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Supersensitivity to Norepinephrine or Dopamine Antagonists After Knife Cuts that Produce Aphagia and Adipsia in Rats[1]

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ALHEID, G. F., J. KELLY, L. MCDERMOTT, A. HALARIS AND S. P. GROSSMAN. *Supersensitivity to norepinephrine or dopamine antagonists after knife cuts that produce aphagia and adipsia in rats.* PHARMAC. BIOCHEM. BEHAV. 6(6) 647–657, 1977. – Aphagia and adipsia of equivalent duration were produced by knife cuts along the lateral border of the hypothalamus (PH cuts), or the medial surface of the globus pallidus (MP cuts) in male albino rats. Striatal dopamine (DA) was reduced by 75% in animals with PH cuts but only 50% by MP cuts. Hypothalamic norepinephrine was reduced 25% by PH cuts and was unaffected by MP cuts. Aphagia and adipsia were positively correlated with DA depletions only in rats with PH cuts. Presurgical catecholamine depletions produced by chronic injections of alpha-methyl-p-tyrosine did not alter the duration of aphagia or adipsia resulting from these knife cuts. However, following recovery of ingestive behavior, rats with PH and MP cuts were supersensitive to the anorexic effects of the dopamine-beta-hydroxylase inhibitor, diethyldithiocarbamate. Exaggerrated anorexia was also observed after DA blockade by haloperidol or alpha-adrenergic blockade by phenoxybenzamine. The most pronounced effects of catecholamine blockade were observed in rats with PH cuts.

Knife cuts Aphagia Adipsia Recovery of function Dopamine Norepinephrine
Diethyldithiocarbamate Phenoxybenzamine Haloperidol Anorexia Supersensitivity

LATERAL HYPOTHALAMIC (LH) lesions result in a complex syndrome of behavioral impairments in the rat which includes aphagia and adipsia. If the animals are kept alive by intragastric intubation of food and water, voluntary ingestive behavior typically reappears after several days, weeks, or months. However, in lesioned animals food and water intake do not appear to be under the control of glucostatic, osmotic, and volumetric mechanisms that regulate intake in the intact rat (see [19,47] for review).

Numerous recent reports have implicated dopaminergic and/or noradrenergic mechanisms in the etiology of the aphagia and adipsia syndrome. Electrolytic lesions in the nucleus of origin of the nigrostriatal pathways [29,49] or along its trajectory through the dorsolateral hypothalamus [41,49] and globus pallidus [23, 31, 38, 39] produce the LH syndrome. That this may be due to an interruption of catecholaminergic fibers is suggested by the fact that microinjections of the neurotoxin 6-hydroxy-dopamine (6OHDA) into the substantia nigra, lateral hypothalamus, or globus pallidus also result in aphagia and adipsia [31, 46, 49]. Intraventricular injections of 6OHDA which preferentially, although not exclusively, destroy catecholaminergic terminals [13, 15, 50], also produce aphagia and adipsia when combined with pharmacological pretreatments that ensure severe dopamine depletions [20, 52, 53].

We have found that surgical transections of the principal efferent or afferent connections of the striatum produce useful models for further study of the role of catecholaminergic pathways in ingestive behavior. Cuts in the parasagittal plane along the lateral border of the hypothalamus, which produce little or no damage to cellular components of the LH, result in aphagia, adipsia, and a complete syndrome of persisting deficits in responding to glucoprivic and hydrational challenges [24,25], but do not produce the severe and often persisting arousal deficits which have been reported after LH lesions or intracranial injections of 6OHDA [7, 32, 33, 48]. The duration of aphagia and adipsia, and some of the persisting regulatory deficits are correlated with the effectiveness of these cuts in depleting striatal and forebrain dopamine (DA) [1,4]. Knife cuts medial or ventral to the globus pallidus also

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produce aphagia and adipsia as well as a variety of persisting deficits [1, 2, 3, 4]. However, the duration or severity of these effects do not seem to correlate with severe striatal DA depletions.

In rats with LH lesions the duration of the aphagia or the probability of survival in the absence of intragastric intubation appears to be modified by various behavioral, physiological, or pharmacological treatments. Starvation [44], glucodynamic hormones [6], electrical stimulation of the lateral hypothalamus [26], nerve growth factor [10], inhibition of catecholamine (CA) synthesis by alpha-methyl-p-tyrosine (AMPT) [21,22], blockade of DA receptors by haloperidol [28], or intraventricular injections of norepinephrine (NE) [9], are effective in modifying the severity of the effects of LH lesions on ingestive behavior. It has also been reported that rats that have recovered from aphagia produced by LH lesions or treatment with 6OHDA are supersensitive to the anorexic effects of AMPT [53].

The effectiveness of electrolytic or chemical lesions in the dopaminergic nigrostriatal projections have led to the suggestion that an interference with dopaminergic components of the striatum may be responsible for the effects of LH lesions or 6OHDA treatments on food and water intake, and that receptor supersensitivity combined with enhanced DA synthesis may be responsible for the recovery of ingestive behavior [21, 22, 47, 53]. Facilitation of recovery by prelesion treatment with AMPT or haloperidol is thought to be the result of DA receptor supersensitivity and/or increased synthesis of DA. Similarly, the heightened anorexia of recovered LH or 6OHDA treated animals after AMPT is thought to reflect the greater dependence of these preparations on catecholamine synthesis in the few remaining DA terminals.

Despite the implication of DA in the etiology of the LH syndrome, aphagia and adipsia have also been observed after treatments which preferentially depleted forebrain norepinephrine [46,47], and a specific role for NE in feeding behavior has been proposed [30].

The present series of experiments represents an extension of our investigation of the behavioral effects of knife cuts on ingestive behavior [1, 2, 3, 4, 34, 35]. To obtain further evidence of the role of dopaminergic or noradrenergic pathways on ingestive behavior, we have examined the effects of pre-surgical treatments that result in CA synthesis inhibition on the duration of aphagia and adipsia subsequent to CA depleting knife cuts. Two different transections were used that we have previously found to have similar effects on aphagia and adipsia but dissimilar effects on striatal DA concentrations [1,3]. In addition, the effects of preferential NE and DA blockers on food intake were observed in rats that had recovered voluntary feeding and drinking after such cuts.

EXPERIMENT 1: EFFECTS OF ALPHA-METHYL-PARA-TYROSINE

METHOD

Animals

Forty adult male albino rats of the Sprague-Dawley strain (Holzman, Madison, WI) were used. The animals weighed approximately 400 g at the time of surgery. They were housed singly, in a temperature controlled vivarium, with food and water available ad lib. Teklad food pellets (6% fat) were placed on the floor of the cage and tap water was always present in inverted bottles equipped with stainless steel spouts. The vivarium was illuminated on a twelve hr light/dark cycle with lights on at 0700 and off at 1900 hr.

Surgery

Knife cuts were made under sodium pentobarbital anesthesia, using a retractable wire knife. The instrument and related surgical procedures have been described elsewhere [45]. Briefly, a 27 gauge stainless steel guide cannula was stereotaxically inserted into the brain at the desired coordinates, and a 150μ spring steel wire was extended through the tip of the cannula, which had been bent at a slight angle in order to guide the wire in a caudal direction. The entire assembly was then lowered a predetermined distance, thus transecting fibers of passage. The wire was then retracted and the guide cannula removed from the brain.

Bilateral parasagittal hypothalamic knife cuts (PH) were made by inserting the guide vertically, at coordinates, AP = 6.0, H = −0.3, L = ± 2.0, de Groot [18]. The wire was then extended 2.5 mm caudally in a parasagittal plane, and the guide lowered 2.5 mm to produce the cut.

Bilateral knife cuts adjacent to the medial surface of the globus pallidus (MP) were made by inserting the guide cannula vertically, at coordinates, AP = 7.3, H = 2.0, L = 1.8 mm, de Groot [18]. The wire was extended 3 mm caudally, at an angle of 45° to the parasagittal plane so that the tip of the cutting wire was located lateral to the axis of the guide cannula. The assembly was then lowered 2.5 mm to produce the desired cut.

Histology

Following completion of all experiments, rats were selected for histology or CA assay in a balanced fashion based on the duration of aphagia after surgery. Animals selected for histology were killed with an overdose of sodium pentobarbital and perfused transcardially with isotonic saline followed by a 10% formol-saline solution. Following further fixation in formalin, brains were sectioned on a freezing microtome. Every fourth 50μm section in the area of the knife cut was mounted on glass slides and stained with cresyl violet.

Biochemical Assays

Rats were sacrificed by decapitation, the brains quickly removed and dissected on ice. Brain regions were weighed and stored in liquid nitrogen until assayed. DA and NE were determined in pooled striata from the right and left hemispheres. The concentration of NE in the hypothalamus was also determined for each animal. Brain regions were homogenized in 15 ml ice cold 0.4 N perchloric acid with 0.25 ml 2% ascorbic acid in each tube. After centrifugation, the supernatant was adjusted to PH 6.5 and passed onto Amberlite (CG-50) columns. The amines were eluted from the columns in 4 ml 1 N hydrochloric acid. Catecholamines were oxidized according to [8].

Brain regions were dissected as follows: after removal of the brain from the calvarium, the cerebrum was placed on its dorsal surface. Perpendicular coronal cuts were then made at the rostral and caudal edges of the olfactory tubercle. The left and right striata were obtained from the resulting section by trimming away the cortex along the corpus callosum, removal of the nucleus accumbens and

olfactory tubercle by a horizontal cut ventral to the striatum, and removal of the septum by cutting along the lateral ventricles. The hypothalamus was dissected by a cut in the coronal plane just caudal to the mammillary bodies which removed the midbrain and remaining brainstem, and a cut in the horizontal plane at the level of the anterior commissure that removed the thalamus. Laterally, remaining parts of the temporal lobes and cerebral peduncles were then trimmed away.

Whole brains of control rats were assayed to assess CA depletion by alpha-methyl-p-tyrosine-methyl-ester (AMPT). The olfactory bulbs and caudal brainstem were removed prior to this assay.

Procedure

Twenty rats were selected at random to receive drug treatments. Twenty additional animals received injections of comparable volumes of isotonic saline. Intraperitoneal (IP) injections of AMPT or isotonic saline were administered on each of the three consecutive days immediately preceeding surgery. An injection of 75 mg/kg AMPT (75 mg/ml of isotonic saline) was given twice daily (12 hr apart) on the first two days. A fifth injection was given on the morning of the third day. On the afternoon of the third day, four AMPT treated rats and four placebo injected controls were selected for CA assay. Six of the remaining AMPT treated rats and six placebo treated controls received PH cuts. Six additional AMPT treated rats and six placebo treated controls received MP knife cuts. Four remaining AMPT treated rats and flour placebo treated rats were retained as unoperated controls.

Aphagia and Adipsia

Following surgery, five food pellets were placed in the home cage and inspected twice daily for tooth marks. The duration of aphagia was defined as the average between the first appearance of tooth marks and the first time a complete pellet (approximately 3 g) was ingested. The latter event always occurred within 12–36 hr of the former. Adipsia was scored as the first day on which 4 ml or more of water were ingested (spillage averaged 1–2 ml per 24 hr).

If a rat failed to eat within 48 hr after surgery, 15 ml of a liquid diet were intubated intragastrically twice daily until voluntary ingestive behavior reappeared. The diet consisted of milk, eggs, sugar, and several drops of a liquid vitamin supplement (Polyvisol).

RESULTS

Histology

Histological data were obtained for 5 of 12 rats with PH cuts and 5 of 12 rats with MP cuts. In this sample, the PH cuts extended from the region just posterior to the optic chiasm to the area just caudal to the subthalamic nucleus. All cuts were bilaterally symmetric and followed the medial surface of the internal capsule. The PH cuts extended from the base of the brain to ventral aspects of the zona incerta. Figure 1 shows schematic representations of the PH cuts projected on a parasagittal section of the deGroot atlas [18].

The MP cuts began at the level of the anterior commissure and extended posteriorly along the lateral

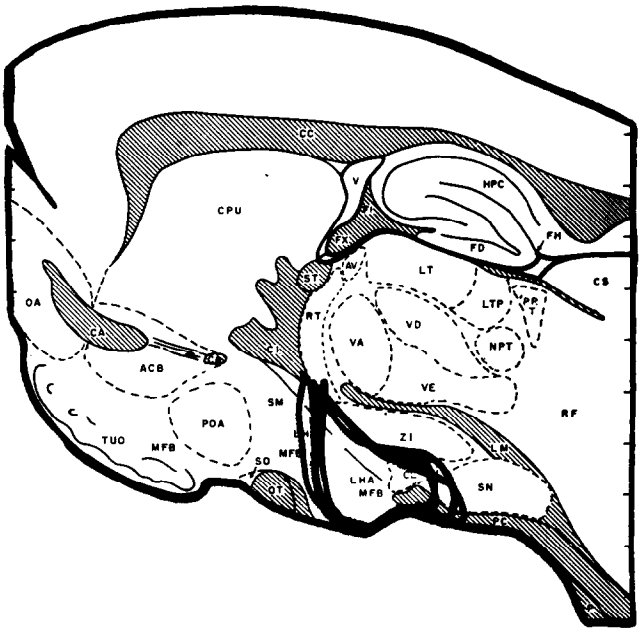

FIG. 1. Parasagittal hypothalamic knife cuts (PSH cuts). Outline represent the common bilateral area intersected by these cuts projected on a sagittal section (lat = 2.0 mm).

border of the internal capsule to the level of the entopeduncular nucleus. The anterior portions of this cut were bilaterally symmetric and extended upward from the nucleus accumbens into the caudate nucleus. Posteriorly, the shape of the MP cut was somewhat irregular due to variable deflections of the wire knife by fascicles of the internal capsule. A schematic representation of a typical MP cut is shown in Figure 2.

Biochemistry

The biochemical effects of AMPT or knife cuts are summarized in Tables 1 and 2. AMPT depleted both catecholamines to about 50% of control levels ($p<0.01$) (Table 1). The PH cuts depleted striatal DA to about 25% of control levels, $t(12) = 6.23$, $p<0.001$. The MP cuts depleted striatal DA to 50% of the control value, $t(12) = 7.11$, $p<0.001$. Caudate NE was less severely affected by both cuts (60% of control after PH cuts, $t(12) = 8.00$, $p<0.001$, and 80% of control after MP cuts, $t(12) = 3.81$, $p<0.01$). The PH cuts reduced hypothalamic NE to 76% of control values, $t(12) = 6.85$, $p<0.001$. The MP cuts did not significantly change hypothalamic NE concentrations.

Aphagia and Adipsia

The duration of aphagia and adipsia is shown in Table 3. No statistically reliable differences in the duration of aphagia and adipsia were observed between cut groups. One rat with MP cuts (in the placebo group) died after 5 days of aphagia. This animal was not included in the scores for his group. In rats with PH cuts, the residual concentrations of caudate DA were negatively correlated with the duration of aphagia, or adipsia (aphagia, $r = -.86$, $n = 7$, $p<0.01$; adipsia, $r = -.91$, $n = 7$, $p<0.01$). Similar correlations were not found in the data from rats with MP cuts.

FIG. 2. Schematic diagram of knife cuts medial to the globus pallidus (MP cuts).

TABLE 1

MEDIAN BRAIN CATECHOLAMINE CONCENTRATIONS (NG/GM) AFTER AMPT

		NE	DA
Control	Median	422	958
(N = 4)	Range	383–492	866–1251
AMPT	Median	230	488
(N = 4)	Range	176–290	228–707

TABLE 2

MEDIAN CATECHOLAMINE CONCENTRATIONS (NG/GM) AFTER KNIFE CUTS

		Striatum		Hypothalamus
		NE	DA	NE
Control	Median	365	9482	2008
(N = 7)	Range	299–448	7779–13302	1745–2050
PH Cuts	Median	224	2343	1532
(N = 7)	Range	172–248	778–4880	1432–1674
MP Cuts	Median	292	4717	2019
(N = 7)	Range	262–353	3787–5548	1841–2402

TABLE 3

MEAN APHAGIA OR ADIPSIA (DAYS) AFTER CENTRAL KNIFE CUTS WITH OR WITHOUT AMPT PRETREATMENT

	PH	MP	PH+MP
	Aphagia ± SE		
AMPT	5.33 ± .502	5.33 ± .620	5.33 ± .375
N	6	6	12
Placebo	3.50 ± .718	5.20 ± 1.15	4.27 ± .672
N	6	5	11
AMPT + Placebo	4.42 ± .497	5.27 ± .588	—
N	12	11	
	Adipsia ± SE		
AMPT	5.67 ± .592	6.83 ± .798	6.25 ± .506
N	6	6	12
Placebo	4.83 ± .644	7.00 ± 1.67	5.82 ± .859
N	6	5	11
AMPT + Placebo	5.25 ± .438	6.91 ± .824	—
N	12	11	

DISCUSSION

Contrary to expectations, catecholamine depletion by AMPT prior to surgery, which should have resulted in denervation supersensitivity, did not reliably alter the duration of aphagia and adipsia in these experiments (rats with PH cuts, in fact, showed apparently paradoxical small effects in the opposite direction). These observations must be considered in conjunction with several reports that pre-operative CA depiction by AMPT, or DA blockade facilitated recovery after LH lesions [21,28].

It has recently been shown that the dose of AMPT may be a critical variable for the demonstration of facilitatory effects on recovery from LH lesions [22]. We did not examine a range of doses in the present experiment but our drug treatment was identical to that which resulted in facilitation of recovery in earlier studies. The CA depletions produced by AMPT in our experiments are also in close agreement with those reported to facilitate LH recovery [21].

It is possible that procedural differences may be responsible for the apparent contradiction. In previous experiments which demonstrated facilitatory AMPT effects, the animals were not fed intragastrically during the period of aphagia and adipsia. Survival (or the lack thereof) was the principal dependent variable. It is not clear, whether under these circumstances a specific action of AMPT on hunger or feeding related brain mechanisms is responsible for the observed beneficial effects on survival. In our experiments, the animals were sufficiently deprived to

initiate inggestive behavior but survival of the animal was not threatened.

It is also possible that our knife cuts may have produced aphagia and adipsia by interfering with pathways that are not catecholaminergic. That this may be true, at least for our MP cuts, it suggested by the observation that medial pallidal cuts which produced relatively small depletions of striatal dopamine and norepinephrine, inhibited voluntary food and water intake as effectively as the PH cuts which had much more severe effects on striatal dopamine. The lack of significant correlations between the biochemical and behavioral effects of MP cuts supports this interpretation. In the case of our PH cuts, consistent, statistically reliable correlations between the duration of aphagia and adipsia and residual striatal dopamine were obtained, which suggest an association between the behavioral and biochemical consequences of these cuts.

These correlations between aphagia, adipsia and the biochemical effects of the PH cuts are in good agreement with the data obtained in other investigations of the effects of parasagittal cuts along the border of the diencephalon [1,3]. Such correlations using similar knife cuts were not observed in several earlier studies from our laboratory (4, 34, 35]. We believe that interference with striatal and/or pallidal functions can produce aphagia and adipsia, but that this effect is correlated with striatal dopamine only when the nigrostriatal afferents are selectively interrupted. The small parasagittal cuts used in the present experiments severely depleted striatal dopamine. Cuts used in earlier investigations were larger or somewhat more lateral, but did not produce significantly greater depletions of DA. These undoubtedly interrupted a greater number of other afferent or efferent connections of the striatum and this may have obscured correlations with striatal DA. It is, of course, also possible that the aphagia and adipsia syndrome may be due to dopamine depletion in restricted regions of the striatum as the work of Neill and Linn [39] suggests. Significant correlations between the behavioral and biochemical consequences of a knife cut (or electrolytic lesion) might then be obtained only when the CA projections to this area are selectively interrupted.

EXPERIMENT 2: EFFECTS OF DIETHYLDITHIOCARBAMATE

The results of the preceeding experiment did not provide evidence for the hypothesis that presurgical CA depletion decreases the duration of aphagia or adipsia after our knife cuts as they do in animals with LH lesions [21,22]. This may, at least in part, reflect the fact that the effects of some of our cuts on food and water intake may not be related to an interruption of CA pathways. However, our PH cuts depleted striatal and hypothalamic CA stores and the biochemical effects of these cuts were significantly correlated with their effects on food and water intake.

To further investigate the contribution of CA pathways to the disturbances in food and water intake that are typically seen after our PH and MP cuts, the anorexigenic effects of central NE or DA blockade were examined in intact controls and in rats which had recovered voluntary ingestive behavior after these cuts.

Rats with LH lesions as well as rats which have been treated with 6OHDA are supersensitive to the anorexigenic effects of AMPT [53]. Since this drug depletes both catecholamines, the specific contribution of DA or NE pathways cannot be established in this experimental paradigm. In the following experiments, we attempted to do so by investigating the effects of several, more selective pharmacological treatments.

In the first of these, diethyldithiocarbamate (DDC) a dopamine-beta-hydroxylase inhibitor was used to selectively deplete NE. To control for possible nonspecific sedative effects of this drug, the anorexic effects of subanesthetic doses of sodium pentobarbital were also examined.

METHOD

Animals

The animals had been previously used in Experiment 1. In the present experiment, all 12 rats with PH cuts and 10 of the 11 rats with MP cuts that survived Experiment 1 were tested. One rat with MP cuts remained hypophagic and required periodic force-feeding. It was not used in the following tests. The 8 unoperated animals were also treated. Half of the animals of each of the groups had received AMPT in the course of Experiment 1.

Procedure

Rats with PH or MP cuts were allowed at least 3 weeks of ad lib feeding and drinking after recovery of voluntary ingestive behavior before the present drug tests were begun. Successive drug tests were separated by a minimum of one week. All animals were first given diethyldithiocarbamate (DDC), a dopamine-beta-hydroxylase inhibitor, which selectively depletes brain norepinephrine [17]. To control for non-specific sedative effects of NE depletion, all animals were subsequently tested with subanesthetic doses of sodium pentobarbital. Each animal was tested with 2 or 3 different doses of DDC and sodium pentobarbital. DDC (125 mg/ml of isotonic saline) was injected IP at doses of 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 and 550 mg/kg. Pentobarbital (Nembutal, diluted to 5 mg/ml of isotonic saline) was injected IP at doses of 1, 5, 9, 13, 17, 21, 25, 30, and 35 mg/kg. Injections were given at the beginning of the dark portion of the lighting cycle. Food intake was measured 3, 6, 15, and 24 hr after the injection. Placebo injections of equivalent volumes of isotonic saline were given on the day preceding each drug test. Doses were given to animals in a balanced fashion, based on the initial period of aphagia. Thus, for any range of drug doses, animals with long or short durations of aphagia are equally represented.

Eating scores were summated across doses to estimate the drug response at each time interval. Median responses were used to represent each dose so that only one score for each dose was used in the estimate of the time course. Statistical comparisons were made between experimental groups by *t*-tests for correlated means, in which control scores were matched with those of knife cut animals on the basis of drug dose.

Dose-dependent curves were estimated from the medians for each dose. Each point (P_n) was computed from the following formula:

$$P_n = (M_{n-1} + 2M_n + M_{n+1})/4$$

where M represents the median for a particular drug dose. This weighted average reduces the discontinuities in the response curve by distributing the average error over several drug doses. Statistical comparisons were made using the raw data for each animal in *t*-tests for independent samples.

RESULTS AND DISCUSSION

Diethyldithiocarbamate reduced food intake in all groups. The time course of this effect is shown in Fig. 3a (median drug dose = 300 mg/kg). Rats with PH or MP cuts ate less than controls in the first 3 hr after DDC. The effect was statistically reliable, $t(10) = 3.27$, $p<0.01$, for the PH group, but failed to reach customary levels of significance for rats with MP cuts ($0.05<p<0.10$). The cumulative food intake in the first 6 hr after DDC was significantly lower for both experimental groups (PH, $t(10) = 2.44$, $p<0.01$; MP, $t(10) = 2.43$, $p<0.05$). The cumulative food intake of rats with MP cuts remained significantly lower than that of controls 15 and 24 hr after the injection of DDC ($t(10) = 3.81$, $p<0.01$ and $t(10) = 2.47$, $p<0.05$, respectively).

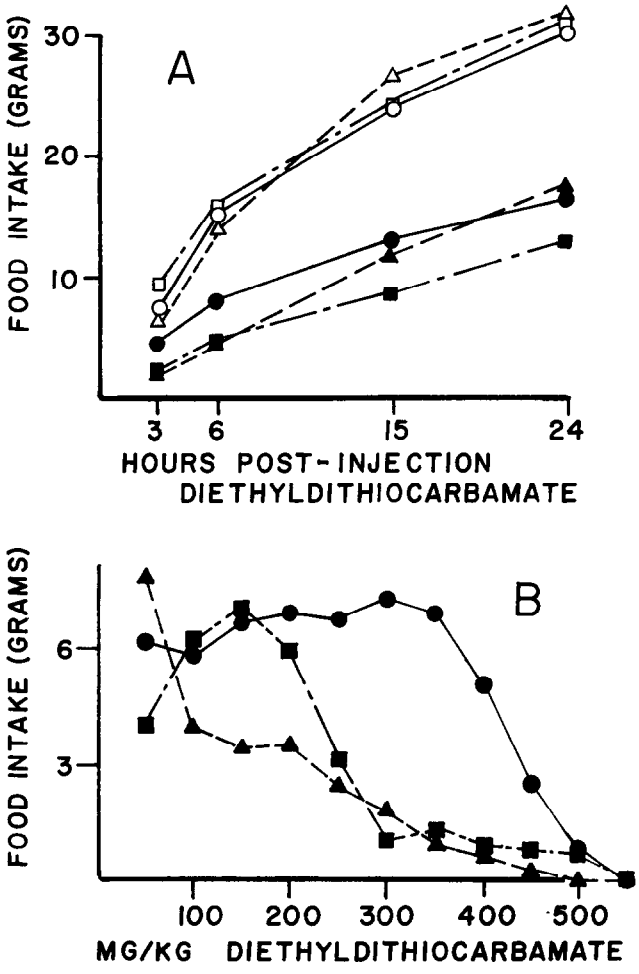

FIG. 3. Food intake after diethyldithiocarbamate. A: cumulative food intake after DDC. B: food intake in the first 3 hr after DDC as a function of dose. ○,● control rats; △,▲ PH rats; □,■ MP rats. Open symbols are used for the placebo injection, filled symbols for eating after DDC injection.

A shift in the dose-dependent curves of both experimental groups is clearly indicated 3 hr after DDC (see Fig. 3b). Both experimental groups displayed pronounced anorexia at 250 mg/kg of DDC whereas the controls required nearly twice as much drug for comparable reductions. The data shown in Fig. 3b also indicates that the overall response of rats with MP cuts did not significantly differ from that of the controls at this interval (preceding paragraph) because of a weak response to the lower doses of DDC. When the food intake of rats with MP cuts was compared with that of controls only for doses of DDC greater than 250 mg/kg these experimental animals were reliably more anorexic, $t(14) = 2.30$, $p<0.05$, in the first 3 hr period after the injection as well as in subsequent measurement intervals.

The results of the barbiturate injections are shown in Fig. 4a (median dose = 17 mg/kg). Three hours after the injection significant decreases in food intake were observed in control animals, $t(9) = 2.31$, $p<0.05$, and in animals with MP cuts, $t(9) = 2.38$, $p<0.05$. Rats with PH cuts also decreased their intake but the effect did not meet customary criteria of statistical reliability. Cumulative food intake in the 6 hr after barbiturate injection was significantly lower than baseline for both rats with PH or with MP knife cuts (PH, $t(9) = 2.30$, $p<0.05$; MP, $t(9) = 2.45$, $p<0.05$).

The cumulative food intake of rats with MP cuts remained low 15 hr after drug injection, ($t(22) = 2.59$, $p<0.05$) but was within the normal range 24 hr post-injection. Neither of the two experimental groups ate reliably less than controls during any interval on either drug or control tests.

The dose-response curve for pentobarbital 3 hr after injection (see Fig. 4b) shows that this sedative decreased eating, but the dose-response curves for both groups of knife cut animals did not differ from that of the controls even though the highest doses of Nembutal produce decreases in eating that were comparable to those seen after DDC.

EXPERIMENT 3: EFFECTS OF HALOPERIDOL OR PHENOXYBENZAMINE

The results of our second experiment indicated that voluntary ingestive behavior after knife cuts which interrupt striatal connections is more dependent on NE synthesis and presumably, NE release than in the normal animal. This is in good agreement with earlier reports of feeding responses to intraventricular injections of NE in animals that were aphagic as a result of LH lesions [9]. Our results suggest that the anorexigenic effects of CA depletion in rats with LH lesions [53] may, at least in part, be due to specific effects on NE pathways. They do not exclude the possibility that DA pathways may also play a significant role in the organization of feeding behavior.

The following experiment was designed to further examine the role of the two catecholaminergic pathways. A relatively specific dopamine receptor blocker (haloperidol) and an alpha-adrenergic receptor blocker (phenoxybenzamine) were administered to rats with PH and MP cuts. All animals had previously received DDC so that a direct comparison of their feeding response to the different treatments can be made.

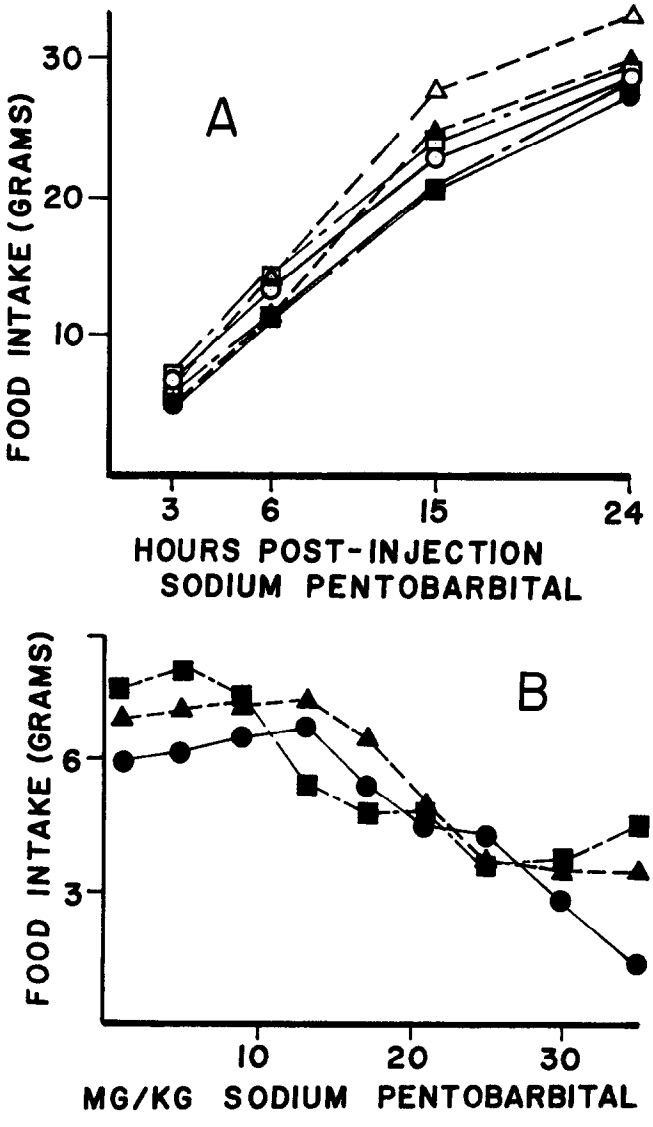

FIG. 4. Food intake after sodium pentobarbital. A: cumulative food intake. B: food intake in the first hr after drug injection as a function of dose. ○,● control rats; △,▲ PH rats; □,■ MP rats. Open symbols represent eating after placebo injection, filled symbols represent eating after sodium pentobarbital injection.

METHOD

Animals

Twelve rats with PH cuts, 10 rats with MP cuts, and 8 control rats from Experiments 1 and 2 were tested.

Procedure

The protocol of the drug tests was identical to that described in Experiment 2, except that haloperidol (HAL), or phenoxybenzamine (PBZ) was injected. Haloperidol (Haldol, McNeal Company, 5 mg/ml, diluted to 1 mg/ml with isotonic saline) was injected IP in the following doses: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, and 1.3 mg/kg. PBZ (10 mg/ml of isotonic saline) was injected IP in doses of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg/kg. Placebo injections of isotonic saline were given on the day preceding each drug test. All injections were given at the beginning of the dark part of the lighting cycle. Food intake was measured 3, 6, 15, and 24 hr after the injection. Statistical comparisons were made in a manner identical to that described for Experiment 2.

RESULTS

The time course of the haloperidol effect on eating is shown in Fig. 5 (median dose = 0.7 mg/kg). Significant reductions in food intake were observed for all animals 3, 6, and 15 hr postinjection of HAL ($p<0.05$). Only rats with PH cuts were significantly hypophagic (relative to their own saline baseline) 24 hr after the drug injection ($t(12) = 4.64$, $p<0.001$). The cumulative effects of HAL were significantly greater in animals with PH cuts than in controls 15 and 24 hr after the injection (15 hr, $t(12) = 2.72$, $p<0.02$; 24 hr, $t(12) = 3.19$, $p<0.01$) although the intake of PH rats on the control test was significantly greater than that of controls 15 hr after the injection, $t(24) = 3.80$, $p<0.001$, and equal to the control baseline at 24 hr postinjection. The cumulative intake of rats with MP cuts was significantly lower than that of the controls 15 or 24 hr after HAL (15 hr, $t(12) = 2.22$, $p<0.05$; 24 hr, $t(12) = 3.17$, $p<0.01$). However, the total intake of rats with MP cuts in 24 hr after HAL did not differ from their own saline baseline.

Figure 5b shows cumulative food intake 3 hr postinjection, as a function of the dose of HAL. Animals with PH cuts were significantly more anorexic at the lowest doses of HAL than the controls (0.1, 0.2, and 0.3 mg/kg; $t(20) = 2.43$, $p<0.05$). No reliable difference was observed between the intake of controls and rats with MP cuts at these or any other doses.

Fifteen hr after HAL (Fig. 6) both experimental groups consumed less food than the controls at high as well as low doses of HAL. There were no reliable differences between the dose-response curves of the two experimental groups although rats with PH cuts tended to eat less than rats with MP cuts at higher doses of HAL.

Rats with PH or MP cuts ate significantly less in the first 3 hr after PBZ than they consumed during related control tests (PH, $t(9) = 5.96$, $p<0.001$; MP, $t(9) = 2.76$, $p<0.05$). Control animals did not decrease their food intake significantly during this interval. The cumulative food intake of all rats was reliably less than on saline control tests 6, 15, and 24 hr after PBZ injection ($p<0.05$) (see Fig. 7a). The cumulative food intake of rats with MP cuts was reliably lower than that of the controls 15 and 24 hr after PBZ injection ($p<0.05$). However, this difference is difficult to interpret since animals with MP cuts also ate reliably less than the controls 15 and 24 hr after control saline injections. The cumulative intake of rats with PH cuts did not differ reliably from that of the controls 6, 15, or 24 hr postinjection.

The effects of different doses of PBZ on food intake during the first 3 hr after the injections are shown in Fig. 7b. Animals with knife cuts appear slightly more anorexic than controls at higher doses of PBZ. This effect was statistically reliable for rats with PH cuts, $t(20) = 2.86$, $p<0.05$, but not for rats with MP cuts ($0.05<p<0.10$) (for doses > 5.0 mg/kg).

The dose-response curve for cumulative food intake 15 hr postinjection of PBZ is shown in Fig. 8. Rats with PH

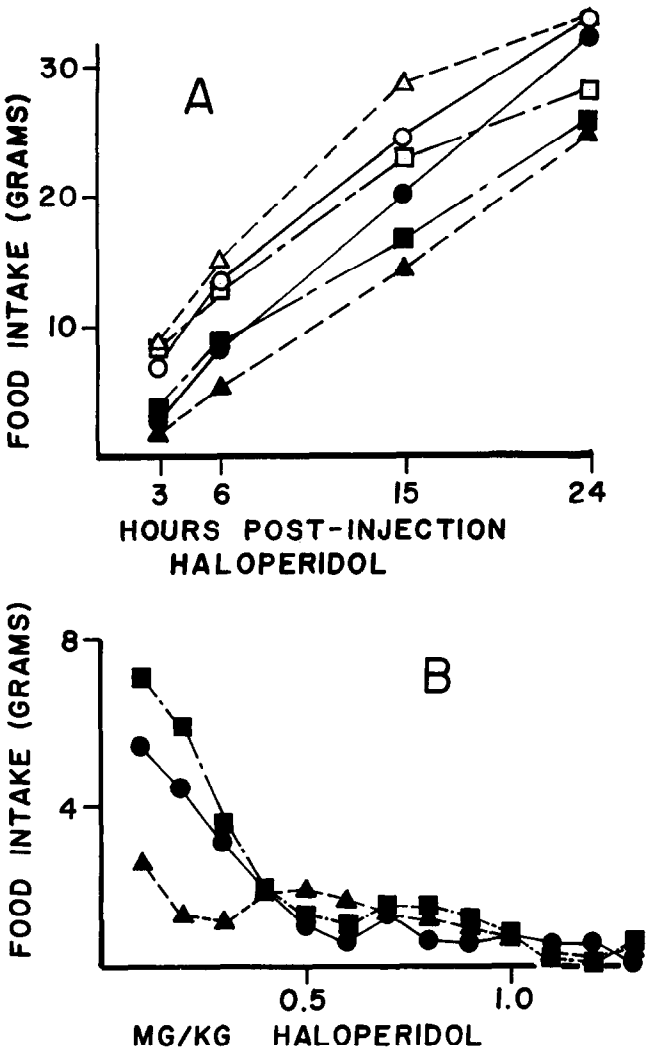

FIG. 5. Food intake after haloperidol. A: cumulative food intake. B: food intake in the first 3 hr after haloperidol as a function of dose. ○, ● control rats; △, ▲ PH rats; □, ■ MP rats. Open symbols represent eating after placebo injections, filled symbols represent eating after haloperidol injection.

cuts tend to eat less than the controls at similar doses of PBZ but the overlap of the dose-response curves at high and low doses prevents this difference from reaching statistical significance.. It should be noted that the control intake of rats with PH cuts was reliably higher than that of the controls, $t(9) = 2.29$, $p<0.05$, 15 hr postinjection. The overall decrease from the baseline food intake was reliably greater for rats with PH cuts than for controls, $t = 3.62$, $p<0.01$, in this time period.

DISCUSSION

The results of this experiment indicate that our PH and MP knife cuts may increase the anorexic effects of both DA and NE blockade. Our PH cuts produced significantly greater depletions of DA than the MP cuts and these depletions were correlated with duration of postoperative aphagia. It may be significant that rats with PH cuts were also most anorexic in response to DA blockade by

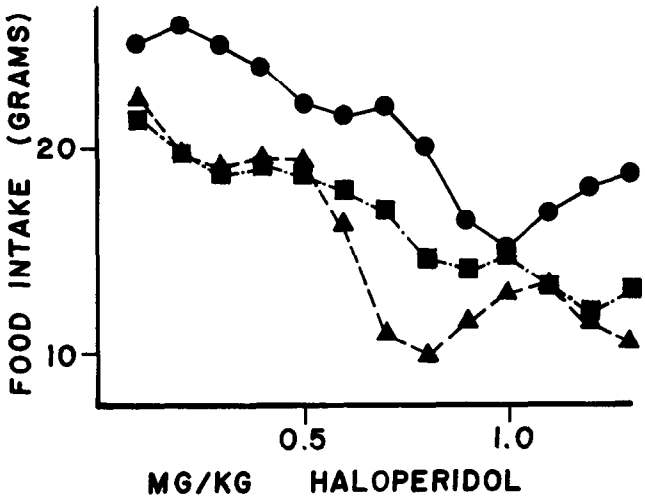

FIG. 6. Food intake in 15 hr after haloperidol as a function of dose. ● control rats; ▲ PH rats; ■ MP rats.

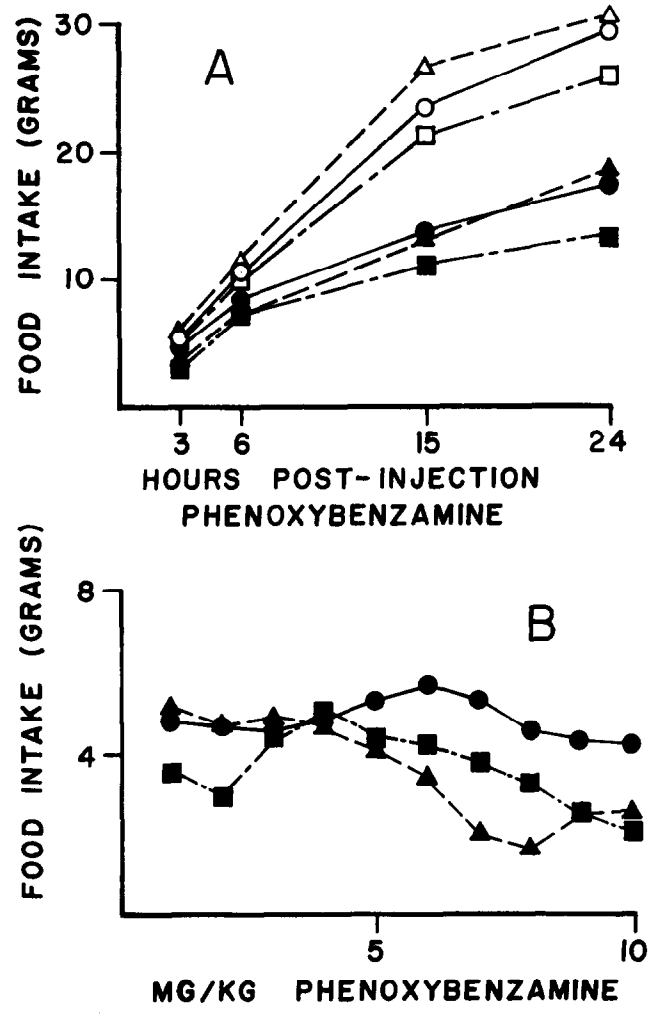

FIG. 7. Food intake after phenoxybenzamine. A: cumulative food intake. B: food intake in the 3 hr after injection as a function of drug dose. ○○, ● control rats; △, ▲ PH rats; □, ■ MP rats. Open symbols represent eating after placebo injections, filled symbols represent eating after phenoxybenzamine injection.

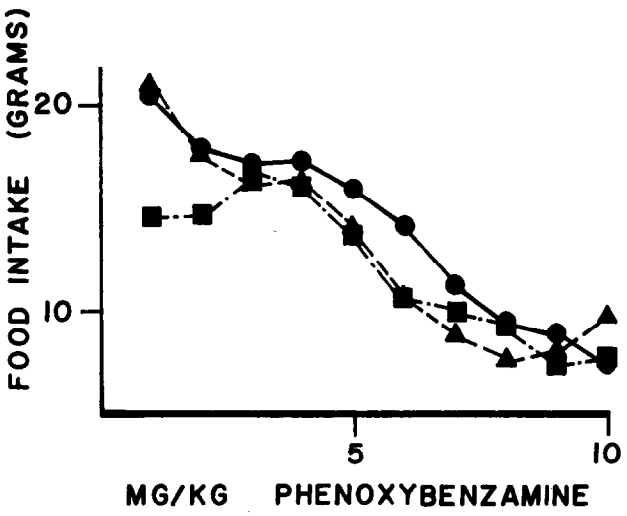

FIG. 8. Food intake in 15 hr after phenoxybenzamine as a function of dose. • control rats; ▲ PH rats; ■ MP rats.

haloperidol. Rats with MP cuts had smaller DA depletions that were not correlated with the duration of aphagia and their food intake did not differ from that of controls during the first 6 hr after the injection of HAL. These observations suggest that the DA system may not be supersensitive in this preparation, at least with respect to the initial effects of DA blockade. The rats with MP cuts did eat less than controls 15 hr after haloperidol but since their baseline food intake was initially lower, the decrease from baseline was not significantly different from that seen in control rats ($p>0.10$). On balance, it appears that the rats with MP cuts responded to haloperidol much like normal animals despite the striatal DA depletions typical of this preparation (see Experiment 1).

Alpha-noradrenergic blockade by PBZ produced a decrease in intake at 15 and 24 hr after the injection that was quantitatively similar to that seen after blockade of NE synthesis by DDC (see Experiment 2). This slow onset, but long-lasting effect of phenoxybenzamine is consistent with the presumed mode of alpha-adrenergic blockade by this compound. Initially, PBZ is thought to act competitively in blocking alpha-receptors. Later, binding to receptor sites results in noncompetitive inhibition [40]. Presumably, higher doses of PBZ might have resulted in complete anorexia within the first three hr after injection, as was seen after high doses of DDC or HAL. High doses of PBZ were not used since doses greater than 5 mg/kg resulted in prolonged anorexia in a few cases. One control, one rat with a MP cut, and four of the animals with PH cuts, ate less than 5 g 24–48 hr after the PBZ injection. In a few cases intragastric feeding was initiated to counter this inanition.

Rats with PH cuts appeared to be supersensitive to PBZ-induced alpha-receptor blockade but the effect was less conspicuous than it had been following DDC. Rats with PH cuts are anorexic sooner than controls, and the effect appears to persist longer in most experimental animals.

Despite the apparent supersensitivity of animals with MP cuts to NE synthesis inhibition by DDC, their response to PBZ did not exceed that of the controls at any time. The PBZ-induced anorexia tended to become apparent earlier in rats with MP cuts (see Fig. 7b) but the lower baseline intake of these animals complicates any interpretation of this effect.

It is interesting to view the differential effects of our pharmacological treatments in conjunction with our earlier observation that the post-operative aphagia observed in rats with MP cuts was as prolonged as that of the most severely afflicted animals with PH cuts. This suggests that normal ingestive behavior is not determined by a single central neurotransmitter system. Rats with PH cuts showed severe DA depletions and increased dependence on DA and NE function. Rats with MP cuts, on the other hand, had only slight changes in striatal DA content and responded normally, or nearly so to drug treatments which reduced central DA functions.

GENERAL DISCUSSION

Several aspects of our results require comment. A comparison of data from rats with PH and MP cuts indicates that aphagia and adipsia may occur as a result of damage to several different pathways. Recent investigations of the LH syndrome [31, 41, 47, 52, 53] have emphasized the involvement of DA axons which pass through the LH enroute to the striatum. The efficacy of PH cuts in depleting striatal DA and producing correlated inhibitory effects on food and water intake indicates that destruction of DA fibers may well produce aphagia and adipsia. However, equally severe effects on food and water intake were seen in rats with MP cuts which produced much smaller depletions of striatal catecholamines. The proximity of the pathways that were severed by our MP knife cuts to the area in which LH lesions are most effective suggest that the aphagia and adipsia after LH lesions may be due to an interruption of several different neural systems that carry afferent as well as efferent information to and from the striatum.

This conclusion is congruent with reports of aphagia and adipsia after lesions which do not involve the nigrostriatal projections, such as lesions in the midbrain [12, 23, 42] posterior to the substantia nigra, and after damage to higher-order projections of the trigeminal nerve that are intermingled with the fibers of the medial forebrain bundle [51].

The differential anorexia shown by our animals to NE antagonism may indicate a general role for this transmitter in the adjustment to destruction of portions of the brain. Considerable plasticity has been found in central NE systems. Damage to anterior portions of adrenergic projections in the CNS has been reported to produce temporary increased growth in the posterior projections of these neurons [43]. Sections of fibers entering the septum from the hippocampus has resulted in takeover of the resulting vacated receptor sites by sprouting and terminal formation by adjacent, undamaged NE projections [36,37]. More relevant to our present observations, aphagia produced by LH lesions may be relieved by central injection of NE [9], or after injection of nerve growth factor [10] which is known to facilitate sprouting in NE axons [11].

The anorexia observed in control as well as experimental animals after NE blockade or after DA antagonism indicates that both of these transmitters participate in the normal maintenance of ingestive behavior. The enhanced anorexia of PH rats after haloperidol suggests that the effects of this cut on ingestive behavior may be due specifically to the

pronounced depletion of dopamine which was observed in rats of this group. The low doses at which PH rats were supersensitive are identical with those that have been reported to be most specific for DA receptor blockade [5].

The increased anorexic effects of CA antagonists does not seem to be due to an increased sensitivity to sedatives since a barbiturate did not produce differential effects. This is of particular interest in the present context in view of earlier suggestions that the increased barbiturate sleeping time after central lesions [27] or after 6OHDA treatments [16] might be due to CA deplation. Our results do not contradict the suggested CA-barbiturate interaction, but indicate that the structures that were depleted by our cuts are not those that interact with the narcotic effects of barbiturates.

The present experiments as well as reports from other laboratories [9, 21, 22, 28] indicate that CA systems (and especially NE) may be important for the recovery and maintenance of eating after central lesions. This does not exclude the possibility that other neurotransmitters may also play an important role in the control of ingestive behavior. Supersensitivity to the anorexic effects of serotonin agonists has been reported after LH lesions [14], and we have recently observed some correlations between the recovery of eating after hypothalamic knife cuts that produce aphagia, and the residual levels of serotonin in some forebrain areas [34].

In conclusion, the central control of eating appears to be related to striatal functions that are mediated, at least in part, by catecholamines. Alterations in CA sensitivity and synthesis appear to be important in the recovery of function after striatal damage, but our data indicate that it cannot be presumed that such changes always occur.

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