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Dopamine Depletion by 6-Hydroxydopamine Prevents Conditioned Taste Aversion Induced by Methylamphetamine but not Lithium Chloride[1]

G. C. WAGNER,* R. W. FOLTIN,* L. S. SEIDEN† AND C. R. SCHUSTER‡

**Department of Biopsychology; Supported by Searle Fellowships,*
†Department of Pharmacological and Physiological Sciences,
‡Department of Psychiatry, University of Chicago, Chicago, IL 60637

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WAGNER, G. C., R. W. FOLTIN, L. S. SEIDEN AND C. R. SCHUSTER. *Dopamine depletion by 6-hydroxydopamine prevents conditioned taste aversion induced by methylamphetamine but not lithium chloride.* PHARMAC. BIOCHEM. BEHAV. 14(1) 85–83, 1981.—Specific lesions of the central dopaminergic system produced by intraventricular injections of 6-hydroxydopamine with pargyline and desmethylimipramine pretreatment significantly attenuated conditioned taste aversions to a sweetened-condensed milk solution induced by methylamphetamine. Identically treated rats formed an aversion to the milk solution when lithium chloride was utilized. These results suggest that the methylamphetamine-induced aversion is dependent upon intact dopaminergic neurons.

Dopamine Conditioned taste aversion 6-Hydroxydopamine Methylamphetamine Lithium chloride

USING a variety of neuropharmacological and psychopharmacological techniques the central dopamine (DA) system has been implicated in the mediation of certain actions of d-amphetamine (AMPH) and methylamphetamine (MA). AMPH has been shown to cause the release of DA [17,21] as well as block the reuptake of DA [11]. The anorexic effects of AMPH were attenuated by depletion of DA induced by 6-hydroxydopamine (6-OHDA) [8, 9, 10] and by pretreatment with DA antagonists haloperidol and pimozide [23]. In addition, the hyperactivity following administration of AMPH was significantly reduced following destruction of the mesolimbic DA system [14] while the induction of stereotypic behavior was dependent upon an intact nigrostriatal DA system [13].

The administration of AMPH subsequent to the consumption of a novel food or fluid has been shown to diminish the intake of that substance when it is again made available [2, 3, 16]. This effect, commonly referred to as conditioned taste aversion (CTA) [1, 4, 16, 18] was attenuated by pretreatment with alpha-methyl-tyrosine (AMT) [6] or by destruction of the catecholaminergic system by 6-OHDA [19]. Neither of these later studies employed methods specific for DA. Rather, both DA and NE were depleted rendering interpretation difficult. However, it has been observed that pimozide, a DA blocker, will also antagonize this effect suggesting that DA is the critical neurotransmitter mediating the aversive effect of AMPH [7].

This study was conducted to further explore the mediation by DA of MA's aversion inducing ability. Specific destruction of DA neurons was achieved by intraventricular 6-OHDA injections with a pretreatment of pargyline (a monoamine oxidase inhibitor) and desmethylimipramine (DMI) (which protects noradrenergic (NE) neurons by blocking their uptake of 6-OHDA) [8,9]. Lithium chloride (LiCl), a substance capable of producing strong conditioned taste aversions independent of the DA system [18], was used as an additional aversion-inducing agent to assure that the 6-OHDA lesion did not affect the subject's ability to form all aversions.

METHOD

Subjects

Thirty-six male Sprague-Dawley rats weighing between 250 and 300 g were housed individually with ad lib access to food (Tekland Co., Winfield, OH).

6-OHDA Injections

All rats were treated IP with pargyline (50 mg/kg) and

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DMI (25 mg/kg) 45 min prior to ether anesthesia. Bilateral holes were drilled 1.5 mm lateral to the midline suture and 0.5 mm posterior to bregma. One-half of the rats were then treated with 200 μg of 6-OHDA (free base) dissolved in physiological saline with 0.1% ascorbate. The remaining rats received the same vehicle without the 6-OHDA. The injection volume was 10 μl per side delivered (over a 30-sec period) via a Hamilton microliter syringe (Hamilton, NV) equipped with a 4.25 mm long 27 ga needle. These coordinates placed the injected volume into the lateral ventricles. Rats were then maintained for 2 weeks with ad lib food and water.

Water Intake Basline

All subjects were maintained at 23.6 hr water deprivation by restricting access to water to a single 20 min presentation occurring at the same time (2:00 to 2:20 p.m.) each day, seven days a week. All fluids were presented in Wahmann (Baltimore, MD) 100 ml calibrated drinking bottles attached centrally to the front of the cage. Baseline intake was determined for 7 days.

Training and Testing

On the eighth day, all rats received a bottle of sweetened condensed milk (Bordon Co., diluted 2:1 water:milk) in place of the 20 min of water. Fifteen minutes after the milk was removed, rats were given an IP injection of methylamphetamine hydrochloride (2.5 mg/kg dissolved in physiological saline at a concentration of 2.5 mg/ml), LiCl (127 mg/kg dissolved in physiological saline to a concentration of 12.7 mg/ml) or physiological saline. There were six control and six 6-OHDA treated rats receiving the MA, LiCl or saline. Three rats from both of the saline-treated groups received the smaller volume of the vehicle while the other three received the larger. On the ninth day, all rats received access to the milk solution for 20 min followed by the appropriate injection 15 min after removal of the milk. On the tenth day, the rats received a 20 min two-bottle choice test. One bottle contained the milk solution while the other bottle contained water with position (left, right) randomized between subjects. Thereafter, rats received ad lib access to food and water.

Assays

One week after the last test, rats were killed and brains removed and dissected according to previously described methods [5,22] to yield samples of caudate nucleus, hypothalamus, cortex, midbrain, and pons-medulla. Brain parts were stored in liquid nitrogen until assayed for DA content (all regions) and norepinephrine (NE) (hypothalamus only). Assays were performed with high performance liquid chromatography with electrochemical detection [12,20]. On the basis of the results, it was evident that three of the 6-OHDA treated rats (one from each experimental group) had near normal DA levels with caudate DA levels greater than three standard deviations above the rest of the lesioned rats. Consequently, these rats were excluded from all calculations for Table 1 as well as Figs. 1 and 2 but not from the correlation coefficient determinations.

RESULTS

There was no significant difference between the 6-OHDA treated and control rats in terms of their baseline water consumption or in the amount of milk consumed during the initial exposure prior to any injection (Fig. 1). Differences in milk intake between the first and second training day were analyzed for each group using the Wilcoxan Signed Rank Test. The amount of milk consumed during the second exposure was increased for the saline treated controls rats $T(6)=1$, $p<0.05$, while there was a significant decrease for those control rats receiving LiCl, $T(6)=0$, $p<0.05$, and a decrease, though not significant, for those rats receiving MA. The saline treated 6-OHDA lesioned rats also showed an increase in amount consumed during their second milk exposure while those receiving the LiCl drank significantly less, $T(5)=0$, $p<0.05$. However, the 6-OHDA treated rats receiving the MA injection displayed behavior similar to those receiving saline, consuming more milk on the second exposure although this did not reach significance (Fig. 1).

Control rats which had received saline drank more milk than water during the two bottle choice test while those control rats which had received MA, $U(6,6)=0$, $p<0.01$, or LiCl, $U(6,6)=0$, $p<0.01$ drank significantly more water than saline controls when presented with the choice. The 6-OHDA treated rats receiving saline also drank more milk during the two bottle choice while those receiving the LiCl drank more water, $U(4,5)=0$, $p<0.02$. However, the 6-OHDA treated rats receiving the MA drank significantly more milk during their two bottle choice test than the saline-treated MA subjects, $U(5,6)=0$, $p<0.01$ (Fig. 2).

Pearson product-moment correlations were calculated comparing the preference ratio from the two bottle choice test (milk intake divided by milk + water intake) as a function of aversion inducing agent with brain levels of DA and NE. There was no significant correlation of brain catecholamines and preference ratio for the groups receiving saline or LiCl. However, for the rats receiving the MA there were significant correlations between caudate DA and preference ratio and cortex DA and preference ratio ($r=-.89$; $p<0.001$ and $r=-.60$, $p<0.05$, respectively).

The catecholamine level assays confirmed that the depletion induced by the 6-OHDA was specific for DA. Comparison of catecholamine levels among each of the three groups of vehicle of 6-OHDA treated rats revealed no significant differences and these within treatment groups were, therefore, combined. The 6-OHDA treatment resulted in DA levels that were significantly depressed in all brain regions while NE levels were not altered (Table 1).

DISCUSSION

Rats with specific lesions of the DA system induced by 6-OHDA responded to both the saline and the LiCl stimuli in a manner indistinguishable from the vehicle treated rats. There was an initial decrease in consumption from baseline water intake levels followed by an increase in amount consumed in those rats recieiving saline while there was a strong aversion in those rats receiving LiCl. The importance of these groups was the verification that the lesion did not disrupt the rat's ability to form an aversion. However, those rats with the specific DA lesion responded to the MA in a manner analogous to those receiving the saline. No aversion was observed towards the milk solution; there was, in fact, an initial decrease in consumption followed by an increase in amount consumed during the second and third exposures. The significant inverse correlation between the DA levels and the extent of the formed aversion produced by the MA

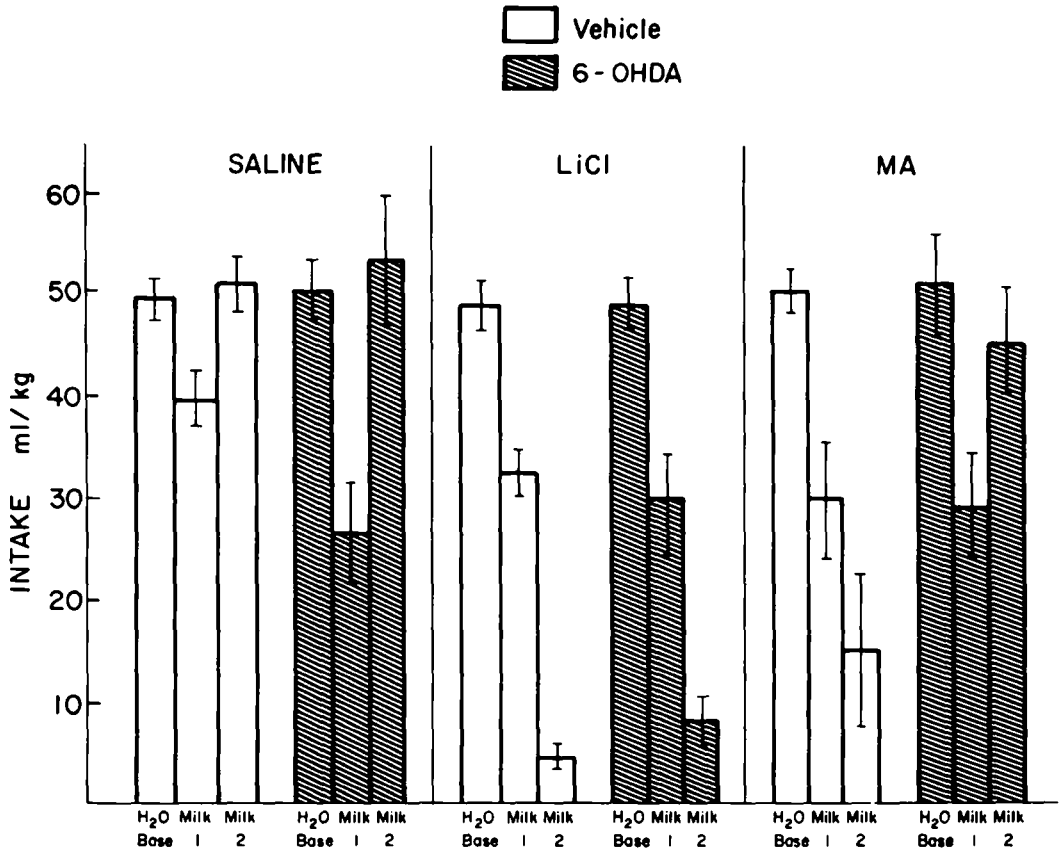

FIG. 1. Mean milk intake ± SEM in ml/kg of water (baseline) and milk (training days 1 and 2) for the 6-hydroxydopamine (6-OHDA) and vehicle treated rats. LiCl=lithium chloride; MA=methamphetamine.

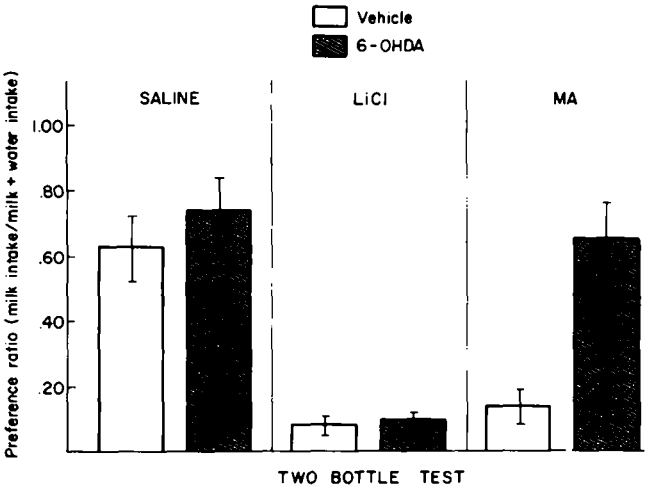

FIG. 2. Preference ratios (milk/milk+water; ±SEM) for the two bottle choice test. 6-OHDA=6-hydroxydopamine; LiCl=lithium chloride; MA=methamphetamine.

concomitant with the lack of any similar correlation in the LiCl groups further suggests the importance of the DA system in the mediation of this effect of MA.

These results extend the observations by others using AMPH [15,19] that an intact DA system was prerequisite for MA to attenuate food intake when the MA was administered post-consumption. In a recent study [15], 6-OHDA alone or in combination with DMI pretreatment was used to destroy DA, NE or both systems. These treated rats were then tested in a paradigm similar to that used in the present study. However, the authors concluded that ". . . the [attenuating] effects of intraventricular 6-OHDA on amphetamine induced aversion was the result of depletion of both NE and DA." This result is contrary to our observation that only DA depletion was required. Two procedural differences may be responsible for this discrepancy. In the above study, the 6-OHDA was injected unilaterally into one ventricle, whereas in the present study, the neurotoxin was delivered bilaterally. The reported DA levels in the above study were depleted to 21% of control, whereas in this study the levels were depleted to 6%. Furthermore, the unilateral injection procedure may result in markedly asymmetric lesions which would mean that the contralateral side may have been depleted to only 40% of control. These differences may be crucial to the interpretative conclusions reached in the two studies.

TABLE 1

DOPAMINE (DA) AND NOREPINEPHRINE (NE) LEVELS IN μg/g OF TISSUE (WITH STANDARD ERRORS) FOR THE VEHICLE AND 6-HYDROXYDOPAMINE (6-OHDA)TREATMENTS

	Caudate DA	Cortex DA	HT DA	Midbrain DA	Pons DA	HT NE
Vehicle						
N=12	8.81	0.27	0.57	0.16	0.07	1.03
	0.34	0.04	0.12	0.05	0.01	0.11
6-OHDA						
N=12	0.56*	0.05*	0.10*	0.04*	0.02*	0.90
	0.17	0.01	0.02	0.01	0.01	0.11

HT—hypothalamus; PONS=pons-medulla; *$p<0.05$.

Although certain of the neurochemical actions of MA may be mediated through a number of transmitter systems or interactions between transmitter systems, the systematic application of psychopharmacological techniques has led to an emphasis upon the DA system [8, 9, 10, 11, 17, 21]. The present demonstration of the specific blocking of MA-induced aversion in the CTA paradigm by brain DA depletions is further support for a role of DA in mediating at least some of the properties of MA.

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