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Morphine Conditioned Taste Aversion Reversed by Periaqueductal Gray Lesions

R. BLAIR AND Z. AMIT

Center for Research on Drug Dependence, Department of Psychology Room H-1060
Concordia University, Montreal, Quebec, Canada H3G 1M8

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BLAIR, R. AND Z. AMIT. *Morphine conditioned taste aversion reversed by periaqueductal gray lesions.* PHARMAC. BIOCHEM. BEHAV. **15**(4) 651–653, 1981.—The role of the periaqueductal gray (PAG) in morphine conditioned taste aversion (CTA) was studied using male Wistar rats as subjects. Following the presentation of a novel saccharin solution, animals with or without a lesion of the PAG were intraperitoneally injected with either morphine, lithium, ethanol or fenfluramine. As evident by the amount of saccharin solution consumed on a subsequent presentation, a PAG lesion reversed a morphine CTA but not CTAs produced by the other drugs used. The results suggest that the PAG may in part mediate morphine CTA.

Conditioned taste aversion Morphine Lithium Ethanol Fenfluramine Amphetamine
Periaqueductal gray Lesion

THERE have been numerous reports indicating that certain drugs when paired with a novel tasting substance produce a conditioned taste aversion (CTA) in rats. CTAs have been shown to be induced by a variety of drugs such as morphine, ethanol, lithium, amphetamine and fenfluramine to mention only a few (e.g. [5, 6, 13]).

Although a wide variety of drugs induce CTAs, it is possible to differentiate in part between CTAs with respect to mediating neurochemicals. It has been shown that both alpha-methyl-para-tyrosine (AMPT, a tyrosine hydroxylase inhibitor) and pimozide (a dopaminergic receptor blocker) blocked the acquisition of both, a morphine and an ethanol CTA, but not a CTA induced by lithium [13]. Similarly, catecholamine depletions induced by intracerebral injections of 6-hydroxydopamine (6-HDA, a specific neurotoxin) or by a systemic injection of AMPT, have been reported to block the CTAs produced by amphetamine and morphine [4, 7, 12]. However, similar catecholamine manipulations by intracerebral injections of 6-HDA did not affect a lithium induced CTA [12,15]. Thus, it appears that catecholamines are involved in the mediation of CTAs produced by morphine, ethanol and amphetamine but not by lithium.

In order to further understand the neural substrates underlying CTAs, other investigators have attempted to identify the site of drug action for certain CTAs. Amit and his colleagues [1] investigated the possibility of a specific interaction between either Δ9-tetrahydrocannabinol (Δ9THC), morphine or ethanol and the dorsal hippocampus in the induction of a CTA. The results indicated that only infusions of Δ9THC into the hippocampus produced a CTA. These findings suggest that some CTAs are centrally mediated and that a CTA may in part result from specific drug-locus interactions.

Taken together, the evidence cited suggests that CTAs may be a function of certain drugs acting at a specific site on certain types of neural tissues. Given this, it seemed of interest to further investigate the neural substrates underlying certain CTAs. In particular, we were interested in examining the site of morphine's action in the production of a CTA by exploring the involvement of the periaqueductal gray (PAG) in a morphine CTA.

Previous research has tentatively implicated the opiate receptor in the production of CTA since naloxone, the opiate receptor antagonist has been reported to attenuate a morphine CTA [11,17]. We have also observed that naloxone (10 mg/kg) reversed a morphine (9 mg/kg) CTA (unpublished observations). It is interesting to note that since naloxone has been found by other investigators to only attenuate and not completely reverse a morphine CTA, it has been suggested that a naloxone-insensitive mechanism may also be involved in the mediation of a morphine CTA [17]. We selected the PAG for investigation since it has been reported to have a high concentration of opiate receptors [10] and it is also known to mediate certain behavioral effects of morphine that are not mediated by the opiate receptor [2, 3, 8, 9]

In order to investigate the role of the PAG, we examined the effect of a lesion of the PAG on a morphine CTA. Furthermore, to assess whether the lesion's effects were specific to a morphine CTA, we also studied the effect of a PAG lesion on CTAs induced by d-amphetamine, fenfluramine, lithium and ethanol.

METHOD

Subjects

The subjects were 96 male Wistar rats (Canadian Breeding Laboratories) weighing approximately 350–450 grams. Animals were housed in stainless steel cages with free access to food and water. The animal colony was illuminated on a 12 hour day/night schedule.

Drugs and Injections

Morphine hydrochloride, ethanol, d-amphetamine sulphate, lithium chloride and fenfluramine hydrochloride were dissolved in Ringer's solution which also served for control purposes. The dose and volume injection of each drug are as follows: morphine, 9.0 mg/kg, 1 ml/kg; ethanol, 1.2 g/kg, 7.5 ml/kg of a 20% solution (v/v); d-amphetamine, 2.0 mg/kg, 1 ml/kg; fenfluramine, 6.0 mg/kg, 2 ml/kg; lithium, 127.2 mg/kg, 20 ml/kg; Ringer's (vehicle control solution) 20 ml/kg. It should be noted that these drug doses have been reported previously to produce CTAs [6,13].

Procedure

At the beginning of the experiment, 48 animals were anesthetized with sodium pentobarbital (60 mg/kg) and given ether supplements when necessary. The 48 subjects then received an anodal electrolytic lesion (2 mA×35 sec) in the PAG. The tip of the lesioning electrode (0.25 mm in diameter) was stereotaxically aimed at the center of the aqueduct of sylvius (6.0 mm posterior to bregma, 0.0 lateral, 5.0 ventral, incisor bar set at +5.0). Control animals were not anesthetized or surgically manipulated. Following surgery, lesioned animals were returned to their home cages and given a 30 day recovery period. During the first few days of recovery, lesioned animals that refused the regular Purina Rat Chow, were given a wet mash composed of Purina Rat Chow and water in a dish in the home cage. If an animal's weight dropped by 20% of the pre-operative weight, the animal received twice daily intragastric tube feedings composed of wet mash diluted with water until the animal's weight stabilized and consumption of the mash commenced. Once animals began to consume dry food pellets, the mash was discontinued.

After recovery, the lesioned and non-lesioned animals were placed on a water deprivation schedule. A single bottle of tap water with a ball-bearing spout was made available to each rat in the home cage for 20 min each day for seven consecutive days. On Day 8 (pairing day), a 0.1% sodium saccharin solution was presented instead of water. The amount of saccharin ingested by each rat was recorded. One minute after the 20 min presentation of saccharin, the lesioned and non-lesioned groups that were divided equally into six groups each, received an IP injection of either morphine, lithium, ethanol, amphetamine, fenfluramine or Ringer's.

For the next seven days (Days 9 to 15) all animals were again given access to water for 20 min daily. On Day 16 (test day) a 0.1% sodium saccharin solution was again presented to the animals for a 20 min period. The amount consumed by each animal was recorded.

At the end of the experiment, all lesioned animals were overdosed with sodium pentobarbital and perfused with saline followed by formal-saline. The brains were removed and fixed in formal-saline. Later, the brains were frozen, cut into 40 micra coronal sections for verification of lesion placement.

RESULTS

Following surgery, two animals required tube feedings for two days. By six days post-surgery, all lesioned animals were eating dry food pellets.

The mean (±S.E.) saccharin consumption on conditioning and test days for lesioned and non-lesioned animals is given in Fig. 1. A three-way analysis of variance of the mean saccharin consumption yielded a significant drug effect, $F=20.4$, $p<0.00001$, a significant day effect, $F=195.8$, $p<0.00001$, a significant drug×days interaction, $F=31.36$, $p<0.00001$, and a significant drug×pretreatment (lesion)×days interaction, $F=2.48$, $p<0.03$.

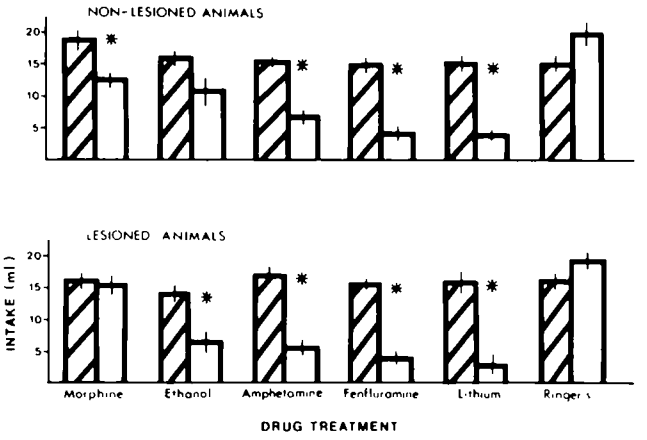

FIG. 1. The mean (±S.E.) saccharin consumption on pairing (bars with diagonal lines) and test (open bars) days for non-lesioned and lesioned animals. The drugs administered on pairing day were either morphine, ethanol, amphetamine, fenfluramine, lithium or Ringer's. Asterisks indicate a significant difference ($p<0.05$) between pairing and test day intakes for each group.

Post hoc Tukey tests revealed that a significant reduction ($p<0.05$) in saccharin intake occurred on test day for the non-lesioned animals that received morphine, lithium, fenfluramine and amphetamine on pairing day. Those non-lesioned subjects that received ethanol on pairing day also showed a reduction of saccharin intake on test day, however, the reduction in amount consumed was equivalent to the critical difference needed for statistical significance. Lesioned subjects that were injected with ethanol, amphetamine, lithium or fenfluramine drank significantly less on test day than pairing day. However, lesioned subjects that received a morphine injection on pairing day consumed similar quantities of saccharin on both pairing and test days.

Histological examination of the lesion site indicated that all lesions damaged the tissue of the midbrain central gray adjacent to the aqueduct of sylvius. Schematic representations of the lesions are displayed in Fig. 2.

DISCUSSION

In agreement with previous studies [6,13] morphine, amphetamine, lithium and fenfluramine were found in the present experiment to produce CTA in non-operated subjects. In contrast to previous studies [13], ethanol did not produce a significant CTA. However, it should be noted that the reduction in drinking on test day by ethanol treated animals was equivalent to the critical difference needed for statistical significance.

Of particular interest are the results obtained from the lesioned animals. Ethanol, amphetamine, lithium and fenfluramine when paired with saccharin all produced a CTA

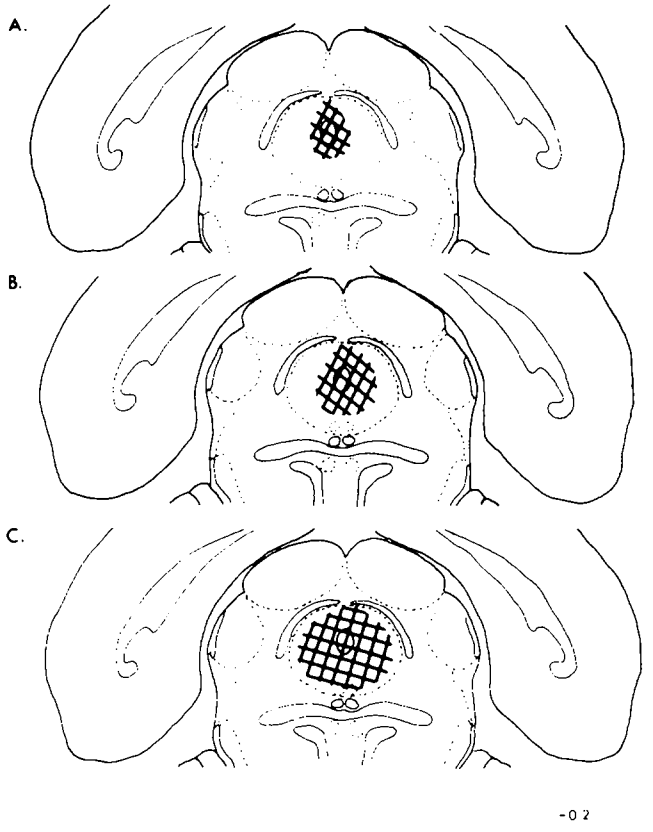

FIG. 2. Schematic representations of small (A), medium (B) and large (C) lesions of the PAG. The cross-hatched lines represent the area damaged by the lesions.

as evident by the reduction in drinking on test day. In contrast, when paired with saccharin, morphine did not produce a significant CTA in PAG-lesioned animals. In fact, these animals drank comparable amounts on pairing day as they did on test day.

It seems unlikely that the reversal of a morphine CTA by a PAG lesion is a function of some general memory deficit since the lesion did not prevent the occurrence of a CTA by the other drugs used. In addition, it does not appear that the blockade of a morphine CTA by PAG lesions resulted from the smaller magnitude of a morphine CTA as compared to CTAs produced by lithium or fenfluramine. A PAG lesion was not observed to block a CTA to ethanol; yet this drug produces a weak CTA as evident by the weak aversion produced by this drug in the non-lesioned animals. Alternatively, the blockade of a morphine CTA by a lesion of the PAG suggests that the PAG is involved in the production of morphine CTA. More specifically, given that previous investigators [11,17] have reported that naloxone attenuates a morphine CTA and that a PAG lesion reverses a morphine CTA in the present study suggests that opiate receptors within the PAG may mediate morphine's action in the production of a CTA.

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