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Dopamine-Norepinephrine Interactions in the Development of Hyperphagia and Obesity Following Medial Hypothalamic Lesions

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NOBREGA, J. N. AND D. V. COSCINA. *Dopamine-norepinephrine interactions in the development of hyperphagia and obesity following medial hypothalamic lesions.* PHARMACOL BIOCHEM BEHAV 25(2) 401–409, 1986.—Conflicting evidence exists on the ability of central 6-hydroxydopamine (6-OHDA) injections to alter the subsequent development of hyperphagia and obesity following medial hypothalamic lesions (MHL) in rats. An initial study [9] found no effects of prior intracisternal (IC) 6-OHDA on the subsequent development of this MHL syndrome, while later work [22,23] reported that dopamine (DA) depletions induced by intracerebral 6-OHDA effectively blocked it. The present study reexamined this issue by investigating the effects of depleting brain dopamine, norepinephrine (NE), or both DA and NE, on overeating and obesity induced by subsequent MH lesions. Different patterns of DA and NE depletions were achieved by IC 6-OHDA in combination with systemic pretreatments designed to protect central NE, DA, or neither amine, respectively. It was found that 6-OHDA regimens that selectively depleted forebrain DA did prevent the development of hyperphagia and obesity following MHL. However, when such forebrain DA depletions were accompanied by NE depletions no such blockade occurred. Manipulations which selectively depleted forebrain NE had no effect on MHL-induced hyperphagia and obesity. These results offer a framework for resolving previous discrepancies in the literature concerning brain monoamines and MHL effects. They also indicate that the effectiveness of brain DA depletions in blocking the MHL syndrome is critically dependent on the functional status of NE systems.

Brain amine depletion Bupropion Dopamine Feeding behavior Hyperphagia
6-Hydroxydopamine Interaction Medial hypothalamic lesions Norepinephrine Obesity

FUNCTIONAL integrity of brain dopamine (DA) systems seems to be essential for the expression of a number of behaviors, including normal feeding and drinking [5, 13, 25, 27]. One area in which the extent of central DA involvement has been controversial is the classical syndrome of overeating and obesity induced by lesions in the medial hypothalamus (MH). An early study by Coscina *et al.* [9] reported no effects of prior intracisternal injections of 6-hydroxydopamine (6-OHDA) on overeating and obesity induced by MH lesions, whereas a subsequent study by Rowland *et al.* [22] reported a complete blockade of MH lesion-induced overeating and obesity in rats depleted of forebrain DA by prior intranigral injections of 6-OHDA. The authors of the latter study suggested that the discrepancy in the results of the two studies might be due to the extent of forebrain DA depletion achieved in each case: more than 90% in the Rowland *et al.* study vs. 60 to 85% in the Coscina *et al.* study. It was therefore conceivable that a certain critical level of forebrain DA depletion would have to be achieved before blockade of the MH lesion syndrome would occur. Evidence contrary to this possibility has been obtained in a more recent study by Rowland and Stricker [23] using rats with different degrees of forebrain DA depletion induced either by varying doses of intraventricular 6-OHDA or by lateral hypothalamic lesions of graded sizes. Such animals still failed to show overeating and accelerated weight gain after MH lesions. The authors concluded that "DA-depleting lesions need not be extensive . . . to interfere with the development of hyperphagia and obesity" ([23], p. 276).

The study by Coscina *et al.* [9] differed from the more recent work by Rowland and coworkers [22,23] in terms of several design variables, including sex of animals, route of intracerebral 6-OHDA injection, and MH lesion technique. An additional and potentially important difference was the amount of relative interference with forebrain norepinephrine (NE) systems as a result of the particular 6-OHDA regimen employed in each case. The intracisternal 6-OHDA injections used by Coscina *et al.* [9] produced a substantial

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depletion of forebrain NE in addition to DA. In contrast, both the intranigral 6-OHDA injections of Rowland *et al.* [22] and the intracerebroventricular injections of 6-OHDA preceded by systemic desipramine used by Rowland and Stricker [23] spared forebrain NE neurons. Since different lines of evidence have implicated NE in the hypothalamic control of feeding [15,19], we sought to reexamine the respective roles of DA, NE and their interactions in the development of the MH lesion syndrome. To achieve this we systematically manipulated depletion of these two monoamines prior to inducing MH lesions. The results obtained provide a framework for resolving discrepancies between the previous studies. In addition, they clarify the specific nature of relationships which appear to exist between DA and NE in the development of the MH lesion syndrome.

EXPERIMENT 1

The purpose of this experiment was to compare the effects of selective DA depletion to the effects of combined DA + NE depletions on the ability of subsequent MH lesions to induce hyperphagia and increased body weight (BW) gain. Simultaneous depletion of both amines was achieved by central injections of 6-OHDA preceded by systemic treatment with the monoamine oxidase inhibitor, pargyline, to potentiate the effects of the neurotoxin [6]. Selective depletion of DA was achieved by central treatment with 6-OHDA preceded by systemic administration of the NE uptake blocker desmethylimipramine (DMI), a regimen designed to protect NE neurons from the toxic effects of 6-OHDA [7].

METHOD

Animals

Female Wistar rats (Woodlyn Labs, Guelph, Ontario) weighing 200 g at the start of the experiment were used. Rats were housed individually in hanging wire-mesh cages in a temperature-controlled room (21°C±1) with lights on between 0700 and 1900 hr daily. Water was always freely available. Except for the phases of the experiment where specific dietary manipulations were implemented, Purina Chow pellets (4% fat; 3.4 kcal/g) were available on cage floors at all times.

Treatment Groups

Following an 8-day adaptation period during which BW was recorded daily, rats were assigned to 5 BW-matched groups. A normal control group was scheduled to receive no injections or lesions. The second group received single intracisternal (IC) injections of 6-OHDA HBr (Sigma, St. Louis, MO) on 3 consecutive weeks; each injection (200 μg 6-OHDA in 20 μl 1% ascorbic acid in deionized water) was preceded by an intraperitoneal (IP) injection of 25 mg/kg BW DMI HCl (Ciba-Geigy) 30 min before the IC treatment (DMI-6OH Group). The third group received the same DMI pretreatment followed by IC injections of the 1% ascorbate vehicle (DMI-VEH Group) at the same times as the second group. The fourth group received single injections of 6-OHDA (200 μg in 20 μl 1% ascorbate) like the second group but each injection was preceded 1 hr by an IP injection of 50 mg/kg BW of pargyline HCl (Sigma, St. Louis, MO) (PAR-6OH Group). The fifth group received the same pargyline pretreatment followed by IC injections of 1% ascorbate vehicle at the same times as the fourth group (PAR-VEH Group). Doses of 6-OHDA are expressed as free base.

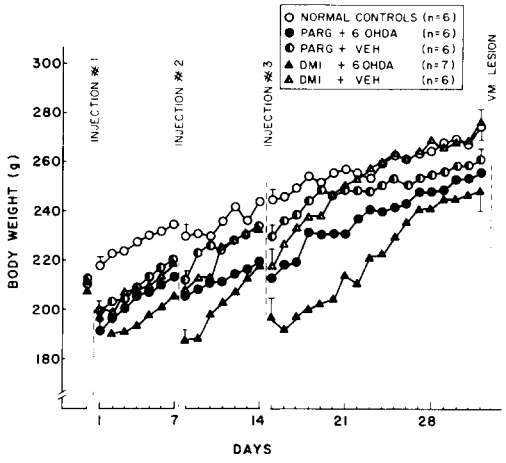

FIG. 1. Effects of repeated IC 6-OHDA on BW gain in Experiment 1. Values represent means and standard errors. See text for description of treatment groups. Numbers in parentheses represent group size.

All IC injections were of a fixed volume (20 μl) and were performed under light ether anesthesia. The design thus included two 6-OHDA groups, one with and one without DMI protection, two vehicle control groups and one untreated control group.

Hypothalamic Lesions

Thirteen days after the last of the 3 IC injections, all rats except the normal control group received bilateral radiofrequency lesions (55°C, 1 min per hemisphere) under sodium pentobarbital anesthesia (35 mg/kg) using a Radionics RFG-4 lesion generator and thermistor probe. The following stereotaxic coordinates were used: 7.0 mm rostral to the interaural line; 0.6 mm lateral to the exposed midsagittal sinus, and 8.5 mm below the surface of the dura, with the skull leveled between lambda and bregma.

Post-Operative Dietary Manipulations

Both food intake (FI) and BW were measured after the MH lesions. All rats were fed regular Purina Chow pellets for three weeks, then a high-fat diet (67% powdered Purina Chow + 33% Crisco shortening by weight; 5.27 kcal/g) for two weeks, and then a high-carbohydrate diet (50% Borden's sweetened condensed milk + 50% water by volume; 2.114 kcal/g) plus powdered Chow for another two weeks. When pellets were used, spillage was collected daily and intake measures were adjusted accordingly. Powdered Chow and high-fat diets were given in modified Wahman feeding cups fastened to the bottom of the cages. Sweetened condensed milk solutions were given in Wahmann graduated drinking tubes fitted with ball-bearing spouts to reduce dripping.

Histology and Biochemical Assays

At the end of the experiment, animals were sacrificed by decapitation. The striatum and neocortex were rapidly dissected on a cold plate and stored at −70°C until assays of DA, NE and serotonin (5-HT) were performed using the HPLC method of Warsh *et al.* [26]. The remainder of the brain was fixed in 10% formalin for at least two weeks.

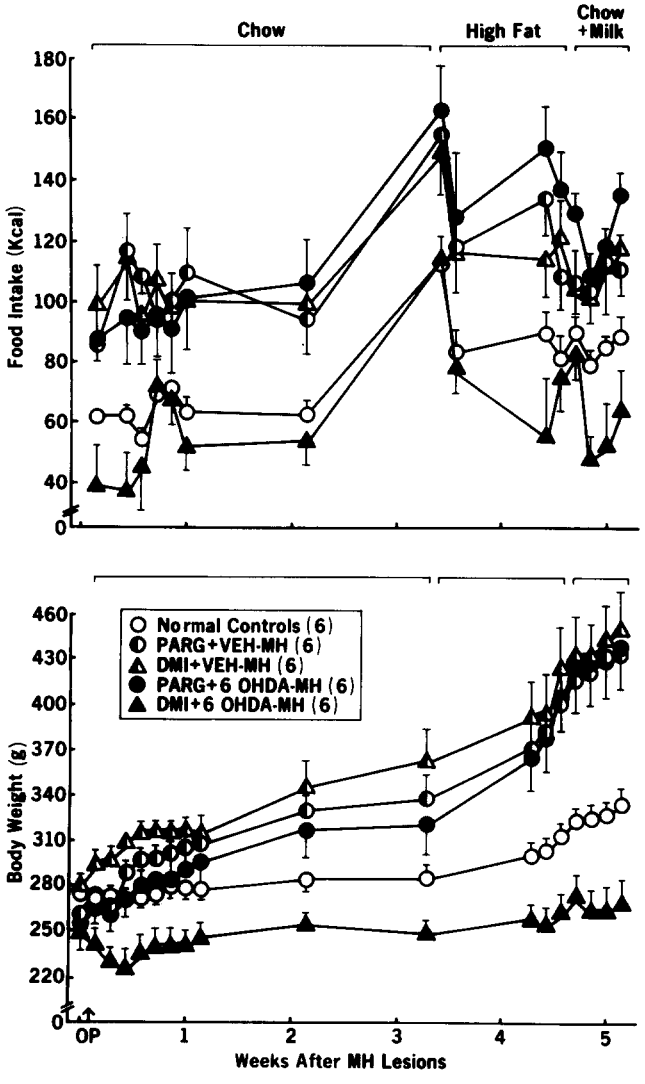

FIG. 2. Body weight gain and food intake following MH lesions in 6-OHDA-and VEH treated groups in Experiment 1. Values represent means and standard errors. See text for description of groups. Numbers in parentheses indicate group size.

Forty-micron sections cut through the lesioned area were used to make photographic prints as described in [24].

Statistical Analyses

Food intake and body weight data were initially analyzed by repeated-measures analyses of variance (ANOVAs) using 6-OHDA (6-OHDA vs. VEH), Systemic Pretreatment (PAR vs. DMI) and Days as factors. Subsequent ANOVAs focussed on the differences between the two target 6-OHDA groups as a function of systemic pretreatment and days. Food intake (FI) data were expressed as caloric intake. Body weights were analyzed both as absolute values and as BW change from baseline. Separate analyses were conducted for each of the three diet phases after the MH lesions.

RESULTS

Effects of 6-OHDA on BW Gain Prior to MH Lesion

As anticipated, each IC injection of 6-OHDA caused temporary decreases in BW. However, as illustrated in Fig. 1, such effects varied as a function of the systemic pretreatment preceding the 6-OHDA injections. Both the PAR-6OH and the DMI-6OH groups lost comparable amounts of weight after the first injection. However, the second and third injections caused much smaller BW losses in the PAR-6OH group, while causing progressively larger losses in the DMI-6OH group. Analyses of Variance using BW changes for the two days following each injection as a dependent variable indicated significant differences in BW loss among groups after the second ($p<0.004$) and third ($p<0.001$) injections. The DMI-6OH group lost significantly more weight than the PAR-6OH group on both occasions ($ts=2.465$ and 2.95, for second and third injections, respectively; $ps<0.05$).

For three days following the third injection, two animals in the DMI-6OH group were fed a mixture of sweetened condensed milk and chocolate chip cookies in an effort to halt their continuing BW loss. This diet supplementation was discontinued after 4 days as the animals regained the ability to maintain BW on Chow pellets. Two weeks after the third injection all groups were eating normally and gaining weight at the same rate. An ANOVA performed at that point indicated no significant differences in BW among the 5 groups ($p>0.1$).

Effects of MH Lesions

As shown in Fig. 2, following surgery all MH-lesioned groups except one began overeating and gaining weight more rapidly than unoperated controls. Rats that had received DMI-6OH injections failed to show either response. Analyses of Variance for the two target 6-OHDA groups in the first post-lesion week confirmed a significant pretreatment effect for FI ($p<0.05$), absolute BW ($p<0.05$), and BW change from weight on lesion day ($p<0.01$). During this first post-operative week, BW differences but not FI differences between the two 6-OHDA groups varied as a function of days, as reflected in significant Systemic Pretreatment × Days interaction ($ps<0.01$). Three weeks after surgery all lesioned groups had gained at least 6 times as much weight as the unoperated normal controls, the exception still being DMI-6OH rats. As a group the latter animals gained 1 ± 13 g during that period, compared to 11 ± 14 g gained by the controls.

After three weeks on Chow pellets, all animals were switched to a high-fat diet. As shown in Fig. 2, caloric intake increased for all lesioned groups substantially beyond the increase shown by the normal controls except for the DMI-6OH group, whose caloric intake and rate of BW gain remained at or slightly below control levels. Analyses of Variance for the two 6-OHDA groups confirmed a significant pretreatment effect on caloric intake ($p<0.001$), absolute BW ($p<0.001$), and BW change ($p<0.001$). As in the case of the first time period analyzed, the BW effects but not the intake effect were found to vary as a function of time, as indicated by significant Systemic Pretreatment × Days interaction for BW and BW change ($ps<0.001$).

Placing the animals on a high-carbohydrate diet produced no alteration in the previous pattern. All lesioned groups except the DMI-6OH group showed caloric intakes and rates of BW gain significantly higher than those shown by unoperated controls. This was confirmed by ANOVAs, which continued to indicate a significant pretreatment difference for the two 6-OHDA groups in terms of caloric intake ($p<0.001$), absolute BW ($p<0.001$), and BW change

TABLE 1

EFFECTS OF 6-OHDA TREATMENTS ON FOREBRAIN AMINE CONTENT IN EXPERIMENT 1[a]

		Striatum			Neocortex		
Group[b]	n	DA	NE	5-HT	DA	NE	5-HT
Normal	6	130.8 (8.5)	0.47 (0.1)	7.5 (0.4)	9.4 (0.4)	3.4 (0.1)	7.7 (0.2)
PAR-VEH	6	141.3 (10.2)	0.37 (.03)	7.3 (0.8)	11.1 (0.9)	3.3 (0.2)	7.8 (0.5)
DMI-VEH	6	120.0 (12.5)	0.46 (.05)	6.5 (0.4)	10.7 (0.4)	3.7 (0.1)	7.9 (0.2)
PAR-6OHDA	6	12.4 (4.2)*	0.19 (.02)*	7.9 (0.6)	2.5 (0.4)*	0.9 (0.2)*	7.8 (0.3)
DMI-6OHDA	6	19.1 (5.0)*	0.28 (.04)	8.9 (0.6)	3.2 (0.5)*	3.4 (0.2)	7.9 (0.6)

[a]Values represent mean (s.e.m.) ng amine/mg protein.
[b]All groups, except Normal, had MH lesions. See text for definition of abbreviations.
*Significantly different from Normal group ($p<0.05$).

($p<0.01$). As in the case of Chow and high-fat phases, the BW changes but not caloric intakes were found to vary as a function time ($p<0.05$ for both interaction effects).

Neurochemical Assays and Histology

As shown in Table 1, none of the manipulations affected 5-HT levels in striatum or neocortex. The data for the VEH groups indicate that MH lesions did not by themselves produce reliable changes in DA, NE or 5-HT concentrations in these two brain areas. The two 6-OHDA groups had equally severe depletions of DA in striatum (range: 84–91%) and neocortex (range: 70–79%), indicating that PAR or DMI pretreatments did not affect this parameter. However, the systemic pretreatments did differentially affect NE concentrations in 6-OHDA-treated rats as expected. While striatal NE was somewhat depleted in both 6-OHDA groups (40 to 50% compared to appropriate VEH controls), cortical NE was depleted only in the PAR-6OH groups (72±6%).

Examination of histological material revealed no differences in lesion placement and/or size that could account for the observed differences in behavioral or neurochemical parameters.

DISCUSSION

Prior DMI-6OH treatment which produced large striatal DA depletions completely blocked the development of hyperphagia and obesity following medial hypothalamic lesions. In contrast, treatment with PAR-6OH, which produced equally large DA depletions plus cortical NE depletion, was without effect on hyperphagia and obesity following MH lesions. These results suggest that central injections of 6-OHDA can block the development of hypothalamic hyperphagia and obesity only when DA is selectively depleted. Rats receiving the PAR-6OH treatment to deplete both DA and NE showed pronounced overeating and obesity after MH lesions despite DA depletions equivalent to those seen in DMI-6OH rats which showed no such responses. Therefore, in agreement with the conclusion reached by Rowland and Stricker [22], the magnitude of DA depletion alone does not appear to be the critical factor in explaining the ability of intracerebral 6-OHDA to block MH lesion effects. The only variable that differed between the two 6-OHDA groups in the present study was the magnitude of brain NE depletion. In the DMI-6OH group such NE depletions were minimal in striatum and non-existent in neocortex, while the PAR-6OH group showed moderate NE depletions in striatum and pronounced depletion in neocortex. The implication is that forebrain DA depletion by itself can indeed block the FI and BW effects of MH lesions, but if additional damage is done to brain NE systems no such blockade will occur.

These findings provide a framework for resolving discrepancies in past research involving this particular preparation. It is quite conceivable that the reason why no effects of 6-OHDA pretreatment were observed by Coscina *et al.* [9] on the subsequent development of hypothalamic hyperphagia and obesity related not to the overall degree of DA depletion achieved, but to the fact that forebrain NE was simultaneously depleted. In contrast, the intranigral or intraventricular 6-OHDA preceded by DMI as used by Rowland *et al.* [22] and Rowland and Stricker [23] resulted in damage restricted to DA systems. Their reports of complete blockade of MH lesion-induced hyperphagia and obesity fully agree with what was shown by the DMI-6OH group in the present study.

In this experiment the effects of dopaminergic lesions were countered, indeed abolished, by a concomitant lesion to noradrenergic systems. Two possibilities may be considered in connection with these findings. It is possible that NE lesions induce a propensity to overeat which simply cancels a propensity to undereat induced by the dopaminergic lesions. Both types of effects have been reported [1, 13, 27]. This would imply a simple additive relationship between these two monoamine depletion effects. Alternatively, it is possible that the obtained results reflect a non-additive, truly interactive relationship between DA and NE depletions. One way to address this point is by investigating the effects of selective NE depletions on MH lesion-induced FI and BW changes. If additive effects were involved, one would expect selective NE depletions, in the absence of DA depletions, to add to the effects of MH lesions on feeding and BW gain. This was the object of Experiment 2.

EXPERIMENT 2

In order to achieve selective depletion of forebrain NE and maintain design compatibility with the first experiment, we used repeated injections of 6-OHDA preceded by systemic pretreatment with bupropion, a clinically useful antidepressant drug which has been shown to block DA uptake and to protect DA cells from the neurotoxic effects of 6-OHDA [6,12].

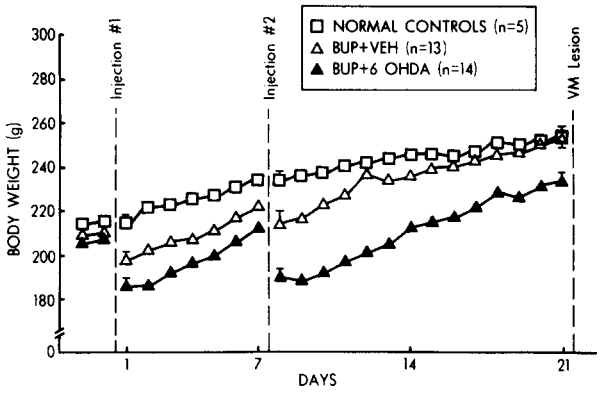

FIG. 3. Effects of systemic bupropion followed by IC 6-OHDA or vehicle on BW in Experiment 2. See text for description of group. Values represent means and standard errors. Numbers in parentheses indicate group size.

METHOD

Female Wistar rats were obtained from the same supplier and housed in conditions identical to those described in Experiment 1. Following one week of daily handling and adaptation to colony conditions, the animals were divided into three groups equated for BW. Group 1 (BUP-6OH, n=14) received an IP injection of 100 mg/kg bupropion HCl (Burroughs Wellcome Co.) 30 min before an IC injection of 6-OHDA HBr (200 μg free base in 20 μl of 1% ascorbic acid-deionized water). Group 2 (BUP-VEH, n=12) received the bupropion treatment followed 30 min later by IC injection of 20 μl ascorbate vehicle. Group 3 (NORMAL CONTROLS) received no injection treatment. These injection procedures were repeated one week later. Two weeks after the second injection, half of the animals in groups 1 and 2 received bilateral MH lesions following the procedures described in Experiment 1. The other half of both groups underwent sham surgery consisting of anesthetization and skull opening but no penetration of brain tissue. All animals were given access to Purina Chow pellets for three weeks, then switched to the high-fat diet described in Experiment 1. Seventeen days after MH lesions all animals were tested for 15 min in an automated open field (a 0.7×0.7 m Plexiglas enclosure with photocells placed at 15 cm intervals along walls). Nine weeks after the MH lesions animals were sacrificed by decapitation. Following the procedures of Experiment 1, striatal and cortical tissues were dissected for neurochemical assays and the rest of the brain was fixed in 10% formalin for histological verification of lesion size and placement. As in Experiment 1, data were analyzed by Repeated Measures ANOVAs.

RESULTS

Effects of Bupropion and 6-OHDA on BW Gain Prior to MH Lesions

Bupropion injections induced temporary but very pronounced gnawing, licking and sniffing, as typically seen after treatments that potentiate DA neurotransmission [12]. As shown in Fig. 3, animals receiving 6-OHDA after bupropion lost more weight than animals receiving the BUP-VEH treatment after each injection (using two-day BW change from injection BW as a dependent variable, $ts(26)=3.62$ for first injection and 4.20 for second, $ps<0.001$). Such weight loss was of the same magnitude after each of the two injections (11% for the BUP-6OH group and 3% for the BUP-VEH group). For six days after the second injection, three animals in the BUP-6OH and one in the BUP-VEH group were given sweetened condensed milk diluted 1:1 with water and/or a powdered Chow and water mash in attempts to halt continuing weight loss. Two weeks after the second injection all animals were gaining weight at comparable rates, but there remained a small (8%) but significant ($p<0.01$) difference in BW between rats that had received 6-OHDA and those that had not.

Effects of MH Lesions

During the first week after MH lesions both lesioned groups consumed more calories than the non-lesioned groups, as indicated by a significant Lesion effect in the ANOVA ($p<0.001$; see Fig. 4). Separate ANOVAs indicated no significant differences in FI ($p>0.05$) between the two target lesioned groups (BUP-6OH-MH vs. BUP-VEH-MH). The small pre-lesion BW differential between these two groups was still detectable ($p<0.05$) during the first post-surgery week. In addition, 6-OHDA and VEH lesioned groups were found to differ in terms of BW change ($p<0.05$). As shown in Fig. 4, VEH-MH rats, showed an immediate increase in BW on post-lesion day 1, while 6-OHDA-MH rats showed no such immediate change. Three weeks after surgery, all lesioned animals were eating 19% more than sham controls, and had gained twice as much weight. At that point no differences remained between the two lesioned groups in terms of caloric intake, absolute BW, or BW change ($ps>0.05$). Placing the animals on a high-fat diet for 4.5 weeks accentuated the differences between lesioned vs. non-lesioned groups in terms of caloric intake, absolute BW and rate of BW gain (all $ps<0.001$), but still did not discriminate between the two lesioned groups on any of the variables measured. Comparisons of sham-lesioned rats that received 6-OHDA to those pretreated with BUP-VEH indicated no differences between those groups at any time in terms of caloric intake or BW parameters ($ps>0.05$).

Two other feeding-related measures were recorded daily and analyzed for each of the two phases of the post-lesion period: the ratio of food eaten/food spilled and the ratio of BW change/unit caloric intake (food efficiency). No significant differences among groups were detected at any point on either of these measures ($ps>0.1$).

The results of the open-field test conducted on post-MH-lesion day 17 are shown in Table 2. No significant differences were found among groups in terms of total number of line crossings during the 15-min test period. Groups did differ on a second measure, number of rearings in the open field. Analyses of variance indicated no difference between MH-lesioned and non-lesioned groups ($p>0.5$) but a significant difference was found between 6-OHDA and VEH-treated rats ($p<0.01$). No significant differences were found between MH-lesioned and non-lesioned 6-OHDA groups ($p>0.5$).

Neurochemical and Histological Assays

Results of neurochemical assays are shown in Table 3. While no alterations were detected in 5-HT levels in cortex, striatal 5-HT levels were somewhat elevated. Analysis of variance indicated that a mean 20% difference in striatal 5-HT levels between MH-lesioned vs. non-lesioned rats was statistically significant ($p<0.003$). The same was true for a

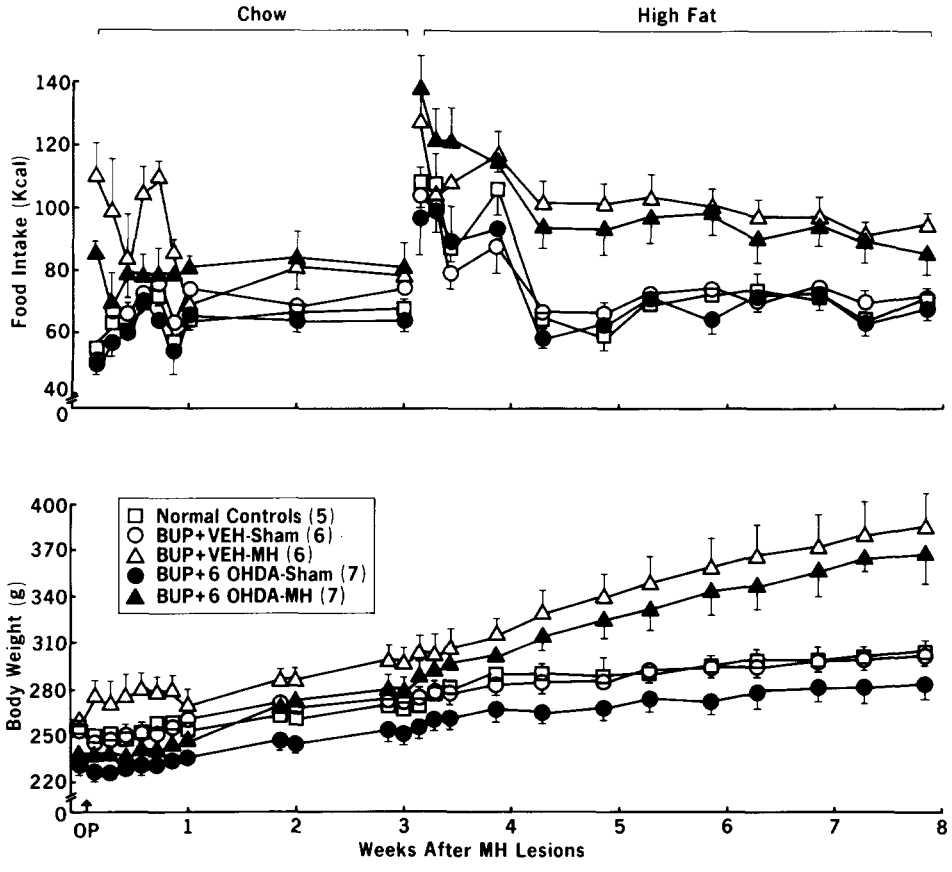

FIG. 4. Food intake and BW gain following MH lesions or sham surgery in Experiment 2. See text for description of group. Values represent means and standard errors. Numbers in parentheses indicate group size.

mean 20% difference between 6-OHDA- and VEH-treated rats ($p<0.001$). However, no difference was found in striatal 5-HT levels between 6-OHDA-MH-lesioned rats and VEH-MH-lesioned rats ($p>0.1$). Similar results were obtained for DA. Cortical DA levels were unaffected by injections or MH lesions, but bupropion treatment resulted in slightly elevated DA levels in the striatum. Such increases were of the order of 16% in lesioned rats compared to non-lesioned rats ($p<0.03$) and 14% in 6-OHDA treated vs. VEH-treated rats ($p<0.058$). However, as in the case of striatal 5-HT levels, no significant differences were found between the two target MH-lesioned groups, BUP-6OHDA-MH vs. BUP-VEH-MH ($p>0.5$). With bupropion treatment 6-OHDA treatment resulted in 20 to 28% NE depletion in striatum and 67 to 74% depletion in cortex.

Examination of lesion placement and size revealed no significant differences among groups on these parameters.

TABLE 2

OPEN-FIELD BEHAVIOR OF 6-OHDA- AND VEH-TREATED RATS 17 DAYS AFTER MH LESION[a]

Group[b]	n	Line Crossings	Rearings
Normal	5	1057 (53)	91 (11)
VEH-SHAM	6	945 (64)	80 (7)
VEH-MH	7	1085 (77)	86 (10)
6OHDA-SHAM	7	849 (113)	55 (8)*
6OHDA-MH	7	1082 (128)	57 (12)*

[a]Values represent mean (s.e.m.) number of occurrences in a 15-min test. Crossings represent machine-recorded total photobeam interruptions. Total number of rearings were counted from playback of video tapes of open field sessions.

[b]All groups, except Normal, had received bupropion. See text for definition of abbreviations.

*Significantly different from Normal group ($p<0.05$).

DISCUSSION

Intracisternal 6-OHDA preceded by systemic bupropion produced substantial depletions of cortical NE while sparing striatal and cortical DA. Prior to MH lesions BUP-6OH animals did not show increased BW gain. If anything these animals showed slower BW gain than BUP-VEH controls. Following MH lesions, BUP-6OH rats showed significant hyperphagia and obesity. These effects, however, were of the same magnitude as those shown by lesioned BUP-VEH rats. These results indicate that NE depletions of the magnitude achieved here do not in themselves cause overeating and obesity, and may in fact result in hypophagia. This is in agreement with reports of hypophagia following selective brain NE depletions induced by intraventricular 6-hy-

TABLE 3

EFFECTS OF 6-OHDA TREATMENTS ON FOREBRAIN AMINE CONTENT IN EXPERIMENT 2[a]

		Striatum			Neocortex		
Group[b]	n	DA	NE	5-HT	DA	NE	5-HT
Normal	5	127.1 (7.7)	0.7 (0.1)	8.2 (0.8)	6.9 (0.7)	3.2 (0.3)	9.1 (0.5)
VEH-SHAM	6	138.1 (10.9)	0.5 (0.05)	8.3 (0.3)	8.9 (0.7)	3.5 (0.2)	9.1 (0.4)
VEH-MH	7	171.5 (8.2)*	0.7 (0.1)	10.8 (0.8)*	7.9 (0.8)	3.0 (0.3)	8.4 (0.6)
6OHDA-SHAM	7	168.6 (7.4)*	0.4 (0.1)	11.0 (0.6)*	7.4 (1.0)	0.9 (0.2)*	9.3 (0.7)
6OHDA-MH	7	184.9 (15.5)*	0.5 (0.1)	12.6 (0.6)*	8.1 (0.7)	1.0 (0.3)*	8.9 (0.5)

[a]Values represent mean (s.e.m.) ng amine/mg protein.
[b]All groups, except Normal, had bupropion pre-treatment. See text for definition of abbreviations.
*Significantly different from Normal group ($p<0.05$).

droxydopa [20] or by treatment with dopamine-beta-hydroxylase inhibitors [18]. On the other hand, these results contrast with reports of increased food intake and BW gain following lesions of the ascending ventral noradrenergic bundle (VNB) [1–3], an effect which was found to summate with the effects of a subsequent MH lesion [3]. The present results are also in contrast to observations of increased food intake and BW gain following NE-depleting 6-OHDA injections into the amygdala [16,17]. The authors of the latter work propose that hyperphagia is associated with lowered NE/DA ratios in the amygdala. The present results suggest that this notion may not hold when other brain structures are involved. Both cortex and particularly the striatum had very low NE/DA ratios as a result of bupropion-6-OHDA treatment, yet no overeating was observed. It must be noted, however, that both the work on VNB and amygdala lesions reported larger NE depletions (>90%) than those achieved here. Therefore, our results do not rule out the possibility that larger NE depletions in certain key structures such as hypothalamus and/or amygdala must be achieved before potentiating effects of NE depletions on feeding behavior become apparent.

While these considerations point to unresolved issues in the general area of brain NE involvement in feeding, they do not affect the specific hypothesis that this study was designed to address. The fact that NE depletions of the magnitude obtained in Experiment 1 neither induced hyperphagia and obesity nor potentiated such effects after MH lesions suggests that the blockade of MH lesion effects in Experiment 1 reflected a true interaction between DA and NE depletion, rather than being due to a mutual cancelling of opposite but independent effects.

This experiment also confirms the usefulness of the bupropion-6OHDA combination as a means to achieve selective depletion of forebrain NE as first documented by Cooper *et al.* [8]. Under the conditions used here the treatment resulted in lowered BW for at least two weeks following the second injection, and in a decrease in open-field rearings, but not in line-crossings, in a test performed four weeks after the last injection. Bupropion treatment was also associated with varying but significant degrees of elevation of striatal DA and 5-HT. Such increases, which ranged as high as 45% for DA and 53% for 5-HT, were not seen in cortical tissue. Since Cooper *et al.* found no elevation of whole-brain DA following bupropion-6OHDA treatment, these localized alterations in striatum might merit further investigation to fully characterize this treatment as a research tool. Nevertheless, the fact that no significant differences were detected in striatal DA levels between the two target MH-lesioned groups suggests that this is probably not a factor in accounting for differences, or lack thereof, between 6-OHDA-MH and VEH-MH groups in the present experiment.

GENERAL DISCUSSION

The results of these two experiments suggest a specific type of interaction between forebrain DA and NE in the expression of a well-defined behavioral syndrome. Prior depletion of forebrain DA completely blocked the overeating and excessive weight gain normally seen after a MH lesion. However, if this DA depletion was accompanied by concurrent depletion of forebrain NE, which by itself has no effect on the syndrome, no such blockade was observed. Stated differently, the effects of a dopaminergic lesion were completely reversed by an additional noradrenergic lesion.

Paradoxical as it may seem, the notion that the effects of a dopaminergic brain lesion can be counteracted by additional brain damage has been documented in a number of behavioral instances by Antelman and Caggiula [5]. These authors proposed an interactive model whereby DA-dependent behavior is modulated by noradrenergic activity, and specifically suggest that often "relatively selective damage to . . . brain DA systems may be more deleterious to the functioning of the organism than similar damage which is also accompanied by a substantial interference with brain NE. Stated another way, depression of NE activity may either prevent or counteract the effects of damage to, or pharmacological intervention in, DA-containing neurons." ([5], p. 648). After reviewing a variety of instances involving behavior such as stereotypies, motor activity, avoidance, aggressive behavior, and stress-induced eating, Antelman and Caggiula observe that "when NE and DA are depressed simultaneously, the behavioral deficits normally seen after more selective DA depletion are either reversed or significantly lessened." ([5], p. 649). Among illustrative examples discussed is a study by Hollister *et al.* [14] on the effects of 6-OHDA on amphetamine-induced stereotypy. They reported that 6-OHDA regimens that preferentially depleted DA by 88% and largely spared NE (20% depletion) completely blocked amphetamine-induced stereotypies. By contrast, a 6-OHDA regimen that produced the same degree of DA depletion (89%) but also depleted NE by 80% had little

effect on amphetamine-induced stereotypy. 6-OHDA treatments which depleted NE by 50% and spared DA (12% depletion) had no effect on amphetamine stereotypy. It is quite conceivable that the exaggerated feeding behavior induced by MH lesions falls in this same category of DA-dependent behaviors which are susceptible to the specific type of NE modulation described by Antelman and Caggiula [5].

That this modulation is not an instance of simple behavioral summation is made clear by the results of the second experiment reported here, where it was found that selective NE-depleting lesions neither induced overeating nor potentiated the feeding-weight gain effects of MH lesions. This is in agreement with other data on the feeding effects of selective but generalized brain NE depletions [20,21]. Together with those data the present findings stand in contrast to reports of increased feeding following damage to local NE systems, such as DMI-6-OHDA lesions of the amygdala [16,17] or VNB lesions [1–3]. It is possible that these discrepancies relate to differences in extent of NE depletion in specific brain regions achieved in each case. Yet other well established evidence indicates that feeding increases when NE is infused into the ventricular system [18] or into localized hypothalamic areas [15]. As a whole these conflicting observations suggest that additional work is needed to clarify the exact role of brain NE systems in FI and BW regulation. In the case of DA systems the picture may be clearer. Ample evidence indicates that selective depletions of brain DA produce feeding and BW deficits. The work of Rowland *et al.* [22] and Rowland and Stricker [23] have shown that such selective brain DA depletions also prevent the overeating and obesity induced by MH lesions. The results reported here indicate that this latter effect of brain DA depletions is conditional on the functional status of brain NE systems. That is, such blockade will occur if and only if NE is not simultaneously depleted. It has been reported that the same type of effect is observed in the case of tail-pinch-induced feeding: this effect is blocked by DA receptor antagonists, but such blockade is reversed by inhibitors of NE synthesis [4,5]. It would be of interest to investigate the generality of this DA-NE interaction to other types of feeding responses (e.g., intake stimulated by chronic hyperinsulinemia as described by Rowland and Stricker [23]), and to fully characterize the neuroanatomical basis of this interaction for feeding as well as other types of behavior.

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REFERENCES

1. Ahlskog, J. E. Food intake and amphetamine anorexia after selective forebrain norepinephrine loss. *Brain Res* **82:** 211–240, 1974.
2. Ahlskog, J. E. Feeding response to regulatory challenges after 6-hydroxydopamine injection into the brain noradrenergic pathways. *Physiol Behav* **17:** 407–411, 1976.
3. Ahlskog, J. E., P. K. Randall and B. G. Hoebel. Hypothalamic hyperphagia: dissociation from hyperphagia following destruction of noradrenergic neurons. *Science* **190:** 399–401, 1975.
4. Antelman, S. M. and A. R. Caggiula. Tails of stress-related behavior: A Neuropharmacological model. In: *Animal Models in Psychiatry and Neurology*, edited by F. Hanin and E. Usdin. Oxford: Pergamon, 1977, pp. 227–245.
5. Antelman, S. M. and A. R. Caggiula. Norepinephrine-dopamine interactions and behavior. *Science* **195:** 646–653, 1977.
6. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J Pharmacol Exp Ther* **174:** 413–420, 1970.
7. Breese, G. R. and T. D. Traylor. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *Br J Pharmacol* **42:** 88–99, 1971.
8. Cooper, B. R., T. J. Hester and R. A. Maxwell. Behavioral and biochemical effects of the antidepressant bupropion (Wellbutrin): Evidence for selective blockade of dopamine uptake *in vivo*. *J Pharmacol Exp Ther* **215:** 127–134, 1980.
9. Coscina, D. V., C. Rosenblum-Blinick, D. D. Godse and H. C. Stancer. Consummatory behaviors of hypothalamic hyperphagic rats after central injection of 6-hydroxydopamine. *Pharmacol Biochem Behav* **1:** 629–642, 1973.
10. Coscina, D. V. and J. N. Nobrega. 6-Hydroxydopamine-induced blockade of hypothalamic obesity: Critical role of brain dopamine-norephinephrine interaction. *Prog Neuropsychopharmacol Biol Psychiatry* **6:** 369–372, 1982.
11. Coscina, D. V., J. N. Nobrega and J. J. Warsh. Brain DA depletion blockade of hypothalamic obesity does not occur with simultaneous brain NE depletion. *Soc Neurosci Abstr* **8:** 712, 1982.
12. Ferris, R. M., R. A. Maxwell, B. R. Cooper and F. E. Soroko. Neurochemical and neuropharmacological investigations into the mechanisms of action of bupropion HCl—A new atypical antidepressant agent. *Adv Biochem Psychopharmacol* **31:** 277–286, 1982.
13. Fibiger, H. C., A. P. Zis and E. G. McGeer. Feeding and drinking deficits after 6-hydroxydopamine administration in the rat: Similarities to the lateral hypothalamic syndrome. *Brain Res* **55:** 135–148, 1973.
14. Hollister, A. S., G. R. Breese and B. R. Cooper. Comparison of tyrosine hydroxylase and dopamine-beta-hydroxylase inhibition with the effects of various 6-hydroxydopamine treatments on d-amphetamine induced motor activity. *Psychopharmacologia* **36:** 1–16, 1974.
15. Leibowitz, S. F. Neurochemical systems of the hypothalamus. Control of feeding and drinking behavior and water-electrolyte excretion. In: eds., *Handbook of the Hypothalamus*, vol. 3, Part A, edited by P. J. Morgane and J. Panksepp, New York: M. Dekker, 1980, pp. 299–437.
16. Lenard, L. and Z. Hahn. Amygdalar noradrenergic and dopaminergic mechanisms in the regulation of hunger and thirst-motivated behavior. *Brain Res* **233:** 115–132, 1982.
17. Lenard, L., Z. Hahn and Z. Karadi. Body weight changes after neurochemical manipulations of lateral amygdala: noradrenergic and dopaminergic mechanisms. *Brain Res* **249:** 95–101, 1982.
18. Lovett, D. and G. Singer. Ventricular modification of drinking and eating behavior. *Physiol Behav* **6:** 23–26, 1971.
19. Martin, G. E. and R. D. Myers. Evoked release of [^{14}C]norepinephrine from the rat hypothalamus during feeding. *Am J Physiol* **229:** 1547–1555, 1975.

20. Richardson, J. S. and D. M. Jacobowitz. Depletion of norepinephrine by intraventricular injection of 6-hydroxydopa: A biochemical, histochemical and behavioral study in rats. *Brain Res* **58:** 117–133, 1973.
21. Rossi, J., A. J. Zolovick, R. F. Davies and J. Panksepp. The role of norepinephrine in feeding behavior. *Neurosci Biobehav Rev* **6:** 195–204, 1982.
22. Rowland, N., J. F. Marshall, S. M. Antelman and D. J. Edwards. Hypothalamic hyperphagia prevented by damage to brain dopamine-containing neurons. *Physiol Behav* **22:** 635–640, 1979.
23. Rowland, N. and E. M. Stricker. Effects of dopamine-depleting brain lesions on experimental hyperphagia in rats. *Physiol Behav* **28:** 271–277, 1982.
24. Skinner, J. E. *Neuroscience: A Laboratory Manual.* Toronto: B. Saunders, 1971.
25. Ungerstedt, U. Aphagia and adipsia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. *Acta Physiol Scand [Suppl]* **367:** 95–122, 1971.
26. Warsh, J. J., A Chiu and D. D. Godse. Simultaneous determination of norepinephrine, dopamine and serotonin in rat-brain regions by ion-pair liquid chromatography on octyl silane columns and amperometric detection. *J Chromatogr* **228:** 131–141, 1982.
27. Zigmond, M. J. and E. M. Stricker. Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats. *Science* **177:** 1211–1214, 1972.