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Alterations in Consummatory Behavior Following Intracisternal Injection of 6-Hydroxydopamine

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BREESE, G. R., R. D. SMITH, B. R. COOPER AND L. D. GRANT. *Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine.* PHARMAC. BIOCHEM. BEHAV. 1(3) 319–328, 1973.– Intracisternal administration of two doses of 6-hydroxydopamine, one with pargyline pretreatment and one without, caused an initial disruption of consummatory behavior. In spite of measures to enhance recovery from these acute effects, 6-hydroxydopamine treated rats were found to maintain body weight at a lower level than control rats. Similar to controls, treated animals were found to drink water in the absence of food and to enhance water consumption in response to the administration of a hypertonic saline solution. However, unlike control rats, animals treated with 6-hydroxydopamine failed to increase food intake following insulin administration. Desoxycorticosterone acetate (DOCA) treatment enhanced saline preference in 6-hydroxydopamine treated rats, but the maximum volume of saline consumed was markedly less than the intake of control rats following DOCA treatment. While control rats drank a large volume of either a sucrose or a saline solution when substituted for water, 6-hydroxydopamine treated animals showed little increase in their intake of these solutions. Preferential depletion of norepinephrine in brain did not alter consumption of a sucrose solution; however, depletion of dopamine produced a significant reduction in sucrose intake. These latter findings suggest that this deficit observed in the 6-hydroxydopamine treated rat involves interruption of dopaminergic pathways.

6-Hydroxydopamine Consummatory behavior Sucrose consumption Saline consumption

ADMINISTRATION of 6-hydroxydopamine into brain has been shown to produce prolonged reductions of brain catecholamines due to destruction of central sympathetic neurons [2, 7, 8, 9, 25, 26]. Similar to other phenylethylamine compounds [3], 6-hydroxydopamine has been shown to affect norepinephrine containing neurons to a greater extent than dopaminergic fibers [8, 9, 25]. In subsequent experiments, depletion of dopamine was found to be enhanced if the 6-hydroxydopamine injections were preceded by pretreatment with pargyline, a monoamine oxidase inhibitor [7, 8, 14, 26, 29].

Treatments combining 6-hydroxydopamine and a monoamine oxidase inhibitor have been used recently to study the role of brain catecholamine-containing fibers in consummatory behavior. Fibiger *et al.* [14], as well as Zigmond and Stricker [29], have reported that the intraventricular administration of 6-hydroxydopamine to animals pretreated with a monoamine oxidase inhibitor produced an acute aphagia and adipsia. Previous studies in which 6-hydroxydopamine was administered in the absence of monoamine oxidase inhibition, have not reported this acute change in consummatory behavior [2, 8, 25] unless the 6-hydroxydopamine was injected intracerebrally into the lateral hypothalamus [21] or into the pathway of the nigro-striatal tract [24]. Interestingly, the consummatory deficits seen after injection into the lateral hypothalamus were interpreted as being due to destruction of norepinephrine fibers [21], while similar effects in the latter study [24] were attributed to destruction of dopamine-containing neurons which are found in the nigro-striatal tract.

The present report provides evidence that intracisternal injections of 6-hydroxydopamine after a monoamine oxidase inhibitor produces the same acute effects on eating and drinking previously observed following intraventricular administration [14, 29]. Previously, little data pertaining to chronic consummatory effects has been reported; this manuscript will describe several chronic deficits in consummatory behavior following treatment with 6-hydroxydopamine, including a reduction in the amount of sucrose solution normally ingested by control rats. In addition, sucrose consumption was examined in rats preferentially

depleted of either norepinephrine or dopamine to determine if one of these amines could be individually implicated in this deficiency.

METHOD

Animals

Male Sprague-Dawley rats (140–180 g) were used in all experiments. Animals were given two doses of 6-hydroxydopamine (200 μg) intracisternally under ether anesthesia, one dose 30 min after pargyline (50 mg/kg) and the second dose one week later without pargyline pretreatment [7,8]. Control animals were injected with pargyline 30 min before the intracisternal administration of 25 μl of ascorbic acid vehicle (0.5%). To reduce dopamine preferentially, rats received 240 μg of 6-OHDA 1 hr after 30 mg/kg desipramine on 2 occasions 1 week apart [8,12]. Depletion of brain norepinephrine with little effect on dopamine was accomplished by administering three 25 μg doses of 6-hydroxydopamine at three day intervals [8,25].

Procedures

In order to examine the acute effects of 6-hydroxydopamine on eating and drinking, rats were individually caged. Water was provided through inverted bottles with metal drinking tubes. Each water bottle was weighed daily and the water consumed was determined by weight difference. Food consumption as well as body weight were also determined each day. Known amounts of food were added to the cage floor each day; the amount of food remaining 24 hr later was determined. Powdered food produced during the act of eating was not included in this weight but large pellets that dropped through the cage were recovered and included in the weight. After 8 days, this experiment was terminated.

In subsequent experimental groups treated with 6-hydroxydopamine, therapeutic measures were taken to alleviate the severe symptoms produced acutely by 6-hydroxydopamine. This treatment consisted of administering 5 ml of a protein hydrolysate and dextrose solution (5% amigen and 5% dextrose injection, Baxter Laboratories, Morton Grove, Illinois) intraperitoneally each day and giving rats fresh slices of oranges and apples. In addition to nutritional supplement which began immediately after the second injection, groups were housed so that three rats occupied a single cage (9 1/2 x 6 x 6 in.) to minimize any possible hypothermia accompanying the 6-hydroxydopamine treatment [5]. With these therapeutic procedures, the acute period of weight loss was lessened and mortality did not exceed 15 percent in the treated rats. It has been found that tube feeding of animals as described by Ungerstedt [24] is also effective. Ten to 20 days after injection, all animals were individually caged. The treated animals appeared active and healthy, but maintained body weight without additional care at a lower level than control rats. At various times after this recovery period, baseline food and water consumption were determined daily for 5 day periods.

Fluid Consumption Tests

After baseline food and water intake was recorded, consumption of several fluids were examined in 6-hydroxydopamine treated rats. In initial studies, water was replaced by one of several solutions using a random cross-over design. The solutions included a 5% sucrose solution, a 0.03% saccharin solution, a 0.01% quinine solution and a 1% saline solution. Consumption of a given test solution was determined at 24 hr intervals during a 4 day period. Following each test period, rats were given water for three days followed by exposure to another solution.

Two preference experiments of fluid consumption were also conducted. Animals were given a choice of a saline solution or water in one group of experiments and a sucrose solution or water in another series of experiments. Bottles containing the solutions were positioned on the cages so that the metal drinking tubes were 1 in. apart and approximately 1 1/4 in. above the floor of the cage. Each day the amount consumed from each bottle was recorded and the bottles reversed. This procedure was subsequently used to determine if 6-hydroxydopamine would alter sodium appetite increased by desoxycorticosterone trimethylacetate administration [18]. Treated and control animals were screened and if after 6 days they were found to ingest primarily the saline solution they were removed from the experiment (N = 3). At this time, the remaining 18 animals were injected with desoxycorticosterone trimethylacetate (DOCA; Percorten, Ciba, Summit, New Jersey) 2.5 mg/kg daily for 6 days and then with 5.0 mg/kg daily for 8 days.

In order to determine changes in the water consumption following an injection of hypertonic salt solution, 2 ml per 100 g body weight of 1 M NaCl solution was injected intraperitoneally [22]. Water consumption was recorded at 2-hr intervals for 6 hr. Total 24 hr water intake was also recorded following this procedure. Treated and control animals were also deprived of food to determine how much water they would drink in the absence of food.

Food Consumption Test

Food intake was measured after subcutaneous injection of insulin (20 units/kg; Iletin, Lilly, Indianapolis, Indiana) [11,16]. Rats were not deprived of food before the test. Insulin was injected at approximately 9:00 a.m. Total food intake was accurately measured at hourly intervals for 5 hr and compared with food intake during the same time period recorded on the day preceding the insulin test. The amount consumed during the subsequent 19 hr was also recorded.

Biochemical Determinations

Following the various treatments, each animal was killed. The brain was immediately removed from the skull and rinsed with cold water. Most brains were then dissected into two halves. One half was frozen immediately on dry ice and stored (−76°C) until homogenized later in 0.1 N HCl for analysis of brain serotonin. Brains were analyzed for serotonin according to the method of Bogdanski, *et al.* [3]. The remaining brain halves were homogenized in 0.4 N perchloric acid and kept frozen until they could be analyzed for catecholamine content as previously described [7]. In other groups of animals treated with 6-hydroxydopamine, brains were dissected to obtain information on the effects of the various 6-hydroxydopamine treatments on brain catecholamine concentration in specific brain areas. Before dissection, the cerebellum was removed. The hypothalamus was dissected rostrally by a cut at the optic chasm, caudally by a cut at the level of the mammillary bodies and dorsally by a cut at the level of the auterior

commissure. The cut at the level of the optic chiasm permitted dissection of the striatum from the forebrain. Tissue referred to as rest of brain was the cortex remaining in the forebrain after removal of the striatum. These tissues were also homogenized in 0.4 N perchloric acid and the homogenates kept frozen (−20°C) until analyzed within 24 hr. After thawing, these homogenate were centrifuged and the supernatant analyzed for catecholamines [7].

Drugs

The 6-hydroxydopamine was obtained from Regis Chemical Co. (Chicago, Ill.). The pargyline was a gift of Abbott Laboratories (Chicago, Ill.). The desipramine was obtained from Geigy Laboratories (Ardsley, N.Y.).

RESULTS

Early Effects of Intracisternally Administered 6-Hydroxydopamine on Food and Water Consumption

After the intracisternal administration of 6-hydroxydopamine (200 μg) to animals treated with pargyline, food and water consumption was interrupted for one day (Fig. 1). The effects of a second injection of 6-hydroxydopamine (200 μg) administered without pargyline 10 days later produced a profound effect on eating and drinking. For the next 8 days, animals showed a total adipsia and aphagia (Fig. 1). At the end of this period, surviving animals were sacrificed. In our experience, if animals are not cared for immediately after the second injection of 6-hydroxydopamine (see Methods), up to 80 percent of the animals within a given group may die. Such observations are in agreement with the recent reports by Fibiger *et al.* [14] and Zigmond and Stricker [29] who administered 6-hydroxydopamine intraventricularly.

Prolonged Effects of 6-Hydroxydopamine Treatment on Water and Food Intake

Survival of 6-hydroxydopamine treated rats was enhanced if they were given nutritional supplements and housed to conserve body heat (see Methods). With this care, animals were severely hypophagic and hypodipsic rather than totally aphagic and adipsic as observed in animals singly caged that received no treatment. After a period of 10–21 days most animals began to eat and drink sufficient amounts of food and water to maintain life without special care. By 30 days, treated rats had reached a stable level of food intake. In Table 1, food and water consumption are shown in two different groups of rats; one group was examined approximately 30 days and the other approximately 180 days after the last injection of 6-hydroxydopamine. The body weights of control and 6-hydroxydopamine treated rats were quite different even though weights were comparable at the time of injection. The fact that this difference is evident 180 days after treatment suggests a chronic impairment in the 6-hydroxydopamine treated rat, which maintains body weight at a subnormal level. Control rats starved for 5 days and then allowed food ad lib reached the weight of control rats within 4–5 weeks indicating that the reduced food intake during the acute period following 6-hydroxydopamine could not account for the changes in body weight observed later. Even though food consumption was reduced slightly at the earlier time period after treatment, the greatest reduction in terms of absolute amount was in water consumption when based on

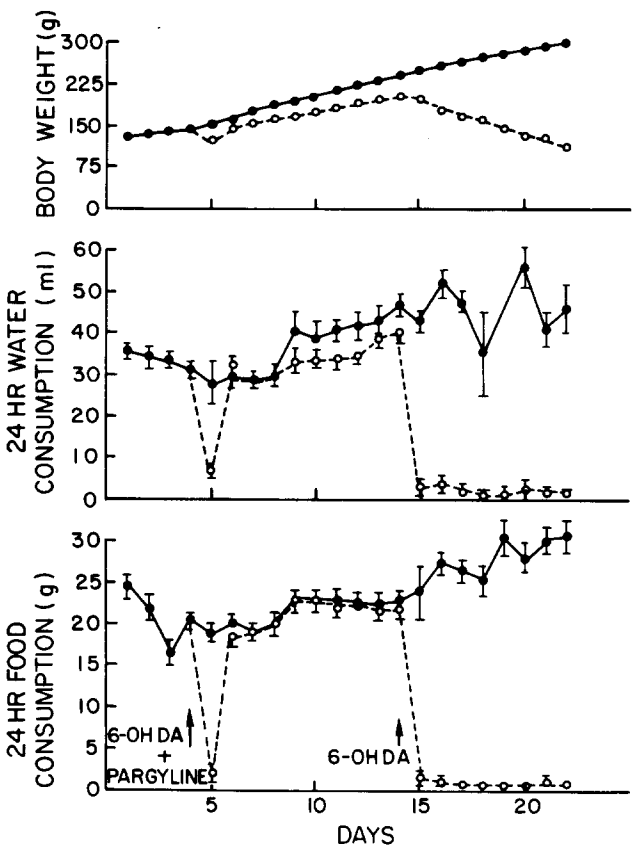

FIG. 1. Effect of 6-hydroxydopamine (6-OHDA) administration on food and water consumption and body weight. Four days after the beginning of the experiment, rats were pretreated with pargyline (50 mg/kg, IP) 30 min before administration of a 200 μg dose of 6-OHDA. The second dose of 6-OHDA (200 μg) was administered 10 days later without pargyline. (●——●) refers to saline treated rats (N = 20); (o- - -o) refers to 6-OHDA treated rats (N = 26).

a 24 hr period (Table 1). However, when food and water consumption were calculated on the basis of body weight in an attempt to correct the body weight difference between the groups, the deficits in consumption in 6-hydroxydopamine treated rats were no longer evident.

In another experiment, 6-hydroxydopamine treated rats were placed on a 22 1/2 hr food deprivation schedule and water intake in the absence and in the presence of food was recorded (Table 2). As above, interpretation is again complicated by differences in body size of control and 6-hydroxydopamine-treated rats. Irrespective of this weight complication, it is clear that 6-hydroxydopamine treatment did not prevent animals from drinking water during food deprivation although the amount of water intake was significantly reduced regardless of the manner in which the data was presented. Whereas the absolute amount of water consumed was significantly reduced when food was present for 90 min, this difference was not present when water intake was corrected for body weight.

Effect of 6-Hydroxydopamine Treatment on the Intake of Sucrose, Saccharin, and Quinine Solutions

From a previous study which indicated that several

TABLE 1

TWENTY-FOUR HOUR FOOD AND WATER INTAKE AFTER 6-HYDROXYDOPAMINE (6-OHDA) TREATMENT

	Food		Water		Body Weight
	(g)	(g/100 g B.W.)	(ml)	(ml/100 g B.W.)	(g)
Group A					
6-OHDA	23.2 ± 0.8*	9.7 ± 0.3	29.4 ± 1.3*	12.4 ± 0.5	240 ± 8*
Controls	27.3 ± 0.7	8.8 ± 0.2	37.7 ± 1.5	12.3 ± 0.5	310 ± 10
Group B					
6-OHDA	24.5 ± 1.4	6.7 ± 0.4	32.6 ± 1.0*	8.9 ± 0.3	367 ± 23*
Controls	28.3 ± 1.6	5.3 ± 0.3	46.7 ± 2.6	8.8 ± 0.5	533 ± 16

6-Hydroxydopamine was administered intracisternally to pargyline pretreated animals as described in Methods; control animals received saline intracisternally and pargyline (50 mg/kg) intraperitoneally. Food and water intake was measured approximately 30 days and 180 days after the last injection of 6-OHDA in Group A and Group B, respectively. Values represent the mean ± S.E. of food and water intakes during a 5 day period presented as g/24 hr or as g/100 g body weight (B.W.)/24 hr. There are 14 to 16 animals per group.

*Values significantly different from control values at $p<0.01$.

TABLE 2

EFFECT OF FOOD DEPRIVATION ON FOOD AND WATER INTAKE IN THE 6-HYDROXYDOPAMINE TREATED RAT

Treatment	Food Present for 90 min		No Food for 22 1/2 hr	24 hr Total
	Food Intake (g)	Water Intake (ml)	Water Intake (ml)	Water Intake (ml)
Control	11.7 ± 1.0	15.9 ± 1.7	22.5 ± 1.5	38.4 ± 2.2
N = 13	(2.4 ± 0.3)	(3.2 ± 0.3)	(4.6 ± 0.25)	(7.8 ± 0.45)
6-OHDA	9.5 ± 0.7	10.6 ± 1.1*	12.2 ± 13*	22.8 ± 1.3*
N = 10	(2.6 ± 0.2)	(3.0 ± 0.3)	(3.4 ± 0.36*)	(6.3 ± 0.36*)

Values in parantheses represent the mean ± S.E. of consumption per 100 g body weight. Animals were treated with 6-hydroxydopamine (6-OHDA) as described in Methods. Rats were placed on a 22 ½ hr food deprivation schedule. The values are from the fifth day of the deprivation schedule. Mean body weight of control group was 490 ± 12 g; weight of 6-hydroxydopamine treated group was 360 ± 12 g before the experiment was initiated.

*$p<0.025$ when compared with consumption of control group.

6-hydroxydopamine treated rats did not drink sucrose [6], a systematic study of this observation was initiated. As shown in Fig. 2, control rats consumed approxmately 3 times as much sucrose solution as water. In contrast to this finding, animals treated with 6-hydroxydopamine showed little increase to the sucrose solution when it was substituted for water (Fig. 2; Table 5). A similar finding was obtained when animals were given a saccharin solution (Fig. 2). In contrast to baseline water intake, correcting values for body weight did not alter interpretation of these findings (Fig. 2; Table 5).

To gain some insight into their ability to taste, rats were given a quinine solution and a choice between water and a sucrose solution. When the quinine solution was substituted for water, control as well as 6-hydroxydopamine treated rats showed an initial decrease in consumption of this solution suggesting that both groups found the taste of the quinine solution aversive (Fig. 2). However, by the second

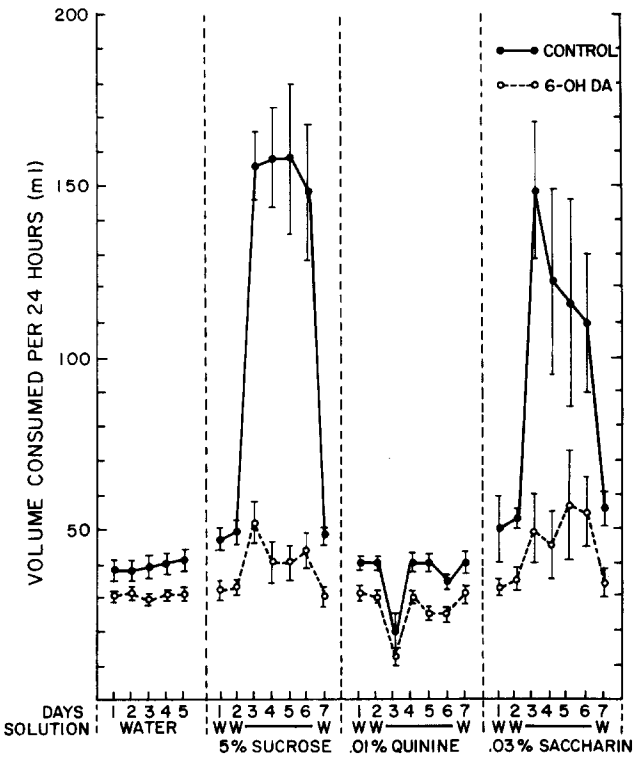

FIG. 2. Effect of 6-hydroxydopamine (6-OHDA) treatment on intake of water, sucrose, quinine, and saccharin solutions. (●——●) refers to fluid consumption of control rats (N = 17); (o- - - -o) refers to fluid consumption of 6-OHDA treated rats (N = 20). Body weight of control rats was 415 ± 9 g. 6-Hydroxydopamine treated rats weighed 300 ± 9 g. All values for sucrose intake and saccharin consumption are significantly different from control values ($p<0.001$). Sucrose consumption for the first day corrected for body weight was 37.8 ± 2.5 ml/100 g for control rats and 17.3 ± 2.2 ml/100 g for 6-hydroxydopamine treated rats ($p<0.001$). Values for saccharin intake for the first day corrected for body weight were 36.1 ± 4.3 ml/100 g for control rats and 15.3 ml ± 3.3 mg/100 g body weight for 6-hydroxydopamine treated rats ($p<0.001$).

day both groups consumed as much quinine as they had previously consumed water. Animals were also allowed to choose between water and a sucrose solution. As before control animals were found to drink far greater amounts of sucrose per 24 hr than water (11 ± 1 ml of water versus 145 ± 16 ml of sucrose; N = 9). The 6-hydroxydopamine treated rats were also found to prefer the solution of sucrose to water (12 ± 1 ml of water versus 54 ± 4 ml of sucrose solution; N = 11). However, in the case of the 6-hydroxydopamine treated rats the volume of fluid ingested was closer to the amount that would have been consumed if water alone had been on the cage. Thus, the results suggest that 6-hydroxydopamine treated rats can differentiate between the taste of water and a solution of sucrose.

Effect of 6-Hydroxydopamine Treatment on Intake of Saline

When control animals were exposed to a 1% saline solution instead of water, a marked increase in fluid intake was observed (Table 3). The 6-hydroxydopamine treated rats exposed to saline, however, showed only a slight increase in intake of this fluid (Table 3). This significant difference was not eliminated when values were corrected for body weight (Table 3).

Other animals were then allowed to choose between water and a 1% saline solution [22]. In this situation, neither control nor 6-hydroxydopamine treated animals showed a distinct preference for either solution after the first two days (Fig. 3). However, following treatment with desoxycorticosterone acetate (DOCA), control animals progressively increased their preference for the saline solution. On the last two days of the experiment, mean intake for control rats increased from a baseline of approximately 22 ml per day of saline to 111 ml per day ($p<0.001$). The administration of DOCA to 6-hydroxydopamine treated rats did not produce an increase as large as that observed in controls. In this experimental group, saline consumed increased significantly from a baseline intake of approximately 20 ml to a high of 46 ml on the last two days that fluid consumption was examined ($p<0.001$). These findings were not changed qualitatively by correcting saline consumption for body weight (22.5 ±

TABLE 3

EFFECT OF 6-HYDROXYDOPAMINE TREATMENT ON THE INTAKE OF A SALINE SOLUTION

Treatment	Water	Saline	Saline	Saline	Saline	Water
Control (ml) N = 17	49.8 ± 4.4	95.3 ± 8.3	99.5 ± 9.3	99.2 ± 7.8	94.0 ± 7.8	44.5 ± 3.2
(ml/100 g)	11.7 ± 1.3	22.4 ± 1.9	23.4 ± 2.2	23.3 ± 2.1	22.1 ± 1.8	10.5 ± 0.7
6-OHDA (ml) N = 20	34.4 ± 2.8*	46.6 ± 5.9*	42.2 ± 6.3*	47.0 ± 5.3*	50.4 ± 5.5*	24.1 ± 3.0*
(ml/100 g)	11.3 ± 0.9	15.3 ± 1.9*	13.9 ± 2.1*	15.1 ± 1.7*	16.5 ± 1.8	7.9 ± 1.0

Saline refers to a 1% sodium chloride solution. Values are expressed as mean ± S.E. of fluid consumed per 24 hr or of intake per 100 g body weight per 24 hr (ml/100 g). 6-Hydroxydopamine (6-OHDA) treatment is described in Methods.

*$p<0.01$ when compared with fluid intake in control rats.

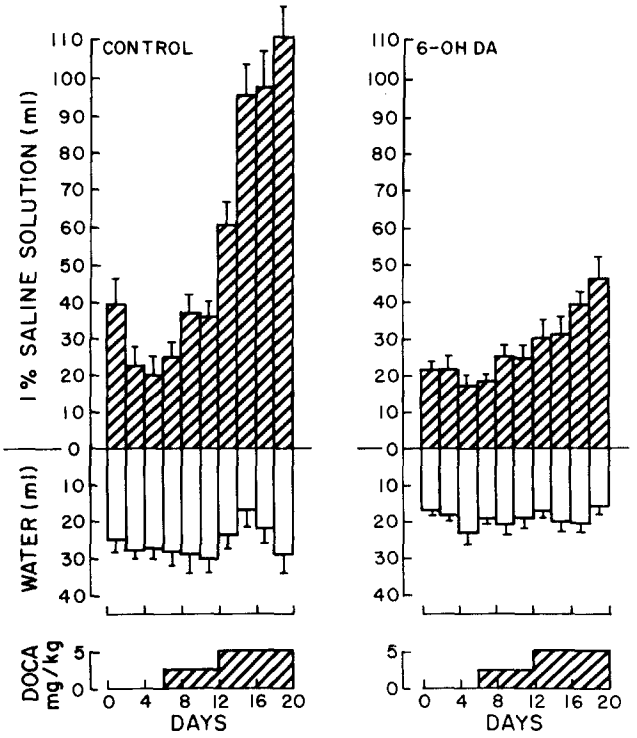

FIG. 3. Effect of desoxycorticosterone acetate (DOCA) on the amount of water or saline consumed by control and 6-hydroxydopamine (6-OHDA) treated rats. The shaded area below the graph indicates the amount of DOCA administered and the duration of treatment. The animals are included in the 6-OHDA treated group; eight rats are included in the control group. Consumption of saline corrected for body weight was 22.5 ± 2.3 ml/100 g/day for control rats and 12.3 ± 1.5 ml/100 g/day for 6-hydroxydopamine treated rats on the last two days saline was measured ($p<0.005$).

2.3 ml/100 g body weight/day for control versus 12.3 ± 1.5 ml/100 g body weight/day for 6-hydroxydopamine treated rats for the final measurement of saline consumption; $p<0.005$).

Effect of 6-Hydroxydopamine Treatment on Water Intake after Hypertonic Saline

In order to examine the drinking response in 6-hydroxydopamine treated and control rats to hypertonic saline, animals were given 2 percent of their body weight of a 1 molar sodium chloride solution. On the basis of absolute water ingested per 2 hr period, 6-hydroxydopamine treated rats did not consume as much as controls, but these differences were not significant (Table 4). When the total amount of water ingested during 24 hr for the two groups was compared, the intake for the control group was significantly greater than for the 6-hydroxydopamine treated group ($p<0.001$). Conversion of these values to the amount of fluid consumed per 100 g of body weight, however, eliminated this difference (Tabie 4).

Effect of 6-Hydroxydopamine Treatment on the Eating Response to Insulin

Previous studies have shown that administration of

TABLE 4

EFFECT OF 6-HYDROXYDOPAMINE TREATMENT ON WATER INTAKE FOLLOWING ADMINISTRATION OF HYPERTONIC SALT SOLUTION

Treatment	Two Hour Totals of Water Consumption 2 hr	4 hr	6 hr	24 hr Total
Control (ml) N = 20	15.4 ± 2.3	13.8 ± 2.4	8.3 ± 1.9	53.7 ± 2.5
(ml/100 g)	3.2 ± 0.4	2.9 ± 0.5	1.7 ± 0.4	11.3 ± 0.5
6-OHDA (ml) N = 20	11.4 ± 1.5	8.8 ± 1.7	6.7 ± 1.5	40.6 ± 2.6*
(ml/100 g)	3.2 ± 0.4	2.5 ± 0.5	1.9 ± 0.4	11.4 ± 0.7

The treatment of rats with 6-hydroxydopamine (6-OHDA) is described in Methods. Control and 6-OHDA treated rats received 2 ml per 100 g body weight of a 1 M NaCl solution. Mean body weight was 476 ± 13 g for the control group and 356 ± 10 g for the 6-OHDA treated group.

*$p<0.001$ when compared with control.

insulin increases food intake [16,19]. This observation is confirmed in the present study (Fig. 4). In contrast to the increased food intake in control rats, animals treated with 6-hydroxydopamine did not increase their food cosumption during the 5 hr period after insulin was administered (Fig. 4). It was also of interest to find that neither the 6-hydroxydopamine treated group nor control rats increased food consumption over preinjection levels for the total 24 hr period after insulin.

Effect of Preferential Depletion of Norepinephrine or Dopamine in Brain on the Consumption of a Sucrose Solution

In an effort to define whether the absence of norepinephrine or dopamine was responsible for the finding that 6-hydroxydopamine treated rats did not drink sucrose, animals with these catecholamines reduced were given a sucrose solution instead of water. These results are shown in Table 5. Note that depletion of norepinephrine did not alter water consumption nor the intake of sucrose solution when based upon body weight. In contrast, when sucrose solution was given to rats preferentially depleted of dopamine, sucrose consumption based on intake per 100 g body weight was significantly reduced when compared with intake in control animals. As observed in 6-hydroxydopamine treated rats with both amines reduced, body weight of the DA Down treated rats was significantly less than control.

Effect of Various 6-Hydroxydopamine Treatments on Concentrations of Norepinephrine, Dopamine and Serotonin in Brain

Following two doses of 6-hydroxydopamine (6-OHDA), one with pargyline and one without pargyline, norepinephrine and dopamine were reduced by approximately 74 and

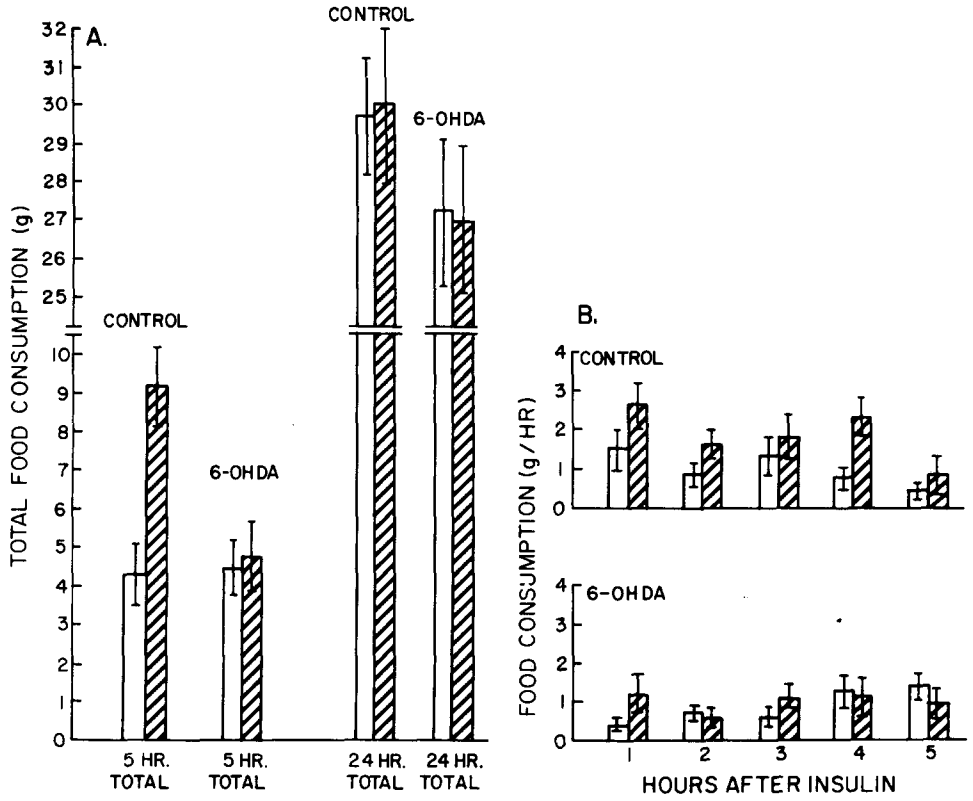

FIG. 4. Effect of insulin (20 units/kg) on food consumption of control and 6-hydroxydopamine treated rats. Open bars refer to food consumption on the day preceding insulin administration. Shaded bars refer to the amount of food consumed after insulin administration. A. Total food consumption 5 and 24 hr after insulin. B. Food consumption (g/hr) for the first 5 hr following insulin. Eleven rats included in the control group and 12 rats included in the 6-hydroxydopamine-treated group.

93 percent, respectively, in whole brain (Table 6). Brain serotonin was reduced by 25 ± 3 percent in the treated rats. The drastic effect of this treatment on brain catecholamines was found to extend to hypothalamus, striatum and rest of brain (Table 6).

Multiple injections of 6-hydroxydopamine in the presence of desipramine (DMI) lowered whole brain dopamine by approximately 85% and norepinephrine by 20% (Table 6). Serotonin was reduced by nearly 40 percent. Dopamine was reduced severely in striatum and rest of brain with norepinephrine concentration much less affected in these areas; norepinephrine was reduced by only 30 percent in the hypothalamus following this treatment.

Injection of three 25 μg doses of 6-hydroxydopamine intracisternally reduced whole brain norepinephrine by 58 percent and brain dopamine by 20 percent. In this case, norepinephrine was reduced by 50 and 68 percent respectivley in the hypothalamus and rest of brain. Whereas dopamine in the striatum was significantly reduced by approximately 50 percent in this group of rats, dopamine in rest of brain was not significantly reduced.

DISCUSSION

In the present study, intracisternal administration of 6-hydroxydopamine in combination with pargyline was shown to cause an acute reduction in eating and drinking similar to that reported by Fibiger and associates [14] and Zigmond and Stricker [29] who administered the 6-hydroxydopamine intraventricularly to animals pretreated with a monoamine oxidase inhibitor. Following recovery from these acute effects on consummatory behavior, animals treated with 6-hydroxydopamine appear to regulate food and water intake normally. While total food and water consumed during a 24 hr period was found to be reduced, this reduction seemed to correlate with the reduced body weight of the treated rats when compared with controls. However, 6-hydroxydopamine-treated rats did gain weight as they matured, but they did not attain the body weight of control rats by 180 days after injection. Similar results have previously been reported to occur following injection of neonatal animals with 6-hydroxydopamine [9,20]. Since rats given no food for 5 days regain weight to control levels, these results suggest that the subnormal weight in the 6-hydroxydopamine-treated rats is not produced by the initial aphagia and adipsia after administration of the drug.

The acute syndrome and recovery found to follow 6-hydroxydopamine appears similar to the acute aphagia and adipsia [1] and recovery [22] observed to occur after bilateral lesions of the hypothalamus. Oltmans and Harvey [17] recently showed that lesions of the lateral hypothalamus reduced brain catecholamines and suggested that the acute symptoms may be due to destruction of catecholamine-containing fibers. Ungerstedt [24] came to a

TABLE 5

EFFECT OF CATECHOLAMINE ALTERATION ON INTAKE OF A SUCROSE SOLUTION

Treatment	Body Weight	Fluid Consumption (ml/100 g Body Weight)	
		Water	Sucrose
Control (N = 18)	413 ± 8	11.9 ± 0.8	37.6 ± 2.5
NE Down (N = 11)	400 ± 11	11.5 ± 0.5	36.8 ± 2.4
DA Down-2x (N = 23)	333 ± 10†	11.3 ± 0.7	22.5 ± 2.1†
6-OHDA (N = 20)	300 ± 9†	11.2 ± 0.9	17.3 ± 2.2†

*Animals were treated as described in Methods. NE Down refers to animals treated with 3 x 25 μg 6-OHDA intracisternally to reduce norepinephrine. DA-Down-2x refers to animals treated with 240 μg 6-OHDA intracisternally 1 hr after desipramine HCl (30 mg/kg, IP) on two occasions. Numbers in parentheses refer to number of animals in each group.

†$p<0.001$ when compared with control.

similar conclusion after injecting 6-hydroxydopamine into the cell bodies of the substantia nigra. However, animals with lesions in the hypothalamus have been found to have persisting deficits in consummatory behavior in spite of their apparent recovery [22]. Since deficits reported in animals recovered from hypothalamic lesions might be present in rats treated with 6-hydroxydopamine, certain of the methods used to examine consummatory behavior in animals with lateral hypothalamic lesions were incorporated into the design of the present studies of recovered 6-hydroxydopamine-treated rats.

Lesions in the hypothalamus have previously been shown to block the increased intake of food following the hypoglycemia from insulin [11] or following administration of 2-deoxy-D-glucose [27]. In the present study, 6-hydroxydopamine was also found to antagonize the feeding response to insulin administration. This similarity has recently been further substantiated by Zigmond and Stricker [29] who reported that 6-hydroxydopamine antagonized feeding produced by 2-deoxy-D-glucose. Similar to previously reported findings in lateral hypothalamic lesioned animals [28], treatment with 6-hydroxydopamine, in addition, antagonized the enhanced appetite for saline following treatment with desoxycorticosterone acetate (DOCA). However, the inability to drink in the absence of food, a deficit described for animals with lateral hypothalamic lesions [22], was not apparent. Furthermore, increased drinking produced by hypertonic saline, which is reported to be eliminated by hypothalamic lesions [23] was not altered by 6-hydroxydopamine treatment. Thus, it would appear that the recovered 6-hydroxydopamine-treated rat is not, in every respect, like the animal which was recovered from lesions of the lateral hypothalamus. These differences may be dependent upon destruction of

TABLE 6

EFFECT OF VARIOUS 6-HYDROXYDOPAMINE TREATMENTS ON NOREPINEPHRINE (NE) AND DOPAMINE (DA) IN WHOLE BRAIN AND VARIOUS BRAIN PARTS

Treatment	Whole Brain mμg/g		Hypothalamus mμg/g		Striatum mμg/g		Rest of Brain mμg/g	
	NE	DA	NE	DA	NE	DA	NE	DA
Control	390 ± 16	732 ± 64	1813 ± 110	300 ± 90	242 ± 8	7715 ± 59	304 ± 18	243 ± 36
6-OHDA	102 ± 8*	53 ± 10*	280 ± 11*	130 ± 27*	38 ± 5*	102 ± 45*	7 ± 2*	19 ± 10*
DA-Down-2x DMI + 6-OHDA (2x)	309 ± 13*	108 ± 17*	1279 ± 233	196 ± 93	240 ± 34	431 ± 270*	210 ± 8*	50 ± 12*
NE Down 3 x 25 μg 6-OHDA	165 ± 7*	580 ± 21	907 ± 45*	215 ± 58	200 ± 26	4051 ± 438*	115 ± 14*	198 ± 36

Each value for whole brain represents the mean ± S.E. of 14–19 determinations; values for brain parts are the mean ± S.E. of 5–12 determinations. Brain dissections are described in Methods. Animals were killed 14–30 days after treatment. Treatments are described in Table 5 and in Methods.

*$p<0.001$ when compared with control.

other neural systems after lesions in the lateral hypothalamus. However, it is also conceivable that slightly greater depletions of brain catecholamines might reveal these deficits. Further work will be required to define these alternatives.

One additional deficiency related to consummatory behavior was the failure of 6-hydroxydopamine treated rats to increase intake of sucrose solution substituted for water. However, it was found that 6-hydroxydopamine treated rats actually preferred sucrose when allowed access to both a sucrose solution and water, but drank less total volume of fluid than control rats. This latter finding would seem to discount taste as a possible explanation for this behavior in 6-hydroxydopamine treated rats.

In subsequent experiments, sucrose consumption was selected to examine the individual role of norepinephrine or dopamine depletion in the consummatory alteration produced by 6-hydroxydopamine. Sucrose intake in animals treated with 3 doses of 25 μg of 6-hydroxydopamine intracisternally, causing a preferential depletion of norepinephrine, was found to be similar to intake in controls. This result is in contrast to work described by Sorensen *et al.* [23] who reported that rats treated with 6 doses of 25 μg 6-hydroxydopamine drank significantly less of a 3% sucrose solution than did controls. At the present time, an explanation of these divergent findings is not apparent. In our experiments, animals in which dopamine was the primary amine depleted displayed a large reduction of sucrose consumption. Similar to earlier proposals [17,24], the latter finding suggests that destruction of dopamine fibers are responsible for the disruption of sucrose intake observed in 6-hydroxydopamine treated rats with both amines reduced. Although analysis of catecholamines in various brain areas indicated that norepinephrine was reduced to some degree in the rats treated with desipramine and 6-hydroxydopamine, animals treated to deplete brain norepinephrine preferentially had greater reductions of norepinephrine in all parts of brain than observed in the dopamine depleted rat. Thus, these results would argue that disruption of dopamine fibers is essential for the sucrose consumption deficit but do not eliminate an involvement of noradrenergic fibers.

In 1962, Grossman [15] observed that injection of norepinephrine into the diencephalon caused the satiated rat to eat suggesting that noradrenergic neurons played a role in the control of eating. In further support of this view, Evetts *et al.* [13] have recently presented evidence that endogenous release of catecholamines by acute administration of 6-hydroxydopamine into the diencephalon also induced eating. As described above, Ungerstedt [24] has recently suggested an involvement of the nigro-striatal dopamine system in the acute aphagia and adipsia following lesions in the substantia nigra. In the present study, preferential depletion of dopamine produced the same deficit observed when both amines were reduced suggesting that chronic deficits in the 6-hydroxydopamine treated rat are related to destruction of dopamine fibers. However, destruction of dopamine fibers in the rat has produced deficits other than in consummatory behavior. For example, responding in an avoidance task has recently been found to be dependent upon dopaminergic fibers [10,20]. Earlier studies have suggested that dopaminergic fibers participated in motor control and that their destruction was responsible for the symptoms of Parkinson's Disease. Whether a common mechanism involving dopaminergic fibers can be responsible for these diverse findings has yet to be determined. Another question to be dealt with in future studies is whether the observation that norepinephrine injected into the diencephalon induces eating is contradictory with the finding that preferential destruction of noradrenergic fibers in brain did not produce chronic deficits in eating and drinking. Understanding of these problems should offer additional knowledge of the mechanisms controlling consummatory behavior.

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REFERENCES

1. Anand, B. K. and J. R. Brobeck. Hypothalamic control of food intake in rats and cats. *Yale J. Biol. Med.* **24:** 123–140, 1951.
2. Bloom, F. E., S. Algeri, A. Groppetti, A. Revuetta and E. Costa. Lesions of central norepinephrine terminals with 6-OH-dopamine: Biochemistry and fine structure. *Science* **166:** 1284–1286, 1969.
3. Bogdanski, D. F., A. Pletscher, B. B. Brodie and S. Udenfriend. Identification and assay of serotonin in brain. *J. Pharmac. exp. Ther.* **117:** 82–88, 1956.
4. Breese, G. R., I. J. Kopin and V. K. Weise. Effects of amphetamine derivatives on brain dopamine and noradrenaline. *Br. J. Pharmac.* **38:** 537–545, 1970.
5. Breese, G. R., R. Moore and J. Howard. Central actions of 6-hydroxydopamine and other phenylethylamine derivatives on body temperature in the rat. *J. Pharmac. exp. Ther.* **180:** 591–602, 1972.
6. Breese, G. R., A. J. Prange, J. L. Howard, M. A. Lipton, W. T. McKinney, R. E. Bowman and P. Bushnell. 3-Methoxy-4-hydroxphenylglycol excretion and behavioral changes in rat and monkey after central sympathectomy with 6-hydroxydopamine. *Nature* **240:** 286–287, 1972.
7. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp. Ther.* **174:** 413–420, 1970.
8. Breese, G. R. and T. D. Traylor. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *Br. J. Pharmac.* **42:** 88–99, 1971.
9. Breese, G. R. and T. D. Traylor. Developmental characteristics of brain catecholamines and tyrosine hydroxylase in the rat: Effects of 6-hydroxydopamine. *Br. J. Pharmac.* **44:** 210–222, 1972.
10. Cooper, B. R., G. R. Breese, J. L. Howard and L. D. Grant. Effects of 6-hydroxydopamine treatments on active avoidance responding: Evidence for involvement of brain dopamine. *J. Pharmac. exp. Ther.* **185:** 358–370, 1973.
11. Epstein, L. V. and P. Teitelbaum. Specific loss of the hypoglycemic control of feeding in recovered lateral rats. *Am. J. Physiol.* **213:** 1159–1167, 1967.
12. Evetts, K. D. and L. L. Iversen. Effects of protriptyline on the depletion of catecholamines induced by 6-hydroxydopamine in the rat. *J. Pharm. Pharmac.* **22:** 540–542, 1970.

13. Evetts, K. D., J. T. Fitzsimons and P. E. Setler. Eating caused by 6-hydroxydopamine induced release of noradrenaline in the diencephalon of the rat. *J. Physiol.* **223:** 35–47, 1972.
14. Fibiger, H. C., B. Lonsbury, H. P. Cooper and L. D. Lytle. Early behavioural effects of intraventricular administration of 6-hydroxydopamine in rat. *Nature New Biol.* **236:** 209–211, 1972.
15. Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am. J. Physiol.* **202:** 872–882, 1962.
16. Mackay, E. M., J. W. Callaway and R. H. Barnes. Hyperalimentation in normal animals produced by protamine insulin. *J. Nutr.* **20:** 59–66, 1940.
17. Oltmans, G. A. and J. A. Harvey. LH syndrome and brain catecholamine levels after lesions of nigrostriatal bundle. *Physiol. Behav.* **8:** 69–78, 1972.
18. Rice, K. E. and C. P. Richter. Increased sodium chloride and water intake of normal rats treated with desoxycorticosterone acetate. *Endocrinology* **33:** 106–115, 1943.
19. Richter, C. P. Increased dextrose appetite of normal rats treated with insulin. *Am. J. Physiol.* **135:** 781–787, 1941.
20. Smith, R. D., B. R. Cooper and G. R. Breese. Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *J. Pharmac. exp. Ther.*, in press.
21. Smith, G. P., A. J. Strohmayer and D. J. Reis. Effect of lateral hypothalamic injections of 6-hydroxydopamine on food and water intake in rats. *Nature New Biol.* **235:** 27–29, 1972.
22. Teitelbaum, P. and A. N. Epstein. The lateral hypothalamic syndrome: Recovery of feeding and drinking after lateral hypothalamic lesions. *Psychol. Rev.* **69:** 74–90, 1962.
23. Sorenson, C. A., G. D. Ellison and D. Masuoka. Changes in fluid intake suggesting depressed appetities in rats with central catecholaminergic lesions. *Nature New Biol.* **237:** 279–281, 1972.
24. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigra striatal dopamine system. *Acta physiol. scand. (suppl.)* **367:** 95–122, 1971.
25. Uretsky, N. J. and L. L. Iversen. Effects of 6-hydroxydopamine on catecholamine neurones in the rat brain. *J. Neurochem.* **17:** 269–278, 1970.
26. Vetulani, J., K. Reichenberg and G. Wiszniowska. Asymmetric behavioral and biochemical effects of unilateral injections of 6-hydroxydopamine into the lateral brain ventricle of the rat. *Eur. J. Pharmac.* **19:** 231–238, 1972.
27. Wayner, M. J., A. Cott, J. Millner and R. Tartaglione. Loss of 2-deoxy-D-glucose induced eating in recovered lateral rats. *Physiol. Behav.* **7:** 881–884, 1971.
28. Wolf, G. Effect of dorsolateral hypothalamic lesions on sodium appetite elicited by desoxycorticosterone and by acute hyponatremia. *J. comp. physiol. Psychol.* **58:** 396–402, 1964.
29. Zigmond, M. J. and E. M. Stricker. Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats. *Science* **177:** 1211–1214, 1972.