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Destruction of Noradrenergic Innervation to the Paraventricular Nucleus: Deficits in Food Intake, Macronutrient Selection, and Compensatory Eating After Food Deprivation

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SHOR-POSNER, G., A. P. AZAR, M. JHANWAR-UNIYAL, R. FILART AND S. F. LEIBOWITZ. *Destruction of noradrenergic innervation to the paraventricular nucleus: Deficits in food intake, macronutrient selection and compensatory eating after food deprivation.* PHARMACOL BIOCHEM BEHAV **25**(2) 381–392, 1986.—Norepinephrine (NE) injected into the paraventricular nucleus (PVN) has a stimulatory effect on feeding behavior and is found to selectively enhance preference for carbohydrate in the rat. The present experiments were conducted to assess the impact of chronic depletion of NE within the PVN on food intake and appetite regulation. The catecholamine (CA) neurotoxin, 6-hydroxydopamine (6-OHDA), when administered into the PVN, produced a significant depletion of PVN NE in association with a variety of behavioral changes. The immediate consequence of the neurotoxin lesion was a dramatic increase in 24-hr food intake, attributed predominantly to a preferential increase in carbohydrate and fat consumption. The long-term effects related to CA depletion were a deficit in daily food consumption, particularly of carbohydrate (−42%). Although animals with diminished PVN NE maintained a normal diurnal feeding pattern, they failed to exhibit the increased ingestion of an energy-rich carbohydrate diet which rats normally show during the dark period of the diurnal cycle. Rats injected with 6-OHDA directly into the PVN exhibited a normal response to glucoprivic challenge, but demonstrated a deficit in their ability to produce compensatory feeding, particularly of carbohydrate and fat, in response to food deprivation. These findings suggest a specific function for PVN noradrenergic mechanisms in normal energy repletion when body energy stores are reduced.

Hypothalamus Paraventricular nucleus 6-Hydroxydopamine Deprivation Glucoprivic feeding
Norepinephrine Appetite regulation Feeding behavior

IT is well documented that the paraventricular nucleus (PVN) has an important function in the regulation of feeding. For example, discrete bilateral lesions of the PVN cause hyperphagia and obesity [2, 26, 43, 44]. In addition, when administered into this area, the α-adrenergic agonist norepinephrine (NE) induces feeding in satiated animals at nearly physiological doses [22] and, when chronically injected, potentiates daily food intake and body weight gain [30,33]. The importance of noradrenergic mechanisms in feeding behavior is supported by biochemical evidence associating enhanced medial hypothalamic NE release with the onset of natural feeding responses [34, 47, 51].

If endogenous NE specifically in the PVN is physiologically active in the regulation of normal feeding behavior, then the destruction of PVN noradrenergic innervation, through 6-hydroxydopamine (6-OHDA) injection, should produce deficits in food intake and body weight. A few studies conducted to date have tested the impact of 6-OHDA injected into the lateral hypothalamus and have shown this manipulation to cause alterations in consummatory behavior. This effect has been attributed to the destruction of catecholamine projections from the midbrain to the neostriatum [38, 45, 50, 54], as well as to the build-up of catecholamines in the lateral hypothalamus proximal to the neurotoxin injection site [52]. There appears to be little evidence concerning the behavioral impact of medial hypothalamic 6-OHDA injection, which is the focus of the present investigation. With administration of 6-OHDA directly into the PVN, we have found [3] that animals exhibit specific disturbances in feeding patterns and nutrient selection, in association with lowered levels of NE in the PVN.

METHOD

Subjects

Experiments were carried out on 105 male Sprague-

Dawley rats weighing 350–400 g at the start of the study. Animals were housed individually, tested in their home cages, and maintained on a constant 12:12 hour light-dark cycle (lights on 0700 hr).

Diet

Freely-feeding animals were permitted to select their food from pure macronutrient diets of protein, carbohydrate and fat, provided simultaneously in three separate glass food cups. The protein diet (3.7 kcal/g) was composed of casein (National Casein Co.) plus 4% minerals (USP XIV Salt Mixture, ICN Pharmaceuticals), 2.97% vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals) and 0.03% cysteine (L-Cysteine hydrochloride, ICN Pharmaceuticals). The carbohydrate diet (3.7 kcal/g) was composed of 37% sucrose, 28% dextrin (ICN Pharmaceuticals), 28% cornstarch, 4% minerals, 2.97% vitamins and 0.03% cysteine. The fat component (7.7 kcal/g) consisted of lard (Armour), mixed with 8% minerals, 5.92% vitamins and 0.08% cysteine. The placement of the food cups within the cage was changed daily to prevent position preference. Water was available ad lib through a glass drinking bottle at the front of the cage.

Surgery

Prior to surgery, animals were permitted to adapt to the macronutrient diets and laboratory conditions for 2–4 weeks. Each rat was stereotaxically implanted under pentobarbital anesthesia and noradrenergic depletion produced by injection of the catecholamine (CA) neurotoxin, 6-hydroxydopamine hydrobromide (6-OHDA, Sigma Chemical), into the PVN (n=44). Immediately before injection, the drug was dissolved in ice-cold sterile physiological saline, containing 0.2–0.4 mg/ml ascorbic acid. A dose of 8 μg of 6-OHDA (calculated as free base)/1.5 μl/side was delivered bilaterally into the PVN. The drug was administered over a 4 minute period at a rate of 0.1 μl/15 sec, through a cannula made from 25-gauge hypodermic tubing with a wire insert for the top. To allow for dispersion of the 6-OHDA, the injector remained in the brain for 2 additional minutes before being removed. An equal volume of the saline-ascorbic acid vehicle was injected into rats of the control group (n=38). The stereotaxic coordinates used were: −0.2 mm anterior to bregma, ±0.4 mm lateral to the midline, and 7.9 mm beneath the surface of the skull, with the top of the incisor bar 3.1 mm above the center of the interaural bars.

Postoperative Measurements

Daily measurements of food intake and diet selection were recorded for some of the rats (n=9 ascorbic acid, n=12 6-OHDA) during the first 2 weeks following surgery. Body weights were taken twice a week. Diurnal feeding patterns were assessed during the third post-operative week (n=19 ascorbic acid, n=16 6-OHDA) by recording food intake measurements twice daily at 0700 and 1900 hr.

Drug Manipulations

During post-operative weeks 4–6, the effects of the glucoprivic agents, 2-deoxy-D-glucose (2-DG) and insulin (INS), on total food intake and diet selection, were tested in 6-OHDA injected rats with marked CA depletion in the PVN (n=14) and ascorbic acid control rats (n=14). All tests were conducted during the daytime (1200–1700 hr), following a 1-hr satiation period with fresh food. At the end of this pretest satiation period, the animals were injected with 2-DG (200 mg/kg) dissolved in sterile physiological saline, or just the saline vehicle alone. This moderate dose of 2-DG was chosen to avoid side effects that may occur with higher doses. The rats received at least 4 tests (2 vehicle, 2 drug), administered in counterbalanced order on separate days. Food intake and diet selection were measured 4 hr after injection, and all scores reported in the Results section represent the average of at least 2 test scores. A test similar to the 2-DG test was conducted with a moderate dose of regular Iletin insulin (Lilly 7 U/kg), except that injections were given subcutaneously. A group of 12 ascorbic acid control rats (4 used in 2-DG test), and 17 6-OHDA animals with marked CA depletion (7 used in 2-DG) were tested with insulin.

Food Deprivation

The impact of the 6-OHDA lesion on compensatory feeding following 24-hr food deprivation was assessed in this experiment. After a 5-day period, during which both food and water were available ad lib and baseline intake scores were obtained, rats (n=18 ascorbic acid, n=16 6-OHDA) were subjected to intermittent 24-hr deprivation. This schedule, carried out over a 10-day period during the 12th to 15th post-operative weeks, alternated 24 hr of food deprivation with 24 hr of food availability. Food intake and diet selection patterns were recorded for the 24-hr periods of food availability and were compared with the baseline feeding scores obtained under freely-feeding conditions. Body weight measurements for the ascorbic acid (n=8) and 6-OHDA rats (n=16) were also taken several times a week during this period as well as during the refeeding week following the food deprivation experiment.

Histochemical Analysis

All animals were confirmed for the extent of their lesion through fluorescence histochemistry. Histochemical procedures to visualize brain CA innervation were carried out according to the Falck-Hillarp fluorescence technique [13]. Under ether anesthesia, the rats were sacrificed and their brains rapidly removed, frozen in liquid propane followed by liquid nitrogen, and then freeze-dried in a vacuum. The freeze-dried brains were exposed to formaldehyde gas and embedded in paraffin, and thin sections through the diencephalon and forebrain were cut to 10 μm. Several hypothalamic areas were scored for depletion of fluorescent varicosities, using a semi-quantitative method based on a rating

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FIG. 1. Fluorescence photomicrographs showing catecholamine varicosities in the hypothalamus. (a) Normal CA innervation of the PVN of an ascorbic acid vehicle-injected rat. (b,d) Similar sections of 2 rats injected with 6-OHDA into the PVN, showing a marked reduction of the CA varicosities. (c) Apparently normal CA fluorescence in the zona incerta of a PVN 6-OHDA injected rat that exhibited a marked CA depletion in the PVN. Other hypothalamic areas also appeared normal, such as the caudal dorsomedial nucleus of a control (e) and 6-OHDA (f) rat, and the caudal perifornical area (level of the ventromedial nucleus), as shown by the control (g) and neurotoxin-injected (h) animal.

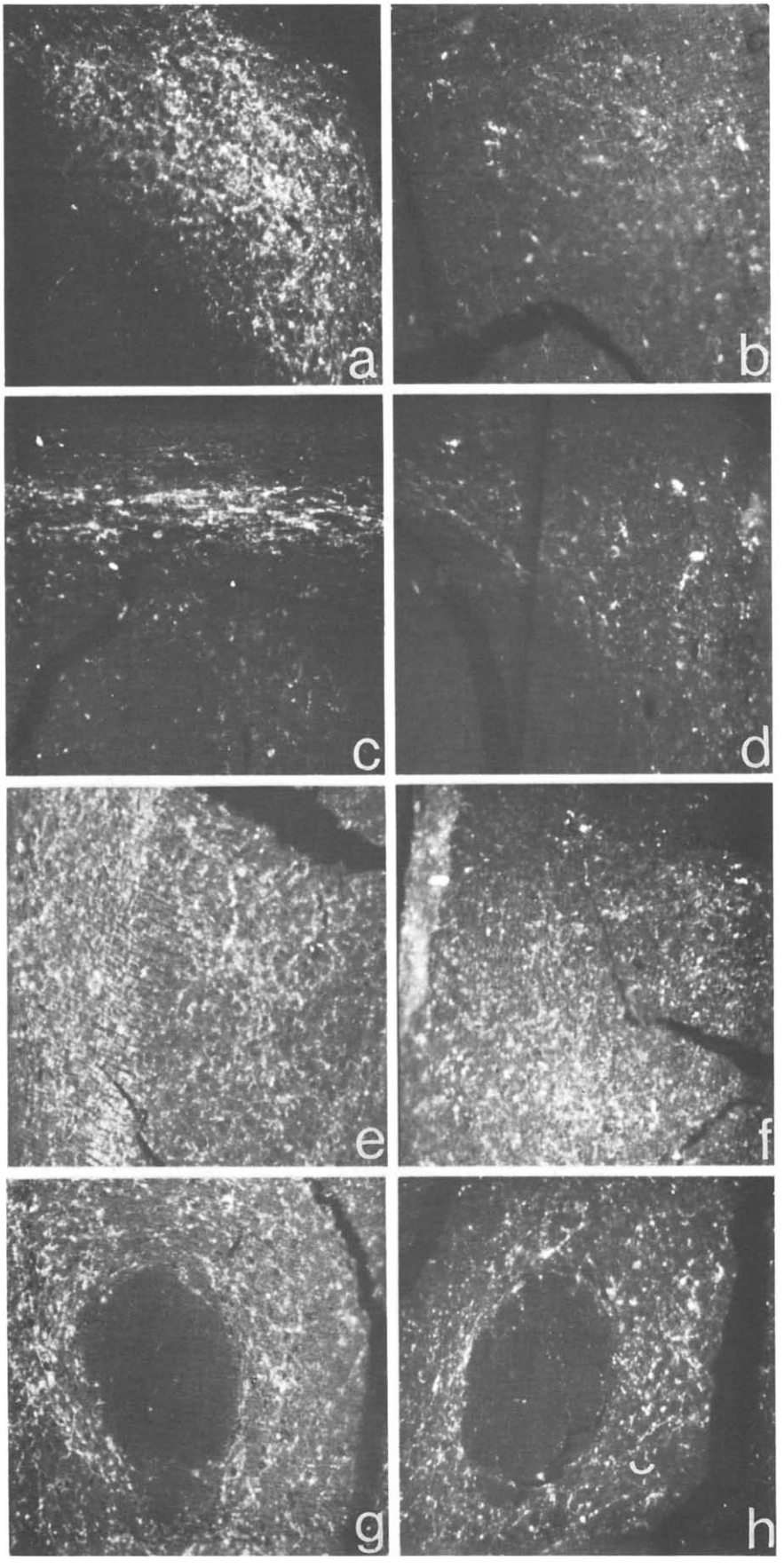
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TABLE 1

EFFECT OF 6-HYDROXYDOPAMINE INJECTED INTO THE PARAVENTRICULAR NUCLEUS ON BRAIN CATECHOLAMINE LEVELS ($\mu g/\mu g$ PROTEIN) AND FLUORESCENCE INTENSITY (RATINGS OF 1–5)

Area	Group	Norepinephrine	Dopamine	Epinephrine	CA Fluorescence
Medial Preoptic Area	Ascorbic Acid	16.3 ± 2.3	1.3 ± 0.2	0.6 ± 0.1	4.0 ± 0.1
	6-OHDA	8.0 ± 1.0	1.5 ± 0.3	0.6 ± 0.1	3.0 ± 0.1
	% change	−51*	+15	0	−25†
Medial Forebrain Bundle	Ascorbic Acid	10.2 ± 0.7	2.6 ± 0.5	0.5 ± 0.1	4.0 ± 0.0
	6-OHDA	5.0 ± 0.4	1.6 ± 0.2	0.3 ± 0.1	4.0 ± 0.0
	% change	−51†	−38	−40	0
Perifornical Hypothalamus	Ascorbic Acid	19.1 ± 1.4	3.1 ± 0.6	0.9 ± 0.2	4.1 ± 0.1
	6-OHDA	9.5 ± 0.8	2.3 ± 0.5	0.8 ± 0.2	4.0 ± 0.0
	% change	−50†	−26	−11	−2
Arcuate Nucleus	Ascorbic Acid				4.0 ± 0.1
	6-OHDA				2.3 ± 0.1
	% change				−43†
Paraventricular Nucleus	Ascorbic Acid	32.0 ± 2.7	3.9 ± 0.5	1.5 ± 0.3	4.3 ± 0.1
	6-OHDA	8.1 ± 1.1	1.7 ± 0.3	0.8 ± 0.2	1.9 ± 0.1
	% change	−75†	−56†	−47	−56†
Periventricular Area	Ascorbic Acid				4.0 ± 0.1
	6-OHDA				2.1 ± 0.1
	% change				−48†
Zona Incerta	Ascorbic Acid				3.9 ± 0.2
	6-OHDA				4.0 ± 0.0
	% change				+3
Dorsomedial Nucleus Anterior	Ascorbic Acid	14.6 ± 1.4	1.8 ± 0.2	0.8 ± 0.1	4.2 ± 0.1
	6-OHDA	9.2 ± 0.9	1.7 ± 0.3	0.7 ± 0.1	2.5 ± 0.1
	% change	−37*	−6	−13	−40†
Posterior					4.1 ± 0.1
					3.6 ± 0.1
					−12*

Values are means ± SEM, based on 10–15 ascorbic acid and 6-OHDA animals for biochemical analyses and n = 17 ascorbic acid and 36 6-OHDA for fluorescence analysis. Values for % change (i.e., the change in CA innervation after 6-OHDA lesion as compared with ascorbic acid rats) are given with the results of statistical analyses (*$p<0.01$, †$p<0.001$).

scale of 1–5 (1 indicating near total lack of fluorescence and 5 indicating high-normal fluorescence), modified after Fuxe [14]. Each area was separately rated by making side-by-side comparisons between ascorbic acid and 6-OHDA injected rats. Photographs of several hypothalamic areas, including the periventricular region, zona incerta, paraventricular and dorsomedial nuclei and the lateral perifornical region, were taken for direct comparisons between 6-OHDA lesion and sham-operated animals. Animals which showed moderate to marked CA depletion, with ratings less than 3, are included in the Results Section described below. A few rats (n=4) showed little or no CA depletion and were excluded from the analysis.

Biochemical Analysis

In order to determine the specificity of the neurotoxin lesion, an additional group of animals (n=15 ascorbic acid, n=12 6-OHDA) were prepared for biochemical analysis 90–120 days after surgery, comparable to the post-surgery time used for the histochemical analysis. Animals were sacrificed by decapitation, and their brains were rapidly removed and frozen on dry ice. Serial sections of 300 μm were cut in a cyrostat and tissue samples microdissected according to the method described by Palkovits [39]. Five hypothalamic nuclei, namely, the PVN, medial preoptic area, dorsomedial nucleus, medial forebrain bundle (at the level of the PVN), and lateral perifornical hypothalamus (at the level of the ventromedial nucleus), were examined and their placement verified via inspection of the brain sections. Norepinephrine, epinephrine and dopamine were measured by high performance liquid chromatography separation using electrochemical detection as described by Levin *et al.* [32].

Statistical Analysis

Results were evaluated by 2-way analysis of variance (ANOVA) for repeated measures, followed by appropriate tests for individual comparisons. Subsequent to these procedures, further analysis of macronutrient preference was determined for food intake after glucoprivic drug administration and following food deprivation; difference scores were calculated and evaluated using single factor ANOVA for repeated measures followed by appropriate post-hoc tests for

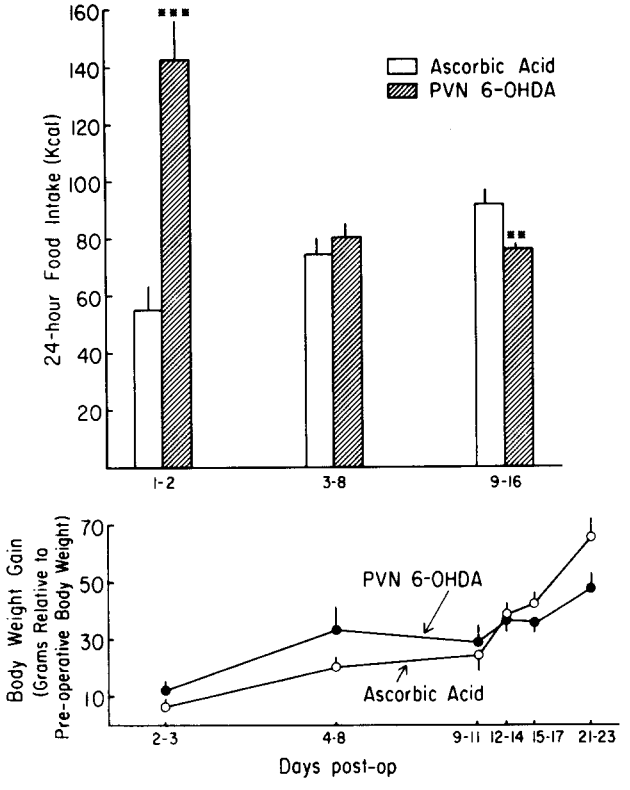

FIG. 2. Animals injected with 6-OHDA into the PVN first demonstrate a dramatic enhancement (***$p<0.001$) in daily food intake that is followed by a period of stability and subsequently, a significant reduction (15–20%) in food intake (**$p<0.05$). A similar trend is shown for body weight gain.

individual mean comparisons [53]. A two-tailed Student's *t*-test for dependent samples (within group) was used to compare relative intakes (percentage scores) and independent samples (between lesion and control group) were compared for measurements of histochemistry, food intake, and body weight gain. *p*-Values exceeding 0.05 were regarded as not significant.

RESULTS

Histochemical Analysis

Histochemical analysis of the ascorbic acid and 6-OHDA rats failed to reveal any neurotoxin-induced change in extra-hypothalamic CA fluorescence observed in the striatum, stria terminalis, caudate and median eminence. Within the hypothalamus, as shown in the photomicrographs (Fig. 1) and Table 1, the damage to the CA innervation was concentrated in the medial aspect of this structure. While several areas were affected in this region, damage appeared to be greatest in the PVN and periventricular area. This effect in the PVN can be seen by comparing an animal injected with ascorbic acid (Fig. 1a) with rats injected with 6-OHDA (Figs. 1b,d). In this nucleus, an average rating of 4.3±0.1 was obtained for the ascorbic acid animals as compared to 1.9±0.1 for the 6-OHDA rats ($p<0.001$). Damage from this lesion clearly extended into the medial preoptic area and the anterior portion of the dorsomedial nucleus. However, CA varicosities within the zona incerta (Fig. 1c, reflecting an average 6-OHDA rating of 4.0±0.0 as compared to 3.9±0.2 for the ascorbic acid rats) and the lateral hypothalamus (medial forebrain bundle area) just lateral to the PVN appeared essentially intact. In contrast to the marked PVN depletion, the dorsomedial nucleus (compare Fig. 1e, average ascorbic acid group rating of 4.1±0.1 versus Fig. 1f, average 6-OHDA group rating of 3.6±0.1), except for its most rostral extent, and the more caudal perifornical area just lateral to the dorsomedial nucleus (Fig. 1g, reflecting average group rating of 4.1±0.1 for ascorbic acid rats versus Fig. 1h, reflecting average 6-OHDA group rating of 4.0±0.0) appeared to retain essentially normal CA fluorescence in 6-OHDA injected animals.

Biochemical Analysis

Biochemical analyses of the neurotoxin-lesion effects are summarized, along with the histochemical results, in Table 1. As indicated in this table, the greatest depletion of endogenous CA was observed in the PVN, consistent with the histochemical findings. Injection of 6-OHDA decreased NE levels in the PVN by 75% compared to the control group, in addition to producing smaller reductions of PVN dopamine (−56%) and epinephrine (−47%). A more complete analysis revealed that other hypothalamic areas, including the nearby medial preoptic area, dorsomedial nucleus and perifornical hypothalamus, retained close to normal dopamine and epinephrine levels, thus substantiating the relatively localized nature of this neurotoxin lesion as indicated by fluorescence histochemistry. In contrast to the strong depletion of NE in the PVN (−75%), a more moderate decline of NE content (−37% to 50%) was observed in the surrounding hypothalamic areas. This damage to NE-containing neurons in surrounding areas was, however, in each case significantly smaller ($p<0.01$) than that observed in the PVN.

Daily Food Intake

The immediate consequence of PVN 6-OHDA injection was a profound enhancement of daily food intake. The total food intake and body weight of the 6-OHDA lesion rats (89.0±2.5 kcal and 472.5±11.0 g) and ascorbic acid control animals (89.3±3.6 kcal and 471.4±8.3 g) were similar prior to surgery. During the first 48 hr after surgery, however, the neurotoxin lesion produced a dramatic increase in 24-hr food intake in the majority of 6-OHDA lesion rats (n=8), relative to (n=9) ascorbic acid animals, $F(1,15=37.07, p<0.001$. As represented in Fig. 2, the total daily calorie intake for animals injected with the neurotoxin was increased 156% above control baseline feeding. This hyperphagic effect lasted for 2–3 days and was associated with a gain in body weight (4.8 g/day lesion vs. 2.9 g/day ascorbic acid), which due to variability of the scores did not reach statistical significance. The initial feeding enhancement produced by 6-OHDA was followed by a few days of stability while body weights returned to normal and then a significant reduction in food intake (Fig. 2). Measurements during the next 2–3 weeks revealed a significant feeding suppression in the 6-OHDA-injected animals (−15–20%), accompanied by a small decrease in body weight below ascorbic acid rats ($p>0.05$).

Diet Selection

The neurotoxin-lesioned rats displayed a distinct modifi-

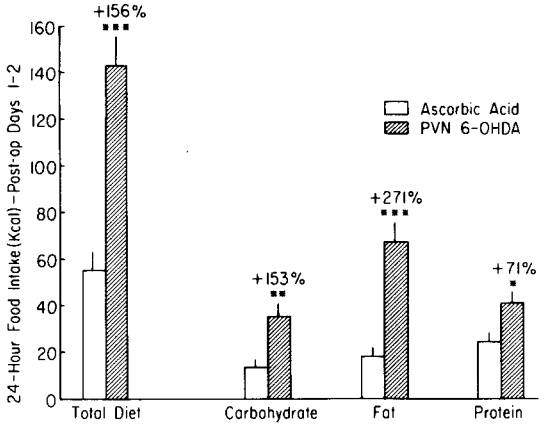

FIG. 3. Analysis of food intake and diet patterns during the immediate post-operative period (days 1–2) reveals that 6-OHDA lesion rats, relative to ascorbic acid control animals, exhibit a strong preference for carbohydrate (**$p<0.01$) and fat (***$p<0.001$) and a modest potentiation of protein intake (*$p<0.05$).

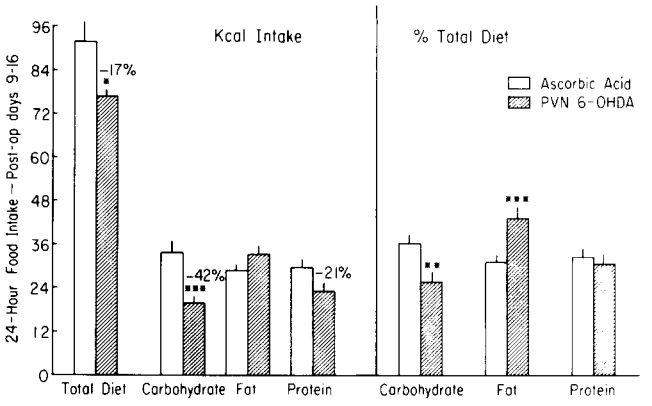

FIG. 4. Neurotoxin-lesion rats, compared to ascorbic acid control animals, demonstrate a significant decrease in total diet intake (*$p<0.05$), associated with a selective reduction in carbohydrate ingestion for measures of kcal intake (***$p<0.01$). This effect is also shown in the relative intake (the percentage of total calories consumed) of each macronutrient. Percent carbohydrate consumption is decreased in 6-OHDA rats (**$p<0.025$), while the percent of total calories consumed as fat is increased (***$p<0.01$); percent protein, however, remained unaffected in these rats.

cation in macronutrient selection, relative to the feeding of ascorbic acid control rats, $F(2,30)=7.80, p<0.002$. As shown in Fig. 3, the initial hyperphagia observed during the first few days after PVN 6-OHDA injection (Fig. 2) was associated with a large enhancement of fat (+271%) and carbohydrate (153%) ingestion along with a smaller enhancement of protein intake (+71%).

This initial increase in carbohydrate intake stands in contrast to the subsequently reduced carbohydrate consumption exhibited by the 6-OHDA rats during the following several weeks. As illustrated in Fig. 4, analyses during days 9–16, after the initial hyperphagia had receded and the long-term hypophagia had developed, $F(1,15)=6.32, p<0.024$, revealed a reliable and selective decrease in carbohydrate intake (−42%), relative to control rats. Representation of the raw score data, in terms of the relative intake of protein, fat and carbohydrate (i.e., the *percentage* of total calories consumed of each macronutrient), showed even more clearly this effect of PVN 6-OHDA injection on diet selection. As indicated on the right in Fig. 4 (% Total Diet), carbohydrate consumption in the ascorbic acid rats accounted for 36% of their total intake, as compared to only 25% of the 6-OHDA rats' total diet. In contrast to this significant decrease in carbohydrate intake, the percent fat consumption was reliably increased and percent protein unaffected in the 6-OHDA rats.

Diurnal Pattern

Analyses of food intake and patterns of daytime/nighttime feeding (during post-operative weeks 2–3) indicated a normal diurnal pattern of feeding in the ascorbic acid control rats, with approximately 84% of their caloric intake consumed at night. A significant day-night shift in macronutrient selection was revealed in this control group. As indicated in Table 2, meals consumed during the daytime, when only a small amount of ingestion was observed, were composed of a significantly higher proportion of fat (48%), as compared to protein (27%) and carbohydrate (25%). Nighttime feeding, in contrast, was associated with a reliable and preferential increase (to 35%) in proportion of carbohydrate consumed and a significant decrease (to 33%) in the proportion of fat ingested. Percent protein was unchanged.

Whereas it was found that PVN 6-OHDA injected animals were able to maintain a diurnal pattern of feeding, certain changes, relative to the ascorbic acid animals, were detected in their feeding behavior, most particularly at night (Table 2). During the dark phase, in contrast to the light, the 6-OHDA lesion rats consumed significantly fewer calories than did the ascorbic acid control rats ($p<0.01$). This feeding suppression was attributed to particular deficits in carbohydrate (−19%) and protein (−29%) ingestion, which explain the significant decrease in 24-hr total caloric intake observed in 6-OHDA lesion rats. Moreover, although these rats with neurochemical lesions were able to exhibit a nighttime shift in percent nutrient selection, with a significant decrease in the proportion of fat ingested as described previously for ascorbic acid control rats, they failed to demonstrate the significant increase in their proportion of carbohydrate selected in the night.

Glucoprivic Feeding

The impact of the PVN 6-OHDA lesion on food intake and diet selection induced by glucodynamic agents was assessed in the following analyses. As shown in Table 3, 2-DG (200 mg/kg) significantly increased total food intake in satiated control rats, $F(1,13)=99.7, p<0.001$. As described in previous studies [20,44], a differential effect on diet selection was associated with a preferential increase in consumption of carbohydrate, relative to fat and protein consumption, $F(1,26)=50.0$, $p<0.01$. This robust stimulatory effect on carbohydrate ingestion significantly increased the relative intake of carbohydrate in total diet from a baseline average of 21% up to 57%. While a smaller but reliable increase in protein intake over baseline feeding was also revealed, the

TABLE 2

DIURNAL RHYTHM OF FOOD INTAKE (Kcal) IN ASCORBIC ACID CONTROL AND 6-HYDROXYDOPAMINE PVN-LESION RATS

	24-hr	Day				Night			
	Total	Total	CARB	Fat	PROT	Total	CARB	Fat	PROT
Ascorbic Acid (N = 19)	97.2 ±3.2	15.6 ±1.4	4.1 ±0.9	7.4 ±0.9	4.0 ±0.5	82.8 ±3.0	28.6 ±1.4	27.2 ±1.4	27.1 ±1.9
	% TOTAL		25.0 ±3.8	48.2 ±3.9	26.9 ± 3.0		35.1 ±1.9	32.6 ±1.9	32.3 ±1.6
6-OHDA (N = 16)	81.1 ±2.0*	13.2 ±1.0	3.6 ±0.6	6.3 ±0.9	3.3 ±0.6	68.0 ±2.4*	23.3 ±1.7+	25.5 ±2.3	19.2 ±1.5*
	% TOTAL		27.1 ±4.2	48.2 ±4.9	24.7 ±3.3		34.6 ±2.4	37.0 ±2.5	28.1 ±2.1

Given are mean (± SEM) daytime and nighttime Kcal intake. The relative intakes of protein (PROT), fat, and carbohydrate (CARB) are also expressed as the percentage of total calories consumed for each macronutrient. Specific comparisons (between lesion and control groups) are significant at *$p<0.01$ and †$p<0.05$. The number of animals used is indicated in parentheses.

TABLE 3

FOOD INTAKE (Kcal) IN RESPONSE TO PERIPHERAL 2-DEOXY-D-GLUCOSE AND INSULIN INJECTION IN ASCORBIC ACID CONTROL AND 6-OHDA PVN LESION RATS

	Total	CARB	Fat	Protein	Total	CARB	Fat	Protein
	SALINE				2-DG			
Ascorbic Acid (N = 16)	3.0 ±0.6	0.7 ±0.3	1.6 ±0.4	0.9 ±0.2	15.6 ±0.8*	8.7 ±0.7*	2.5 ±0.4	4.4 ±0.3*
6-OHDA (N = 14)	4.5 ±0.7	2.5 ±0.6†	1.2 ±0.3	0.8 ±0.3	14.1 ±1.6*	8.3 ±1.4*	2.2 ±0.4*	3.9 ±0.3†
	SALINE				INSULIN			
Ascorbic Acid (N = 12)	8.9 ±1.0	3.6 ±0.7	2.6 ±0.4	2.7 ±0.4	18.0 ±1.0*	9.5 ±0.8*	4.0 ±0.5*	4.4 ±0.5*
6-OHDA (N = 17)	6.7 ±0.9	2.3 ±0.5	1.9 ±0.4	2.4 ±0.5	16.2 ±1.2*	8.7 ±0.6*	3.4 ±0.6†	4.0 ±0.6†

Results for each group are expressed as mean Kcal ± SEM. The number of animals used is indicated in parentheses. CARB = carbohydrates.

Comparisons between saline and drug scores yielded significant differences at *$p<0.01$ and †$p<0.05$. Comparisons between the lesion and control group scores yielded significant differences † in a saline score.

percent concentration of this macronutrient was not altered. Fat ingestion was unchanged by 2-DG administration.

Neurotoxin lesion of the PVN appeared to have no significant impact on 2-DG induced feeding (Table 3). Rats injected with 6-OHDA exhibited a comparable increase in total diet, $F(1,13)=33.05$, $p<0.001$, and a similar alteration in diet selection. Analysis of difference scores indicated that the stimulatory effect of 2-DG on carbohydrate intake was reliably greater than the effect of this drug on protein and fat consumption, $F(1,26)=12.8$, $p<0.01$.

Similar to the glucoprivation induced by 2-DG, injection of INS (7 U/kg) stimulated feeding behavior in ascorbic acid control rats (Table 3). The drug-induced enhancement of total diet intake, $F(1,11)=76.4$, $p<0.001$, produced a preferential increase in carbohydrate consumption, $F(1,22)=52.2$, $p<0.01$, that consequently altered the percent concentration

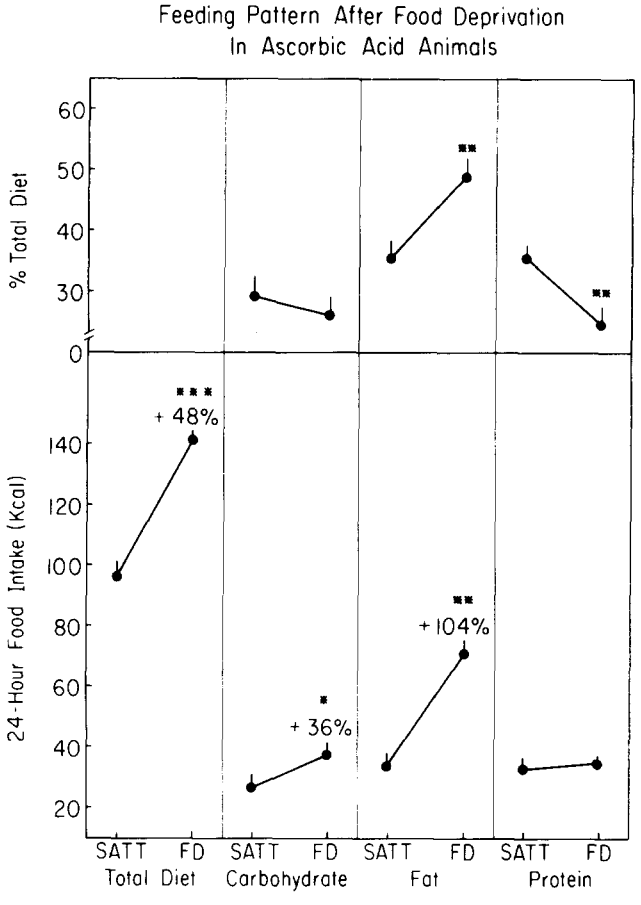

FIG. 5. Feeding pattern after food deprivation in ascorbic acid animals. Analysis of the rats' diet selection pattern after 24-hr food deprivation indicates that control animals show a robust compensatory feeding response (***$p<0.001$) associated with a selective increase in fat (**$p<0.01$) and significantly more carbohydrate (*$p<0.05$) than protein intake. This trend is also reflected in the relative intake, the percentage of total calories consumed, for each diet. Whereas the percentage of fat consumed is significantly increased (**$p<0.01$) and carbohydrate unaltered after deprivation, the percent protein is dramatically reduced (**$p<0.01$).

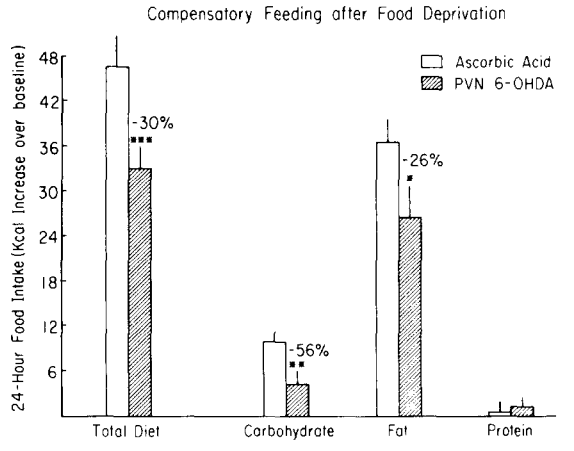

FIG. 6. Compensatory feeding after food deprivation. Compared to ascorbic acid control rats, animals with PVN 6-OHDA lesions exhibit a disturbance in their ability to respond to 24-hr food deprivation. The neurotoxin lesion reduced compensatory feeding of total diet (deprivation-induced eating increase over ad lib baseline scores) by 30% (***$p<0.01$). This deficiency was associated with a large reduction in compensatory carbohydrate feeding (**$p<0.025$) and a smaller deficit in fat intake (*$p<0.05$).

of carbohydrate in total diet from a baseline average of 40% up to 53%. Insulin also produced a reliable increase in the consumption of protein and fat; these effects were smaller than the carbohydrate enhancement, however, and the relative intake of these nutrients, in fact, decreased significantly from 31% to 23% for fat and from 30% to 24% ($p>0.05$) for protein intake.

As shown in Table 3, INS injected into 6-OHDA rats significantly potentiated total diet intake. Similar to the results obtained with ascorbic acid rats, a preferential increase in carbohydrate consumption, relative to fat and protein ingestion, $F(1,32)=46.8$, $p<0.01$, was observed in these neurotoxin-lesioned rats.

Food Deprivation

To determine the importance of the PVN CA innervation in permitting animals to compensate following food deprivation, we fasted animals for 24 hr and assessed their refeeding responses during the next 24-hr period. At this time (12 weeks) post-operative, 24-hr food intake under ad lib baseline conditions was somewhat, but not significantly, reduced in the 6-OHDA lesion group (89.4±2.0 kcal) relative to the ascorbic acid group (95.9±3.6 kcal). Under conditions of food deprivation, however, the 6-OHDA fasted rats showed a clear deficit in their ability to compensate on days when food was provided.

When deprived of food for 24 hr, ascorbic acid control animals on refeeding days exhibited a 48% increase in total caloric intake relative to baseline ad lib feeding scores, $F(1,17)=160.2$, $p<0.001$. As shown in the lower part of Fig. 5 (Kcal intake), this compensatory feeding was associated with a preferential increase in consumption of fat, and significantly greater carbohydrate ingestion relative to protein intake. Representation of these Kcal data in terms of the relative intake of protein, fat and carbohydrate, shows a similar alteration in diet selection. As indicated in the top part of Fig. 5 (% Total Diet), ascorbic acid animals after deprivation significantly increased the percent of fat selected to 49.0±2.7%, maintained a constant proportion of carbohydrate (26.4±2.7%) and reliably reduced their percent protein intake to 24.6±1.0%.

Relative to ascorbic acid rats, the 6-OHDA rats after a 24-hr fast demonstrated a significant disturbance in their ability to respond to food deprivation. Although 6-OHDA rats significantly increased their caloric intake following 24-hr food deprivation, $F(1,15)=112.5$, $p<0.001$, this potentiation was clearly attenuated relative to that shown by ascorbic acid control rats (Fig. 5). Representation of the raw Kcal data in terms of compensatory feeding difference scores (i.e., deprivation-induced eating minus ad lib baseline scores) illustrates this effect most clearly in Fig. 6. Following 24-hr food deprivation, 6-OHDA lesioned animals increased their caloric intake by 32.0±3.0 kcal over their normal ad lib feeding scores. Compared to the 45.7±3.7 Kcal increase for the control rats, this represents a 30% reduction ($p<0.01$) in the compensatory feeding of total diet in PVN-lesion rats.

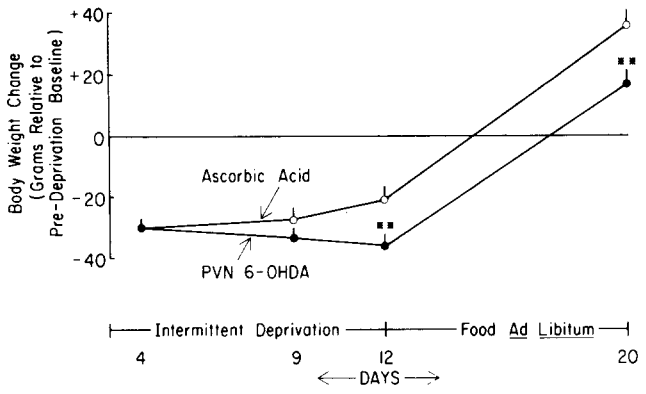

FIG. 7. The deficiency in compensatory feeding displayed by 6-OHDA lesion rats after 24-hr fasts is reflected in their greater loss of body weight, relative to ascorbic acid control animals (**$p<0.025$) and their slower body weight recovery.

Moreover, the compensatory feeding of carbohydrate was most particularly affected by this neurotoxin (−56%, $p<0.025$), as compared to the smaller deficit in fat intake (−26%, $p<0.05$) and no change in protein intake.

This deficiency in compensatory feeding exhibited by 6-OHDA lesion rats was reflected in their loss of body weight during this period (Fig. 7). Prior to deprivation, body weight was reliably different for the control and 6-OHDA lesion groups. During the first week of intermittent deprivation, both groups lost approximately 5% of their body weight. In subsequent days of deprivation, however, only the 6-OHDA-injected rats continued to show a significant loss in weight (−5.9%). Furthermore, during the week of ad lib food following the deprivation period (Fig. 7), the 6-OHDA animals demonstrated a slower body weight recovery (to only +17.5±4.6 g) compared to a +36.3±4.8 g weight gain for the control rats.

DISCUSSION

Neurotoxin damage to the catecholaminergic innervation of the PVN resulted in a pronounced reduction of endogenous norepinephrine (75%) and dopamine (56%) in this nucleus, along with significantly smaller or negligible neurochemical deficits in surrounding medial and lateral hypothalamic areas. This pronounced change in endogenous CA function was associated with specific disturbances in the feeding patterns of freely-feeding and food deprived rats. Initially, animals given bilateral PVN infusions of 6-OHDA demonstrated a profound enhancement of 24-hr food intake, associated with an increase in preference for energy-rich foods, as well as a gain in body weight. Chronic infusion of NE into the PVN has similarly been shown to potentiate daily food intake, particularly carbohydrate, and body weight gain in rats [28–30, 33]. Consistent with an earlier report [12], this suggests that the initial increase in food intake following 6-OHDA injection is most likely due to the neurotoxin-induced release of endogenous neurotransmitter.

Subsequent to this immediate effect of 6-OHDA, there developed the opposite pattern of feeding behavior associated with the disruption of noradrenergic innervation to the PVN. In the present experiment, animals with diminished PVN NE exhibited a reduction in daily food intake along with a selective decrease in carbohydrate ingestion. These effects were only moderate in size but were long-lasting, occurring over several weeks. The particular disturbance in carbohydrate regulation in animals with 6-OHDA PVN lesions is consistent with other evidence indicating that PVN NE mechanisms are involved in the modulation of carbohydrate appetite [28–30, 49]. In those studies with pure macronutrient diets, carbohydrate consumption was shown to be selectively enhanced after acute, as well as chronic injection of NE into the PVN. A similar preference for carbohydrate has also been seen with PVN and peripheral injection of the α 2-noradrenergic agonist clonidine, in addition to the tricyclic antidepressant drugs which are believed to act through the release of endogenous NE [24,30]. It may be noted that, under some conditions, particularly in rats which have a relatively strong baseline preference for fat, NE may be found to enhance fat consumption possibly in addition to carbohydrate intake [29]. Our results of the macronutrients, showing an alteration in fat intake in neurotoxin-lesioned rats, may possibly reflect this NE-induced change in fat intake. They may also reflect possible damage to other neurotransmitter or endocrine systems, which modulate intake of fat [7].

Analysis of diurnal rhythms of macronutrient selection revealed interesting patterns in ascorbic acid rats and specific disturbances in 6-OHDA lesion rats. It is clear from this work and previous studies [18, 21, 48], that rats have the ability to alter meal composition depending on the time of day. In the present investigation, ascorbic acid control rats exhibited a specific increase in the proportion of carbohydrate ingested during the dark period. This is in contrast to the small amount of carbohydrate intake observed during the minimal daytime feeding and is in accord with recent findings from this laboratory [48], that have established an initial preference for large carbohydrate meals during the early hours of the night in freely-feeding rats. These results with pure macronutrient diets are also in agreement with, and extend the findings of earlier studies [18,21], that have reported an increase in the proportion of non-protein energy consumed at night in rats provided with a choice of two diets containing either 60% or 0% protein.

Injection of 6-OHDA into the PVN specifically disturbed this feeding pattern by affecting nighttime food intake. Neurotoxin-lesioned rats displayed a deficit in total feeding during the active dark phase of the diurnal cycle and furthermore, failed to exhibit the significant increase in carbohydrate feeding that was shown by ascorbic acid control animals during this period. These findings suggest a specific function of PVN noradrenergic mechanisms in monitoring and replenishing energy stores during the nocturnal feeding period. This proposal is consistent with recent evidence demonstrating a sharp increase in PVN α 2-noradrenergic receptor sites [27], in addition to an increased sensitivity to α-noradrenergic receptor stimulation [6], specifically during the early hours of the dark period. Neurotoxin-injected rats also exhibited a reduction in nighttime protein consumption, suggesting that the lesion may have affected other neurochemical systems reported to be involved in the modulation of protein appetite [23]. It is of particular interest that the diurnal feeding rhythm was maintained in rats receiving injections of 6-OHDA directly into the PVN. This contrasts with observations of electrolytically lesioned PVN rats who exhibit a disruption in daytime feeding [2,44], suggesting that

the neurotoxin did not damage these processes involved in the generation of a daytime/nighttime feeding rhythm.

With regard to the effects of insulin and 2-deoxy-D-glucose, it has been suggested by numerous studies that brain CA systems are involved in mediating the stimulatory effect of these glucoprivic agents on food intake [5, 9, 10, 25, 36, 41]. As in previous reports with a self-selection feeding paradigm [19, 20, 44], our present findings demonstrate that these agents affect nutrient selection in a manner similar to that produced by PVN noradrenergic stimulation. In the ascorbic acid control animals injected with moderate doses of INS or 2-DG, we observed a robust enhancement of total food intake, associated with a dramatic and specific increase in carbohydrate consumption. This effect, however, was not diminished in animals with reduced CA innervation to the PVN, nor was it affected by electrolytic PVN lesions [44]. While other doses need to be tested before final conclusions can be drawn, our findings, together with the PVN electrolytic lesion result, argue that the PVN itself, in addition to the α-noradrenergic receptor system within it, is not critical for glucoprivic feeding to occur. This does not negate, however, the possibility that noradrenergic innervation of areas just outside the PVN, such as the periventricular nucleus or the dorsomedial nucleus [4], may be involved in the 2-DG and INS-induced feeding responses.

While the glucoprivic feeding tests produced essentially negative results, the deprivation tests distinguished the 6-OHDA rats as having specific deficits in feeding regulation under conditions involving rapid energy expenditure. As revealed in this investigation, and in previous studies using separate dietary components [1, 37, 40, 42], deprivation-induced feeding is associated with alterations in macronutrient feeding patterns. After 1 24-hr fast, ascorbic acid control rats in the present study compensated for this deficit by increasing both fat and carbohydrate intakes. This modification in macronutrient selection after 24 hr of deprivation confirms earlier findings of Piquard *et al.* [40], who demonstrated a similar deprivation-induced enhancement of carbohydrate and fat in rats that had been subjected to a considerably longer, 5-day period of starvation. The present results are also in agreement with earlier reports showing that food restriction specifically reduces protein intake [35,37].

The functional integrity of the PVN appears to be essential for normal compensatory eating of energy-rich nutrients. Deprived of food for 24 hr, animals with decreased CA innervation to the PVN, compared to control rats, demonstrated a 30% reduction in compensatory feeding of total diet (Fig. 5). Furthermore, this deficit was associated with decreased compensatory feeding particularly of carbohydrate, and to a lesser extent fat. This is in agreement with earlier studies reporting reduced compensatory food intake in food-deprived rats that received either intraventricular injections of 6-OHDA [11] or midbrain lesions of ascending catecholaminergic innervation to the diencephalon [25]. As shown in the present study, this deficiency was reflected in significantly greater loss of body weight in the PVN 6-OHDA rats during deprivation and a subsequent slower body weight gain during the recovery period [25]. The disturbance in compensatory feeding observed in these rats provides additional support for a regulatory function of the PVN α 2-noradrenergic system in normal energy repletion after food deprivation. Animals with PVN electrolytic damage exhibit a similar disruption in their ability to produce compensatory feeding of carbohydrate, in response to both short and long-term food deprivation [44]. Additional evidence for the involvement of endogenous PVN NE and α-2-receptors in carbohydrate repletion is provided by the findings that circulating glucose concentration and hypothalamic noradrenergic activity are closely related [46], that NE turnover within the medial hypothalamus is significantly increased in fasted animals [16,47], and that α-2-receptors in the PVN are dramatically down-regulated by food deprivation [17].

The findings of this study strongly implicate a role for PVN NE in the regulation of carbohydrate ingestion, particularly during the dark period of the diurnal cycle as well as in response to food deprivation. Although the 6-OHDA PVN lesion leaves diurnal feeding basically intact, nocturnal diet patterns and eating after food deprivation are clearly disrupted, in particular, with respect to the control of appetite for carbohydrate. The magnitude of these effects, small-to-moderate in size, may reflect the fact that noradrenergic innervation to the PVN was only partially destroyed and/or regenerated. It may also be due to some additional damage (significantly smaller than that in the PVN) sustained by CA systems in surrounding medial and lateral hypothalamic areas. In our interpretations of the data, we have focused on the PVN noradrenergic system, since it was most dramatically affected by this neurotoxin and has been most extensively studied. With greater and more anatomically focused depletion of NE, we would expect more pronounced effects to occur.

In addition to hypothalamic NE, there is evidence that NE in the amygdala may also be involved in the mediation of feeding behavior. Reduced CA innervation to the amygdala has recently been shown to produce disturbances in food consumption [31]. Furthermore, NE injection into this area is associated with modulation of food preferences [8] and potentiation of feeding in food-deprived animals [15]. Through further research, it will be interesting to determine the relationship between amygdalar noradrenergic innervation and the hypothalamic system in the control of food intake, macronutrient selection and eating patterns.

REFERENCES

1. Andik, I., S. Donohoffer, I. Moring and J. Szentes. The effect of starvation on intake and selection. *Acta Physiol Acad Sci Hung* **2**: 363–368, 1951.
2. Aravich, P. F. and A. Sclafani. Paraventricular hypothalamic lesions and medial hypothalamic knife cuts produce similar hyperphagia syndrome. *Behav Neurosci* **97**: 970–983, 1983.
3. Azar, A. P. G. Shor-Posner, R. Filart and S. F. Leibowitz. Impact of medial hypothalamic 6-hydroxydopamine injections on daily food intake, diet selection and body weight in freely-feeding and food-restricted rats. *Proc Eastern Psychol Assoc* **55**: 106, 1984.
4. Bellinger, L. L., L. L. Bernardis and S. Brooks. Feeding responses of rats with dorsomedial hypothalamic lesions given ip 2DG or glucose. *Am J Physiol* **235**: R168–R174, 1978.
5. Berthoud, H. R. and G. J. Morgenson. Ingestive behavior after intracerebroventricular infusions of glucose and 2-deoxy-D-glucose. *Am J Physiol* **233**: R127–R133, 1977.
6. Bhakthavatsalam, P. and S. F. Leibowitz. Studies on the influence of adrenal glucocorticoids on the alpha-noradrenergic and opiate feeding effects of different drugs in rats. *Proc Eastern Psychol Assoc* **55**: 106, 1984.

7. Blundell, J. E. Problems and processes underlying the control of food selection and nutrient intake. In: *Nutrition and the Brain*, vol 6, edited by R. J. Wurtman and J. J. Wurtman. New York: Raven Press, 1983, pp. 164–220.
8. Borsini, F. and E. T. Rolls. Role of noradrenaline and serotonin in the basolateral region of the amygdala in food preferences and learned taste aversions in the rat. *Physiol Behav* **33**: 37–43, 1984.
9. Breese, G. R., R. D. Smith, B. R. Cooper and L. D. Grant. Alterations in consummatory behavior following intracisternal injection of 6-hydroxydopamine. *Pharmacol Biochem Behav* **1**: 629–642, 1973.
10. Coscina, D. V., C. Rosenblum-Flinick, 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.
11. Ellison, G. D. and D. E. Bresler. Tests of emotional behavior in rats following depletion of norepinephrine, of serotonin, or of both. *Psychopharmacologia* **34**: 275–288, 1974.
12. Evetts, K. D., J. T. Fitzsimmons 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.
13. Falck, B., N. A. Hillarp, G. Thiem and A. Torp. Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Histochem Cytochem* **10**: 348–354, 1962.
14. Fuxe, K. Cellular localization of monoamines in the medial eminence and the infundibular stem of some mammals. *Z Zellforsch* **61**: 710–724, 1964.
15. Grossman, S. P. Behavioral effects of chemical stimulation of the ventral amygdala. *J Comp Physiol Psychol* **57**: 29–36, 1964.
16. Jhanwar-Uniyal, M., B. Dvorkin, M. H. Makman and S. F. Leibowitz. Distribution of catecholamine receptor binding sites in discrete hypothalamic regions and the influence of food deprivation on binding. *Soc Neurosci Abstr* **6**: 2, 1980.
17. Jhanwar-Uniyal, M., F. Fleischer, B. E. Levin and S. F. Leibowitz. Impact of food deprivation in hypothalamus α-adrenergic receptor activity and norepinephrine (NE) turnover in rat brain. *Soc Neurosci Abstr* **8**: 711, 1982.
18. Johnson, D. J., E. T. S. Li, D. V. Coscina and G. H. Anderson. Different diurnal rhythms of protein and non-protein intake by rats. *Physiol Behav* **22**: 777–780, 1979.
19. Kanarek, R. B., R. Marks-Kaufman and B. J. Lipeles. Increased carbohydrate intake as a function of insulin administration in rats. *Physiol Behav* **25**: 779–782, 1980.
20. Kanarek, R. B., R. Marks-Kaufman, R. Ruthazaer and L. Gualtieri. Increased carbohydrate consumption by rats as a function of 2-deoxy-D-glucose administration. *Pharmacol Biochem Behav* **18**: 47–50, 1983.
21. Leathwood, P. D. and L. Arimanana. Circadian rhythms of food intake and protein selection in young and old rats. *Annu Rev Chronopharm* **1**: 255–258, 1984.
22. Leibowitz, S. F. Adrenergic stimulation of the paraventricular nucleus and its effects on ingestive behavior as a function of drug dose and time of injection in the light-dark cycle. *Brain Res Bull* **3**: 357–363, 1978.
23. Leibowitz, S. F. Brain monoamines and peptides: Role in the control of eating behavior. *Fed Proc* **45**: 1396–1403, 1986.
24. Leibowitz, S. F., A. Arcomano and N. J. Hammer. Potentiation of eating associated with tricyclic anti-depressant drug activation of α-adrenergic neurons in the paraventricular hypothalamus. *Prog Neuropsychopharmacol* **2**: 349–358, 1978.
25. Leibowitz, S. F., N. J. Hammer and L. L. Brown. Analyses of behavioral deficits produced by lesions in the dorsal and ventral midbrain tegmentum. *Physiol Behav* **25**: 829–843, 1980.
26. Leibowitz, S. F., N. J. Hammer and K. Chang. Hypothalamic paraventricular nucleus lesions produce overeating and obestity in the rat. *Physiol Behav* **27**: 1031–1040, 1981.
27. Leibowitz, S. F., M. Jhanwar-Uniyal and C. R. Roland. Circadian rhythms of circulating corticosterone and α 2-noradrenergic receptors in discrete hypothalamic and extrahypothalamic areas of rat brain. *Soc Neurosci Abstr* **10**: 294, 1984.
28. Leibowitz, S. F., P. Roosin and M. Rosenn. Chronic norepinephrine injection into the hypothalamic paraventricular nucleus produces hyperphagia and increased body weight in the rat. *Pharmacol Biochem Behav* **21**: 801–808, 1984.
29. Leibowitz, S. F., G. F. Weiss, F. Yee and J. Tretter. Noradrenergic innervation of the paraventricular nucleus: Specific role in control of carbohydrate ingestion. *Brain Res Bull* **14**: 561–567, 1985.
30. Leibowitz, S. F., O. Brown, J. R. Tretter and A. Kirchgessner. Norepinephrine, clonidine, and tricyclic antidepressants selectively stimulate carbohydrate ingestion through noradrenergic system of the paraventricular nucleus. *Pharmacol Biochem Behav* **23**: 541–550, 1985.
31. 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.
32. Levin, B. E. Retrograde axonal transport of beta-adrenoreceptors in rat brain: effect of reserpine. *Brain Res* **300**: 103–112, 1984.
33. Lichtenstein, S. S., C. Marinescu and S. F. Leibowitz. Effects on ingestive behavior of chronic infusion of norepinephrine and clonidine into the hypothalamic paraventricular nucleus of the rat. *Brain Res Bull* **13**: 591–595, 1984.
34. Martin, G. E. and R. D. Myers. Evoked release of (14C) norepinephrine from the rat hypothalamus during feeding. *Am J Physiol* **229**: 1547–1555, 1975.
35. McArthur, R. A. and J. E. Blundell. Effects of age and feeding regime on self-selection of protein and carbohydrate. *Appetite: J Intake Res* **3**: 153–162, 1982.
36. McCaleb, M. L., R. D. Myers, G. Singer and G. Willis. Hypothalamic norepinephrine in the rat during feeding and perfusion with glucose, 2-DG, or insulin. *Am J Physiol* **236**: R312–R321, 1978.
37. McDonald, D. G., J. A. Stern and W. W. Hahn. Effects of differential housing and stress on diet selection, water intake and body weight in the rat. *J Appl Physiol* **18**: 937–942, 1963.
38. Myers, R. D. and G. E. Martin. 6-OHDA lesion of the hypothalamus: interaction of aphagia, food palatability, set-point for weight regulation and recovery of feeding. *Pharmacol Biochem Behav* **1**: 329–345, 1973.
39. Palkovits, M. Isolated removal of hypothalamic or other brain nuclei of the rat. *Brain Res* **59**: 449–450, 1973.
40. Piquard, F., A. Schaefer and P. Haberey. Influence of fasting and protein deprivation on food self-selection in the rat. *Physiol Behav* **20**: 771–778, 1978.
41. Ritter, R. C. and M. Neville. Hypothalamic noradrenaline turnover is increased during glucoprivic feeding. *Fed Proc* **35**: 642, 1977.
42. Schutz, H. G. and F. J. Pilgrim. Changes in the self-selection pattern for purified dietary components by rats after starvation. *J Comp Physiol Psychol* **47**: 444–449, 1954.
43. Sclafani, A. and P. F. Aravich. Macronutrient self-selection in three forms of hypothalamic obesity. *Am J Physiol* **244**: R686–R694, 1983.
44. Shor-Posner, G., A. P. Azar, S. Insinga and S. F. Leibowitz. Deficits in the control of food intake after paraventricular nucleus lesion. *Physiol Behav* **35**: 883–890, 1985.
45. Smith, G. P., A. J. Strohmayer and D. G. Reis. Effect of lateral hypothalamic injections of 6-hydroxydopamine on food and water intake in rats. *Nature New Biol* **235**: 27–29, 1972.
46. Smythe, G. A., H. S. Grunstein, J. E. Bradshaw, M. V. Nicholson and P. J. Compton. Relationships between brain noradrenergic activity and blood glucose. *Nature* **308**: 65–67, 1984.
47. Stachowiak, M., J. Bialowas and M. Jurkowski. Catecholamines in some hypothalamic and telencephalic nuclei of food-deprived rat. *Acta Neurobiol Exp* **38**: 157–165, 1978.
48. Tempel, D. L., P. Bhakthavatsalam, G. Shor-Posner, D. Dwyer and S. F. Leibowitz. Nutrient self-selection at different periods of the light-dark cycle in free feeding and food deprived rats. *Appetite* **6**: 294, 1985.

49. Tretter, J. R. and S. F. Leibowitz. Specific increase in carbohydrate consumption after norepinephrine (NE) injection into the paraventricular nucleus (PVN). *Soc Neurosci Abstr* **6:** 532, 1980.
50. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigra-striatal dopamine system. *Acta Physiol Scand* **367:** 95–122, 1971.
51. Van der Gugten, J. and J. L. Slangen. Release of endogenous catecholamines from rat hypothalamus in vivo related to feeding and other behaviors. *Pharmacol Biochem Behav* **7:** 211–219, 1977.
52. Willis, G. L. and G. C. Smith. A role for amine accumulation in the syndrome of ingestive deficits following lateral hypothalamic lesion. *Appetite* **5:** 239–262, 1984.
53. Winer, B. J. *Statistical Principles in Experimental Design*, 2nd edition. New York: McGraw-Hill, 1971, pp. 261–271.
54. Zigmond, M. J. and E. M. Stricker. Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats. *Science* **177:** 1211–1213, 1972.