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Lack of Cross-Tolerance Between Morphine and Autoanalgesia[1]

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CHANCE, W. T. AND J. A. ROSECRANS. *Lack of cross-tolerance between morphine and autoanalgesia.* PHARMAC. BIOCHEM. BEHAV. 11(6) 639–642, 1979.—The acquisition of autoanalgesia (behaviorally-induced antinociception) was investigated in morphine-tolerant and non-tolerant rats. Tolerance to morphine did not affect analgesia acutely-elicited by a brief (15 sec) schedule of footshock. Similarly, analgesia elicited by classically conditioned fear was not attenuated by morphine tolerance. These data suggest that endorphins may not be the principle mediators of autoanalgesic phenomena.

Tolerance Cross-tolerance Morphine Endorphin Autoanalgesia Stress Conditioned fear Analgesia

RECENT experiments have suggested that antinociception, as assessed by a variety of procedures, can be reliably elicited by several stressful manipulations. Thus, Rosecrans and Chance [17] observed that hyperemotionality-producing brain lesions or classically conditioned fear elevated tail-flick latencies in the rat [4,8]. Other researchers reported similar data using both acute [14] and chronic [1] schedules of footshock. Subsequently, analgesia, as assessed by the flinch-jump procedure, was reported following cold water swim-induced stress [2]. Although each of these procedures undoubtedly involve activation of the pituitary-adrenal system, systemic administration of adrenalcorticotropic hormone does not elicit analgesia [5], nor does hypophysectomy attenuate analgesia induced by acute footshock or conditioned fear [5,6].

Opiate binding experiments have, however, suggested a relationship between autoanalgesia and opiate peptide activity in the brain. Thus, CNS binding of ^{3}H-naloxone was reduced by a chronic schedule of analgesia-producing footshock [1,16]. Similarly, binding of ^{3}H-etorphine [9,10] and ^{3}H-N-Leu-enkephalin [9,12] to rat brain homogenate was reduced in animals rendered analgesic by fear conditioning procedures. Furthermore, a significant negative correlation between analgesia and opioid binding was reported [9,12], suggesting that more endogenous ligand was released and bound in animals exhibiting analgesia.

The lack of naloxone antagonism, however, presents a particular problem for interpreting endorphin mediation of autoanalgesic phenomena. Analgesia elicited by acute footshock [7,14] or conditioned fear [7, 9, 11] is not affected by doses of naloxone much larger than necessary to antagonize morphine analgesia. Similarly, analgesia induced by chronic schedules of footshock [1] or cold water stress [2] is only partially reduced by high doses of naloxone.

Therefore, the purpose of the present experiment is to investigate opiate-autoanalgesic interactions from the aspect of tolerance. Since analgesia induced by classically conditioned fear does not exhibit tolerance, but rather increases to an asymptote by the fourth or fifth day of conditioning, autoanalgesia was assessed in morphine-tolerant rats. Evidence of cross-tolerance would, therefore, be suggestive of similar mechanisms mediating the two phenomena.

METHOD

Subjects

Thirty adult, male, Sprague-Dawley rats (Flow Laboratories, Dublin, VA) served as subjects in this experiment. These animals were individually-housed with food and water available ad lib under a 12 hr light/dark cycle.

Apparatus

Antinociception was assessed using a modification of a radiant-heat tail-flick apparatus [13] 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 rat 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 the present experiment was adjusted to elicit responses within 3 to 4 sec in non-drugged control rats. To prevent tail damage, a latency cut-off criterion of 9 sec was also maintained. Nonscrambled shock was delivered by a Lafayette shocker (A 615C) to a 21×21

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cm grid platform. These grids were 3 mm in diameter and were spaced 15 mm apart. The grid platform was elevated (8 cm) and was used to support a subject during the tail-flick tests.

Acquisition and Assessment of Opiate Tolerance

On the first day of the experiment the subjects were randomly assigned to 3 groups of 10 rats each. Groups MT (morphine-tolerant) and MNT (morphine-nontolerant) were each injected (IP) with 10 mg/kg morphine sulfate (Mallinkrodt, St. Louis, MO), while group SC was injected (IP) with an equal volume of normal saline. The tail-flick latencies were determined for each rat 1 hr after the injection. Across the next 8 days the dose of morphine was gradually incremented for group MT to an asymptote of 60 mg/kg for Days 7–9, while groups MNT and SC received injections of saline. Tail-flick latencies continued to be assessed 1 hr after the injections throughout this period. To assess tolerance, 10 mg/kg of morphine was administered to groups MT and MNT on day 10, while group SC again received saline. To maintain the tolerance, group MT again received 60 mg/kg of morphine 1 hr after the tolerance test.

Acquisition of Autoanalgesia and Assessment of Cross Tolerance

Autoanalgesia was elicited as previously reported [4] by the classical conditioning of fear to the environmental stimuli associated with the tail-flick procedure. In the present experiment, the effects of acute shock on the analgesic response of tolerant and non-tolerant rats was also investigated. On the day following the tolerance test (Day 11), each rat was removed from the home cage, placed on the grid and the tail-flick latencies were determined. Ten to fifteen sec later, each rat in groups MT and MNT was shocked (0.9 mA), while being held on the grid, for 15 sec. Rats in group SC were held on the grid for the same period of time but no shock was administered. To assess the analgesic effects of this acute footshock, tail-flick latencies were again determined for each rat 10 to 15 sec after the termination of the footshock. To assess acquisition of fear-induced analgesia, tail-flick latencies continued to be assessed on each of the next 6 days for groups MT and MNT 10 to 15 sec prior to the footshock. No determinations of analgesia, however, were made acutely following the shock. To assure the continuation of tolerance in group MT, 60 mg/kg of morphine was administered on each of the above days, while groups MNT and SC received saline 1 hr after the tests. Throughout these tests, group SC was treated in a similar manner to groups MT and MNT but was never shocked. During the above tests, the experimenter had no prior knowledge of subject-group assignment. Overall statistical evaluations were accomplished using analyses of variance techniques, with individual comparisons made by t tests.

RESULTS

The mean tail-flick latencies 60 min following the injection of saline (SC) or morphine (10 mg/kg) in tolerant (MT) and non-tolerant (MNT) rats is presented in Fig. 1. To allow comparison, the analgesic response to the initial treatment of Day 1 (open bars) and following the chronic schedule of morphine injection (stippled bars) on Day 10 is presented. Although there was no difference between groups MT and MNT following the initial injection of morphine, repeated

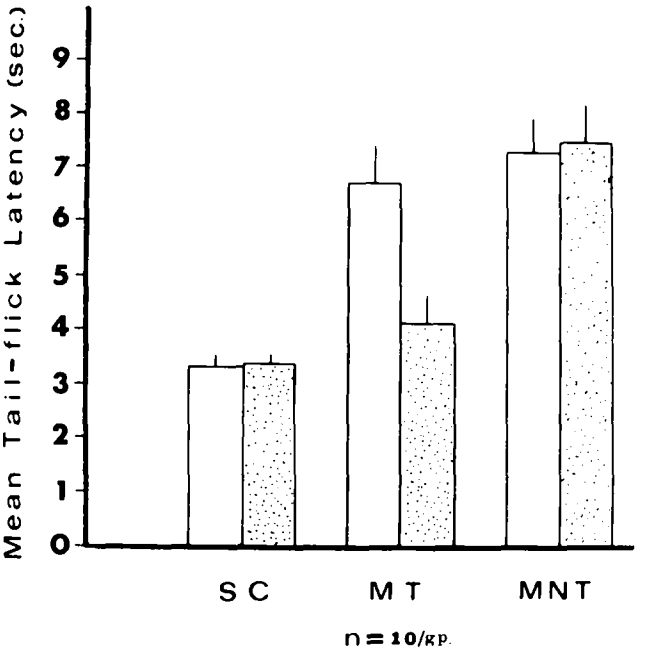

FIG. 1. Mean (SEM) tail-flick latencies of rats before and after chronic administration of morphine sulfate or saline. The analgesic effects of 10 mg/kg of morphine (MT, MNT) or saline (SC) were assessed 60 min after the initial (open bars) as well as following the chronic (MT, 9 days) schedule (stippled bars) of administration.

daily administration of incremented doses of morphine induced a state of tolerance in group MT, $t(18)=3.91$, $p<0.01$: MT vs MNT; $t(18)=1.24$, n.s.: MT vs SC.

The mean tail-flick latencies of morphine tolerant and non-tolerant rats prior to (open symbols, Day 1) and following (closed symbols, Day 1) footshock as well as following acquisition of conditioned fear (Days 2–7) are presented in Fig. 2. Although there was no difference in baseline tail-flick latencies between the groups, $F(2,27)=2.99$, n.s., 15 sec of footshock significantly elevated the response latencies of both groups MT and MNT, $F(2,27)=15.8$, $p<0.01$, to the same degree, $t(18)=0.20$, n.s.: MT vs MNT. Thus, morphine tolerance had no effect on analgesia acutely elicited by footshock.

Within the fear conditioning paradigm of the next 6 days the response latencies of groups MT and MNT continued to increase, as compared to the non-shocked controls (group SC), to an asymptote of approximately 7 sec. A repeated measures analysis of variance indicated a significant difference between groups, $F(2,27)=23.74$, $p<0.01$, a significant increase in latencies across trials, $F(6,167)=19.03$, $p<0.01$, and a significant group × trials interaction, $F(12,167)=2.95$, $p<0.01$. The total lack of difference between groups MT and MNT on any trial, emphasize the inability of opiate tolerance to attenuate autoanalgesia.

DISCUSSION

Analgesic tolerance has been demonstrated to β-endorphin as well as cross-tolerance between β-endorphin and morphine [21]. In addition, analgesic cross-tolerance between morphine and β-endorphin [18,20] has been observed. Furthermore, cross-tolerance between morphine and en-

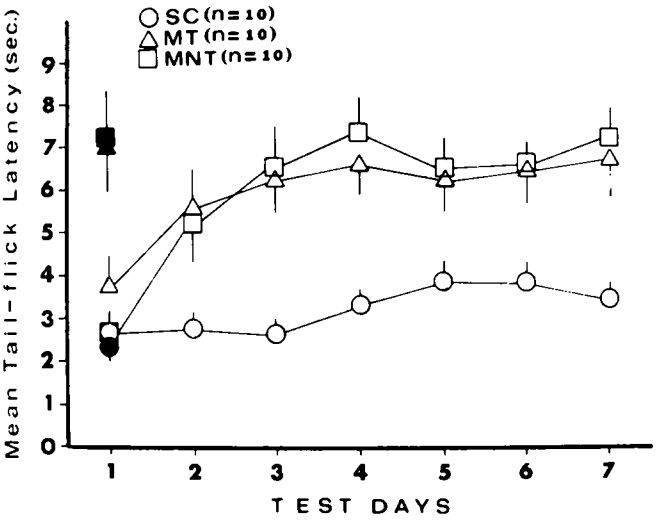

FIG. 2. Mean (SEM) tail-flick latencies of morphine tolerant (MT), non-tolerant (MNT) and control (SC) rats at baseline conditions (open symbols, Day 1) and following acute footshock (MT, MNT: closed symbols, Day 1) or control manipulation (SC: closed symbols, Day 1). To assess acquisition of autoanalgesia in tolerant and non-tolerant rats, tail-flick latencies were determined across the next 6 days with each test of groups MT and MNT being followed by footshock.

kephalin has been demonstrated for inhibition of spontaneously-active cortical neurons [23] and inhibition of contraction of smooth muscle [22]. Although the neuronal basis of opiate tolerance is presently unclear, demonstration of cross-tolerance is an accepted procedure for illustrating similar mechanisms of drug action. Thus, various endorphin peptides apparantly share to some extent with morphine similar mechanisms for elicting analgesia, inhibiting neuronal activity and inhibiting smooth muscle.

In the present experiment, the involvement of endorphin systems in the mediation of autoanalgesia was investigated by assessing acquisition of autoanalgesia in morphine-tolerant rats. As illustrated in Fig. 2, there was no reduction in analgesia acutely-following footshock. Similarly, acquisition of autoanalgesia was not attenuated in morphine-tolerant rats across the 6 days of fear conditioning, even though their tolerance was being maintained by daily injections of morphine. Although previous research reported changes in CNS opiate binding, which correlated well with the analgesia observed in these behavioral paradigms [1, 9, 10, 12, 16], the total lack of cross-tolerance between morphine and autoanalgesia argues against endorphins as the sole mediator of autoanalgesia. Similar results have been reported using cold water stress to elicit analgesia. Thus, no cross-tolerance was observed between morphine and cold water stress or between cold water stress and morphine [3]. Additional evidence counter to the role of endorphins in mediating autoanalgesia is the failure of even large doses of naloxone to antagonize the antinociciptive effects of these behavioral manipulations [7, 9, 11, 14]. If, as suggested by the binding experiments, endorphins are involved in autoanalgesia, they may do so by acting on non-typical opiate receptors that have a low affinity for naloxone. Thus, leu-enkephalin exhibits saturable, high-affinity binding at brain receptor sites that are not readily competed for by naloxone [15,19].

Alternatively, considering the lack of cross-tolerance and lack of naloxone antagonism, the stress induced by conditioned fear or acute footshock could activate parallel opiate and non-opiate neuronal systems. Blockade or tolerance of either one individually would not suppress the analgesic effect, but might explain the reports of partial antagonism by naloxone across varying behavioral procedures [1, 2, 7, 9, 11, 14]. At present, no pharmacological manipulations have successfully antagonized autoanalgesic phenomena. Lesions of the nucleus raphe magnus, a descending serotonergic system, have partially reduced both acute footshock 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 autoanalgesia and warrant further pharmacological investigations into the phenomena.

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