presented in Table 1. Although there were no differences in baseline response latencies (tail-flick 1) between any groups, 15 sec of footshock (tail-flick 2) elicited significant analgesia, $t(16)=3.98$, $p<0.01$: SE vs. SC; $t(15)=7.07$, $p<0.01$: NE vs. NC, in the shocked groups as compared to controls. Neither baseline tail-flick latencies nor the acutely elicited analgesia were significantly affected by pretreatment with 20 mg/kg of naloxone.

In Fig. 1, the acquisition of autoanalgesia across the next 4 days is presented as well as the combined baseline (open symbols) and acute shock (filled symbols) data of Day 1. The rats that had previously (24 hr) been shocked (S) exhibited significantly longer tail-flick latencies on day 2 then did the non-shocked control (NS) rats, $t(33)=4.22$, $p<0.01$. This difference further increased on day 3 to an asymptote of approximately 7 sec in the shock (S) group. On the day after the last acquisition trial (Day 6), the effect of naloxone (20 mg/kg; IP) was again tested. As in the preceding drug test, naloxone failed to reduce the tail-flick latencies of control (SC=3.62 ± 0.6; NC=3.13 ± 0.2) or fear conditioned (SE=5.83 ± 0.6; NE=7.90 ± 0.7) rats. In the fear conditioned rats, there was an apparent potentiation of tail-flick latencies by naloxone, $t(16)=2.24$, $p<0.05$.This difference appears to be due to sampling error, however, since the difference in the means of these two groups approached significance on the preceding day in the absence of drugs (5.99 vs. 7.12 sec).

The mean (± SEM) tail-flick latencies prior to and following acute footshock or control treatments in mice 15 min after the injection of naloxone (4.0 mg/kg; IP) or saline are presented in Table 2. Although the mice demonstrated lower baseline tail-flick latencies and less acute analgesic effects of shock than rats, 15 sec of footshock still elicited significantly increased antinociception under both saline, $t(20)=3.70$, $p<0.01$, and naloxone, $t(22)=3.70$, $p<0.01$, conditions. Again, naloxone was ineffective in antagonizing

TABLE 1

EFFECT OF NALOXONE ON SHOCK-INDUCED ANTINOCICEPTION IN RATS

Groups*	N	Tail-Flick 1	Tail-Flick 2
SC	8	5.51 ± 0.5	4.12 ± 0.4
NC	9	4.44 ± 0.7	3.70 ± 0.3
SE	10	4.04 ± 0.3	†7.85 ± 0.8
NE	8	4.25 ± 0.4	†8.68 ± 0.6

*Groups NC and NE were pretreated with naloxone (20 mg/kg) before the tail-flick tests, while groups SC and SE received saline. Only groups NE and SE were shocked between the two tests of antinociception.

†$P<0.01$, as compared to controls.

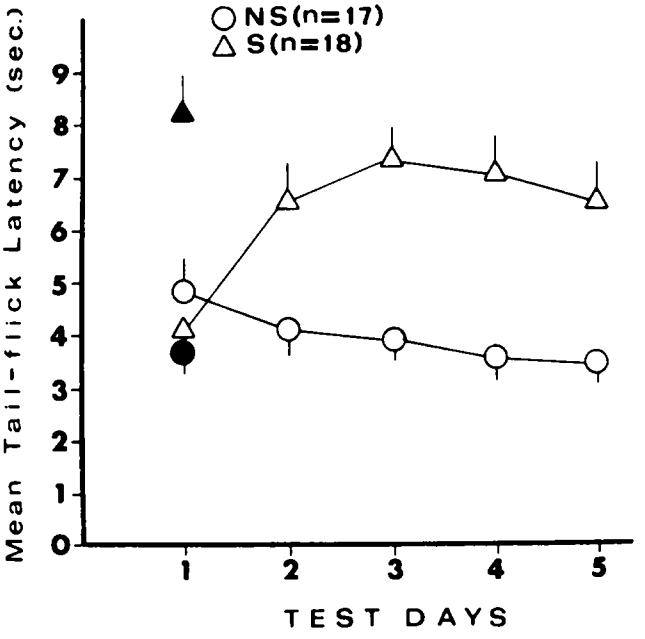

FIG. 1. Acquisition of autoanalgesia by rats, expressed as mean (± SEM) tail-flick latencies, for both acute footshock (S, Day 1) or conditioned fear (S, Days 2–5) paradigms. On Day 1 the tail-flick latencies were determined for group S prior to (open symbols) and 10 sec following (filled symbols) footshock. Tail-flick latencies continued to be determined for group S prior to footshock for Days 2–5. Antinociception was also assessed for group NS at these times, but shock was never administered.

TABLE 2
EFFECT OF NALOXONE ON SHOCK-INDUCED ANTINOCICEPTION IN MICE

Groups*	N	Tail-Flick 1	Tail-Flick 2
SC	12	2.69 ± 0.2	2.07 ± 0.2
NC	12	2.42 ± 0.3	2.04 ± 0.1
SE	10	2.89 ± 0.4	†3.71 ± 0.3
NE	12	2.88 ± 0.3	†4.09 ± 0.6

*Groups NC and NE were pretreated with naloxone (4 mg/kg) before the tail-flick tests, while groups SC and SE received saline. Only groups NE and SE were shocked between the two tests of antinociception.

†$P<0.01$, as compared to controls.

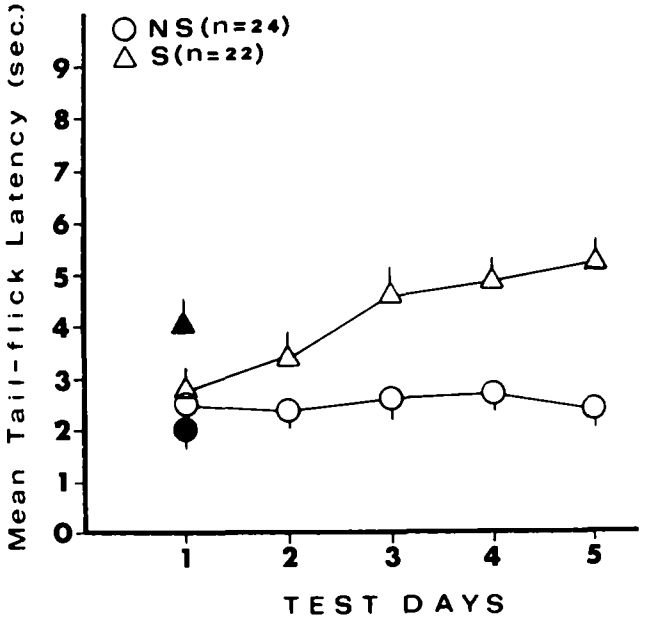

FIG. 2. Mean (± SEM) tail-flick latencies of mice prior to (open symbols, Day 1) and following (filled symbols, Day 1) acute shock (S) or control (NS) treatment. On Days 2–5, acquisition of autoanalgesia was assessed by determining tail-flick latencies prior to shock (S) or control (NS) treatment.

the increased tail-flick response latencies acutely elicited by footshock.

Acquisition of autoanalgesia within the conditioned fear paradigm (Days 2–5) as well as the combined data of baseline (open symbols, Day 1) and acute shock (filled symbols, Day 1) tests are presented in Fig. 2. Significant antinociception was acquired within one day, $t(44)=3.16$, $p<0.01$: Day 2, and continued to increase to approximately 5 sec on Day 5. Thus, mice exhibited a pattern of autoanalgesia that was very similar to that previously observed in rats.

DISCUSSION

In the present experiments antinociception was elicited in rats and mice by a brief period (15 sec) of footshock (0.9 mA) and by classically conditioned fear. Naloxone, at doses (20 and 4 mg/kg) much greater than necessary to antagonize morphine analgesia, was ineffective in reducing antinociception elicited by these behavioral procedures.

These results agree with previous experiments in which naloxone did not antagonize acute shock-induced antinociception [17] and only partially blocked chronic shock-induced antinociception [1], while naltrexone had no effect on antinociception elicited by classically conditioned fear [9]. A similar array of effects of naloxone has been reported for analgesia elicited by electrical stimulation of midbrain structures, with naloxone totally [20], or partially [2] reversing or not affecting [21,24] stimulation produced analgesia (SPA). Similarly, naloxone has been reported to have no effect [15], augment or reduce [5] pain perception in humans.

Reversibility of effects by naloxone is a universally-accepted test of opioid peptide activity [16]. Recent experiments, however, have suggested that the various endorphin peptides have varying affinities for a variety of opiate and opioid receptors [3, 18, 23]. Thus, leu-enkephalin exhibits saturable, high affinity binding at brain receptor sites that are not readily antagonized by naloxone [3, 18, 23]. Conversely, β-endorphin appears to bind to typical morphine sites [18], showing a high affinity for opiate receptors [4]. β-endorphin also exhibits strong antagonism of ^{3}H-naloxone binding, while leu-enkephalin shows only weak antagonisn [18]. Furthermore, in the mouse vas deferens test, which has been characterized as similar to brain leu-enkephalin receptors [18], 11 times the dose of naloxone is required to antagonize leu-enkephalin as normorphine-induced inhibition of contraction. In addition, naloxone has been reported to elevate the ED50 analgesic doses of morphine and methadone by a factor of 5, while actually reducing the ED50 analgesic dose of leu-enkephalin [6]. Therefore, autoanalgesia may be partially mediated by endorphin receptors which are resistant to the antagonistic effects of naloxone.

Alternatively, considering the inability of high doses of naloxone to even slightly reduce autoanalgesia, one must assume that the final pathway for analgesic expression is non-opiate in nature. Thus, the stress induced by conditioned fear or acute shock could activate parallel opiate and non-opiate systems. Blockade of either one individually would not suppress the analgesic effects, but would explain the variability observed with naloxone reversal of analgesia induced by stress or focal brain stimulation.

To date no pharmacological manipulations have successfully antagonized autoanalgesic phenomena. Lesions of the nucleus raphe magnus, a descending serotonergic system, have, however, partially reduced both morphine and conditioned fear-induced analgesia [11]. These data suggest that a serotonergic system descending to the cord from midbrain levels may partially mediate the inhibitory activity of both autoanalgesia and morphine.

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