Pharmacology Biochemistry & Behavior, Vol. 33, pp. 847–851. Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Interaction of Haloperidol and Area Postrema Lesions in the Disruption of Amphetamine-Induced Conditioned Taste Aversion Learning in Rats

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Received 23 January 1989

RABIN, B. M. AND W. A. HUNT. *Interaction of haloperidol and area postrema lesions in the disruption of amphetamine-induced conditioned taste aversion learning in rats*. PHARMACOL BIOCHEM BEHAV **33**(4) 847–851, 1989.—Two experiments were run to determine the mechanisms underlying the acquisition of an amphetamine-induced conditioned taste aversion. In the first experiment, it was shown that pretreatment with haloperidol (0.1–0.5 mg/kg, IP) attenuated, but did not prevent, taste aversion learning produced by amphetamine (3 mg/kg, IP). In the second experiment, combining area postrema lesions with haloperidol (0.5 mg/kg) pretreatment completely blocked the acquisition of an amphetamine-induced taste aversion. The results are interpreted as indicating that amphetamine-induced taste aversion learning has both a central component, which is mediated by dopaminergic receptors, and a nondopaminergic peripheral component, which is mediated by the area postrema.

Conditioned taste aversion Amphetamine Haloperidol Dopaminergic Area postrema

A conditioned taste aversion (CTA) can be produced by pairing a novel tasting solution with a variety of unconditioned stimuli, including ionizing radiation, lithium chloride (LiCl), amphetamine and morphine (6,13). The neural mechanisms underlying the acquisition of a CTA to toxic treatments appear to differ from those underlying taste aversions produced by self-administered compounds (6). In contrast to radiation- and LiCl-induced taste aversions, which require the mediation of the area postrema (AP) (11, 14, 16), AP lesions have no effect on the acquisition of a CTA produced by injection of higher doses of amphetamine (>1.5 mg/kg) (15,16) or morphine (19). Conversely, manipulation of specific catecholaminergic systems disrupts CTA learning following injection of amphetamine, but not that induced by LiCl (10, 17, 20, 21).

Since amphetamine is a dopamine agonist, most research into the neural mechanisms of amphetamine-induced CTA learning has focused on the role of the dopaminergic system in this behavior. However, this research has shown that disruption of dopaminergic activity causes only an attenuation of amphetamine-induced CTA learning rather than its complete elimination (5, 10, 17, 20, 21), as would be expected if the dopaminergic properties of amphetamine formed the basis for the acquisition of the CTA. Since the observation of an attenuated response, instead of its elimination, may reflect an incomplete disruption of dopaminergic activity, the first experiment in this series was designed to further evaluate the role of dopamine in the acquisition of an amphetamine-induced CTA by using subjects that had been pretreated with the dopaminergic antagonist haloperidol. Haloperidol was selected because it has a high binding capacity for dopamine receptors (1), and should, therefore, block dopaminergic activity at doses that produce few CTA-related side-effects (4).

GENERAL METHOD

Subjects

The subjects were male Crl:CD BR VAF/Plus rats (*Rattus norvegicus*) weighing 300–400 g at the start of the experiment. Rats were quarantined on arrival and screened for evidence of disease before being released from quarantine. They were main-

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tained in an AAALAC accredited facility in plastic Microisolator cages on hardwood chip contact bedding, and provided commercial rodent chow. Water was also available except as required by the experimental protocol. Animal holding rooms were maintained at 21 ± 1°C with 50 ± 10% relative humidity using at least 10 air changes per hour of 100% conditioned fresh air. The rats were maintained on a 12-hr, light:dark, full spectrum lighting cycle with no twilight.

Procedure

Rats were placed on a 23.5-hr water deprivation schedule for 10 days during which water was available for 30 min during the early light phase of the diurnal cycle. On the conditioning day, the rats were presented with two calibrated drinking tubes, one containing tap water and the other containing a 10% sucrose solution, and intake of each measured. Immediately following the 30-min drinking period, the rats were injected with either haloperidol or saline. Thirty min later, they were injected with either amphetamine or saline. On the test day (24 hr later), the rats were again given access to the calibrated tubes and intake of water and sucrose solution measured.

The data are presented as preference scores: sucrose intake divided by total fluid (water + sucrose) intake. A preference score of less than 0.50 indicates an aversion to the normally preferred sucrose solution. For data analysis, the preference scores were transformed using the arcsin transformation to normalize the distributions (7) and subjected to mixed two-way analyses of variance.

EXPERIMENT 1

This experiment was designed to determine whether or not pretreatment with the dopamine antagonist haloperidol could disrupt the acquisition of a CTA produced by treatment with high doses of amphetamine.

Procedure

The subjects were 66 rats divided into 7 groups of 6–13 rats/group. Three of the groups received injections of haloperidol (0.1, 0.2, and 0.5 mg/kg, IP) followed 30 min later by injection of isotonic saline. Another 3 groups received one of the three doses of haloperidol followed by injection of amphetamine (3.0 mg/kg, IP) 30 min later. The final group received a saline injection (IP) followed by injection of amphetamine.

Results

Pretreatment with haloperidol alone did not produce any consistent changes in test day fluid intake. The results of the treatment on the acquisition of a CTA are summarized in Fig. 1. As shown in the top panel, none of the doses of haloperidol produced a CTA when administered by itself. In contrast, aversions were observed in all groups that were given injections of amphetamine, whether they were pretreated with saline or with haloperidol. The initial analysis of variance showed that the main effect for dose for the comparison across the haloperidol and saline treatments was not significant, $F(3,37) = 2.059$, $p > 0.10$. The main effect for day for the comparison between conditioning and test days, $F(1,37) = 45.342$, $p < 0.001$, and the dose-by-day interaction, $F(3,37) = 3.436$, $p < 0.05$, were both significant. This analysis indicates that there was a reduction in test day sucrose intake in all groups regardless of the dose of haloperidol (0.0–0.5 mg/kg) utilized, but that the nature of the reduction varied as a function of the dose.

Two additional analyses were performed to determine the basis

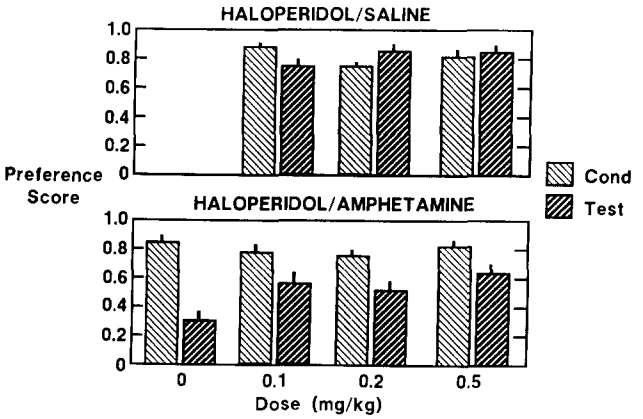

FIG. 1. Taste aversion learning produced by administration of one of several doses of haloperidol followed by isotonic saline (upper panel) or haloperidol followed by amphetamine, 3 mg/kg (lower panel). The dose of haloperidol is given on the X-axis. Error bars indicate the standard error of the mean.

for the significant dose-by-day interaction above. The first analysis was for the data of the three groups that received haloperidol. Only the main effect for day, $F(1,29) = 19.088$, $p < 0.001$, was significant, indicating that amphetamine produced an equivalent reduction in test day sucrose intake across all three doses of haloperidol. Since the main effect for dose in this analysis was not significant, $F(2,29) = 1.122$, $p > 0.10$, the data of all three haloperidol groups were combined and compared to those obtained from animals pretreated with saline. Again, the main effect for day for the comparison between conditioning and test days was significant, $F(1,39) = 50.949$, $p < 0.001$, as was the drug-by-day interaction, $F(1,39) = 10.239$, $p < 0.01$. The main effect for drug for the comparison between the haloperidol- and saline-treated animals, $F(1,39) = 3.039$, $p < 0.10$, did not achieve significance. This analysis, therefore, indicates that although a test day reduction in sucrose intake was observed in both the haloperidol- and saline-treated animals, the animals given haloperidol responded differently to the amphetamine injection than did the animals given saline.

Discussion

The present results showing that injection of haloperidol does not produce a CTA by itself confirms previous research reported by Giardini (4). In addition, the attenuation of the amphetamine-induced CTA by pretreatment with haloperidol is consistent with a large number of studies implicating the involvement of dopaminergic mechanisms in this response (5, 17, 20, 21).

More specifically, the present results showing a dose-independent attenuation of an amphetamine-induced CTA by the dopamine antagonist haloperidol is consistent with the results obtained by Grupp (5), who reported that pretreating rats with the dopamine antagonist pimozide produced a similar dose-independent attenuation of amphetamine-induced CTA learning. However, in contrast to the present results which showed that haloperidol attenuated a CTA following injection of amphetamine at a dose of 3 mg/kg, pimozide only attenuated a CTA produced by 1 mg/kg, but not 2 mg/kg, amphetamine (5). This observation would be consistent with the greater binding capacity of haloperidol at dopaminergic synapses (1).

As indicated above, the attenuation of the amphetamine-induced CTA was independent of the dose of haloperidol over a range of 0.1–0.5 mg/kg. This observation may be due to the fact that, for amphetamine-induced CTA learning, there is no differ-

ence between the minimum dose needed to produce an attenuation and the dose that produces a maximal effect. Alternatively, it may also reflect the fact that the lowest dose tested was sufficient to produce maximal attenuation of the amphetamine-induced CTA. In the latter instance, the use of even lower doses may be necessary to show dose-dependent haloperidol effects on amphetamine-induced CTA learning. The present data do not allow an evaluation of these alternate hypotheses.

However, it is not clear why, if the amphetamine-induced CTA depends upon the action of the drug at dopaminergic synapses, the disruption of dopaminergic activity produces only an attenuation of CTA learning and not the complete loss of the response. One possibility, suggested by Lorden *et al.* (10), is that the intraventricular injection of 6-hydroxydopamine, which attenuates amphetamine-induced CTA learning, produces depletion of both dopamine and norepinephrine. In contrast, intranigral administration of 6-hydroxydopamine causes depletion only of dopamine and does not affect the acquisition of an amphetamine-induced CTA, suggesting that the combined depletion of both dopamine and norepinephrine is necessary for the attenuation of an amphetamine-induced CTA under these conditions. This hypothesis, however, is not consistent with the observation that treatment with the dopamine antagonists pimozide and haloperidol are effective in attenuating amphetamine-induced CTA learning in a manner similar to that observed following intraventricularly-administered 6-hydroxydopamine, because the effects of these drugs are restricted to the dopaminergic system. It is possible that intranigral injection of 6-hydroxydopamine does not produce the same pattern of dopamine loss as produced by intraventricular injection, such that specific structures that may be important in mediating the CTA response may not have been affected by the treatment (8).

Another possibility may involve the area postrema. Although taste aversions produced by high doses of amphetamine (>1.5 mg/kg) are not affected by AP lesions (15,16), aversions produced by lower doses of amphetamine do seem to be mediated by the AP (15). AP involvement in mediating the CTA response to amphetamine may reflect the operation of two possible mechanisms. First, it may result from activation of dopaminergic receptors located in the AP (9,12). Consistent with this hypothesis are data showing that intracranial injections of amphetamine, unlike LiCl, into the vicinity of the AP will produce a CTA (2,18), and that lesions of the AP can alter the motor responses of rats to injection of amphetamine (3). However, this hypothesis is not consistent with the present results because the haloperidol injection should have blocked all dopaminergic activity, both in the AP as well as centrally.

Alternatively, the AP may be involved as the result of its activation by an endogenous, nondopaminergic, peripheral factor, in a manner similar to that involved in the acquisition of LiCl- and radiation-induced aversions (13). This hypothesis would suggest, therefore, that amphetamine-induced CTA learning involves both a peripheral mediator as well as a central dopaminergic component. It may, therefore, be that the manipulation of dopaminergic synapses by neurotoxins or by use of dopamine antagonists affects only the central mechanisms involved in amphetamine-induced CTA learning, but not the peripheral mechanisms involving the AP. This results in an attenuation of the CTA response rather than its complete disruption following manipulation of the dopaminergic system. If this hypothesis is correct, then it should be possible to produce a complete disruption of amphetamine-induced CTA learning by combining haloperidol treatment with lesions of the AP.

EXPERIMENT 2

As indicated above, this experiment was designed to evaluate the possibility that amphetamine-induced CTA learning involves both a central component mediated by the dopaminergic system and a peripheral component mediated by the AP.

Procedure

Histologically confirmed lesions were made in the AP of 13 male albino rats. An additional 14 rats served as unoperated controls. The details of the surgical procedures have been published previously (14,15). Briefly, the rats were anesthetized with sodium pentobarbital (35 mg/kg, IP), the AP exposed and cauterized under direct visual control. Following surgery, all animals were given a prophylactic injection of Bicillin (60,000 units), returned to their home cages and allowed to recover from the effects of the surgery for 3–4 weeks before beginning the behavioral testing.

The behavioral procedures were identical to those detailed above except that, because there were no dose effects, only a single dose of haloperidol was used (0.5 mg/kg, IP). The intact rats were divided into two groups ($n=7$ each), one of which was given haloperidol followed 30 min later by amphetamine (3 mg/kg, IP), while the second group was given an equivolume injection of saline followed by amphetamine. All the rats with AP lesions were treated with haloperidol followed by amphetamine. These procedures were similar to those utilized in a previous study of the role of the AP in the acquisition of an amphetamine-induced CTA (15), with the exception that the control rats in that study were not given an injection of isotonic saline prior to the amphetamine injection.

At the conclusion of the experiment, the operated animals were sacrificed with an overdose of pentobarbital (50 mg/rat, IP) and perfused intracardially with isotonic saline and 10% formalin saline. The brains were removed, fixed in formalin saline and 50 μm sections taken from the brainstem at the level of the AP. Representative sections of the AP and a lesion are presented in Fig. 2. Lesion size was somewhat variable, involving only the AP in some cases, but impinging on the dorsal parts of the nucleus of the solitary tract in others. However, no behavioral differences were observed as a function of lesion size.

Results

There were no significant differences in average fluid intake between the three experimental groups. As shown in Fig. 3, pretreatment with haloperidol in intact animals attenuated the CTA produced by injection of amphetamine, but did not eliminate it. In the rats with AP lesions, however, pretreatment with haloperidol produced a complete disruption of amphetamine-induced CTA learning. This finding contrasts with previous research (15) showing that AP lesions do not, by themselves, produce a significant attenuation of a CTA following injection of 3 mg/kg amphetamine.

Statistical analysis of the data using an analysis of variance followed by orthogonal comparisons (7) indicated that for the intact animals the saline-treated rats showed a significantly reduced test day sucrose intake compared to the haloperidol-treated rats, $F(1,23)=6.67$, $p<0.05$. The comparison between the intact rats and those with AP lesions was also significant, $F(1,23)=29.21$, $p<0.01$, indicating that both groups of intact rats showed a greater test day aversion to the sucrose than did the group with AP lesions.

Discussion

These results clearly indicate that the AP is involved in the acquisition of an amphetamine-induced CTA. As was observed in

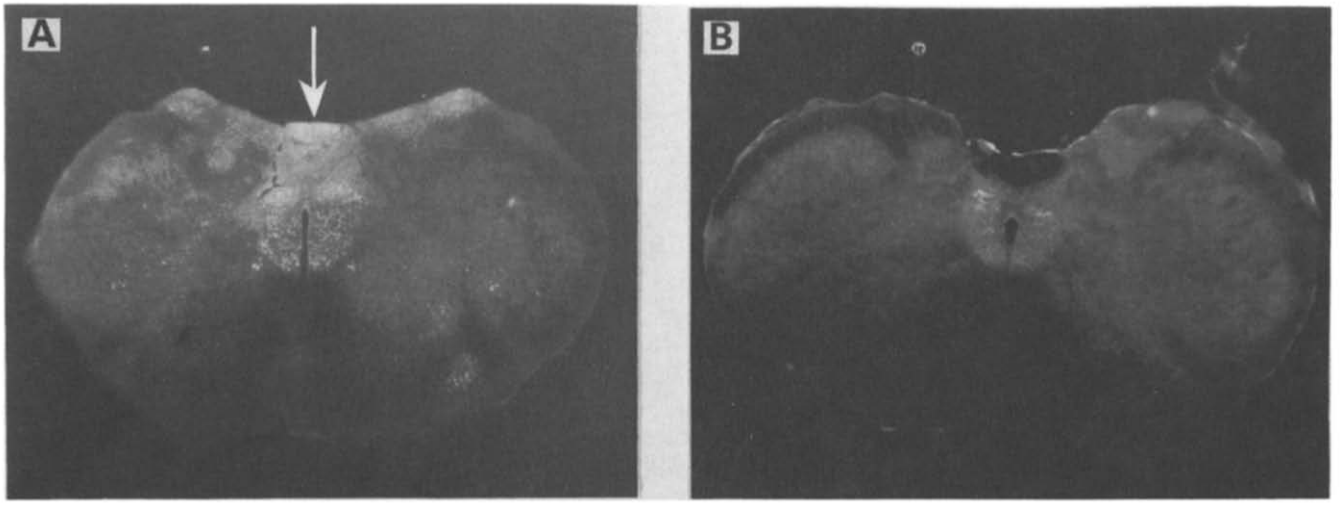

FIG. 2. Photomicrographs of sections of the brainstem of an intact rat showing the area postrema (A, arrow) and a representative lesion (B).

the first experiment of this series, haloperidol pretreatment attenuated the CTA produced by amphetamine, but did not completely block it. Previous research has shown that lesions of the AP do not affect the acquisition of an amphetamine-induced CTA when higher doses of amphetamine are used (15). A complete disruption of the amphetamine-induced CTA was obtained only when the haloperidol was combined with lesions of the AP.

A possible explanation for the present observation of AP involvement in the CTA following administration of 3 mg/kg amphetamine may be that, in the untreated animal, the response of the central dopaminergic system to the large dose of amphetamine overshadows a relatively weak response mediated by the AP, leading to the acquisition of a CTA that is apparently not modulated by the destruction of the AP. In contrast, when the activity of the central dopaminergic system has been reduced by treatment with the dopamine antagonist haloperidol, as in the present experiment, or by other means (5, 10, 17, 20, 21), then the contribution of the AP-mediated mechanisms are expressed in behavior. Thus, the complete elimination of amphetamine-induced CTA learning requires both the disruption of the mechanisms mediated by the dopaminergic system as well as those mediated by the AP, which are independent of the dopaminergic system.

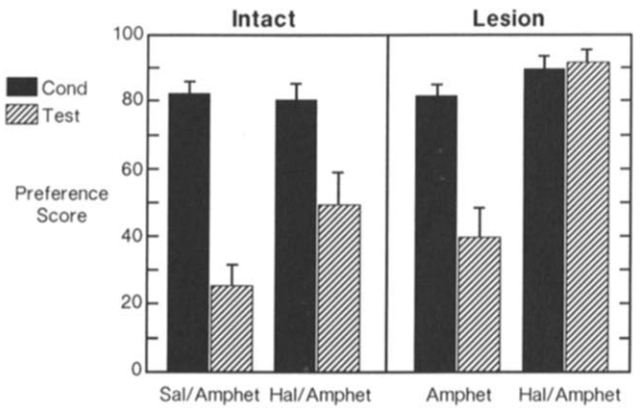

FIG. 3. Effects of haloperidol and area postrema lesions on the acquisition of an amphetamine-induced CTA. Preference scores from the two groups of intact animals are shown in the left-hand panel; from rats with AP lesions, in the right-hand panel. The data for the amphetamine-only group has been recalculated from (15). Error bars indicate the standard error of the mean.

GENERAL DISCUSSION

The present results indicate that the acquisition of a taste aversion following injection of the dopamine agonist amphetamine involves two distinct mechanisms: a central mechanism mediated by the dopaminergic system as well as a peripheral mechanism mediated by the AP. Complete disruption of amphetamine-induced CTA learning requires manipulations that affect both mechanisms simultaneously.

In this regard, amphetamine seems to be somewhat unique. For both radiation- and LiCl-induced aversions, destruction of the AP is sufficient to produce the complete disruption of CTA learning (11, 14, 16). Manipulation of the dopaminergic system by intraventricular injection of neurotoxins (10, 17, 20) or by lesions of the dorsolateral tegmentum (21) have no effect on the acquisition of a LiCl-induced CTA. Similarly, pretreatment with haloperidol (0.1–0.5 mg/kg) has no effect on the acquisition of a radiation-induced CTA (Rabin and Hunt, unpublished results). These findings suggest that, with the exception of amphetamine, a dopamine agonist, the dopaminergic system is not routinely involved in the acquisition of a CTA with these toxins. Therefore, the present results would suggest the hypothesis that the acquisition of a CTA may involve distinct brainstem pathways, depending upon the specific nature of the unconditioned stimulus.

ACKNOWLEDGEMENTS

We wish to acknowledge the support of the Computer Science Center Facilities of the University of Maryland Baltimore County. This research was supported by the Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, under work unit 4320. Views presented in this paper are those of the authors; no endorsement by the Defense Nuclear Agency has been given or should be inferred. This research was conducted according to the principles described in the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Research, National Research Council.

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