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Clonidine Hyperphagia: Neuroanatomic Substrates and Specific Function

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SHOR-POSNER, G., A. P. AZAR, M. VOLPE, J. A. GRINKER AND S. F. LEIBOWITZ. *Clonidine hyperphagia: Neuroanatomic substrates and specific function.* PHARMACOL BIOCHEM BEHAV **30**(4) 925–932, 1988.—Recent studies have indicated that the α_2-noradrenergic agonist clonidine (CLON), when peripherally and centrally administered, potentiates feeding in satiated rats in a manner similar to that observed following injection of norepinephrine (NE) into the hypothalamic paraventricular nucleus (PVN). The present experiments examined the effects of CLON on meal patterns and macronutrient selection and compared these findings to earlier NE-stimulated feeding studies. Administration of CLON (25 nmoles), directly into the PVN (n=5), similar to PVN injected NE, produced an increase in meal size (190%) and feeding duration (164%), with no change in meal frequency. Additional tests were conducted in rats with PVN electrolytic or 6-hydroxydopamine lesions. In Sham rats (n=16) peripheral CLON (0.05 mg/kg), like NE, produced an increase in food intake and particularly potentiated carbohydrate ingestion. Discrete electrolytic lesions of the PVN (n=5) abolished this CLON-induced feeding and carbohydrate preference, suggesting that the PVN may be a primary site for CLON-stimulated hyperphagia. Neurotoxin lesions of the PVN (n=17), which reduced PVN NE levels by 75%, failed to alter peripheral CLON-induced feeding. This and other evidence indicates that this agonist may be acting via postsynaptic α_2 receptors in the PVN to potentiate carbohydrate intake, rather than via presynaptic release of NE from nerve endings in the PVN.

Feeding behavior Clonidine Paraventricular hypothalamus Lesions

NORADRENERGIC receptor mechanisms within the hypothalamic paraventricular nucleus (PVN) have been suggested to have a physiological role in the control of food intake [11]. When administered into this area norepinephrine (NE) elicits feeding in satiated rats and enhances eating in hungry rats [10,23]. The α_2-noradrenergic agonist clonidine (CLON) has similarly been shown to stimulate feeding when injected intraperitoneally in different species [16, 19, 24, 25, 35], or directly into the PVN of rats [4, 19, 33]. Long-term feeding patterns, moreover, are altered by chronic infusion of NE or CLON directly into the PVN, which produces a potentiation of daily food intake, associated with an increase in meal size but not the number of eating bouts, and an enhanced body weight gain [15,18].

Additional similarities between these two noradrenergic agonists have been revealed in a variety of pharmacological, biochemical and behavioral studies. It has been shown, for example, that both NE and CLON stimulate feeding behavior through α_2-type adrenoreceptors [4, 20, 26], and produce the strongest eating response at the start of the dark period [1], when a sharp increase in PVN α_2-receptor binding sites occurs [7]. Destruction of the PVN, through electrolytic lesions, effectively blocks feeding elicited by either central NE or peripheral CLON injection [14,33]. In other studies, administration of CLON and NE has been associated with an alteration in macronutrient selection, namely, an increase in carbohydrate ingestion [13, 16, 17]. These findings indicate that CLON and NE may be acting through the same central mechanisms, and suggest that the PVN may be a primary site in the mediation of CLON-induced hyperphagia and macronutrient selection.

To examine this further, the effect of CLON on spontaneously motivated feeding has been investigated through an examination of the meal-taking patterns of freely feeding rats after PVN injection of CLON. In addition, the importance of the PVN and particularly catecholaminergic (CA) innervation to this nucleus, in eliciting the feeding responses and macronutrient selection induced by CLON, has been assessed in PVN-lesioned animals. The results of these studies, portions of which have been presented in preliminary form [27], demonstrate that CLON enhances feeding, particularly of carbohydrate, through an alteration in meal

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size and feeding duration. Destruction of the PVN abolishes these effects of CLON, which appear to occur via postsynaptic α_2-receptor activity in the PVN rather than presynaptic release of endogenous NE.

METHOD

Subjects

Male albino Sprague-Dawley rats (n=43) weighing 350–450 g, were used in these experiments. The animals in Experiment 1 (n=5) were individually housed in Plexiglas cages with floors of stainless steel rods, located in noise-attenuating chambers under a 12:12 light-dark cycle (lights on at 5:00 a.m.). The animals of Experiments 2 (n=10) and 3 (n=28) were housed separately in standard wire mesh cages and maintained under a similar lighting cycle (0700–1900 hr).

Surgery

Those rats requiring acute drug injections directly into the PVN (Experiment 1) were stereotaxically implanted, under pentobarbital anesthesia, with unilateral 23 gauge cannulas with a screw-on top [9]. The stereotaxic coordinates used were: −0.4 mm caudal to bregma, 0.3 mm lateral to the midline, and 8.2 mm beneath the surface of the skull, with the top of the incisor bar 3.1 mm above the center of the interaural line. At the end of the experiments, the animals were sacrificed, and their brains histologically prepared (50 μ frozen sections were cut and stained with cresyl violet) for analysis of cannula placements.

To produce bilateral PVN electrolytic lesions, the rats in Experiment 2 were anesthetized with pentobarbital, and the lesions were made with stainless steel electrodes (size 00 insect pins), which were insulated with Epoxylite and bared to a 0.5 mm conical tip. With the top of the upper incisor bar raised 3.1 mm above the interaural line, the electrode was lowered according to the following stereotaxic coordinates: 0.4 mm posterior to bregma, 0.4 mm lateral to midline, and 8.7 mm ventral to skull surface. After lowering the electrode into the hypothalamus, the lesion was made using a 1 mA direct anodal current (for durations of 10–15 sec) and a rectal cathode. Sham-lesioned rats received identical treatment except that no current was passed through the lesioning electrode.

An additional group of rats was studied in Experiment 3, to determine the effect of neurotoxin-induced catecholamine (CA) depletion on feeding elicited by CLON. Each rat was stereotaxically implanted under pentobarbital anesthesia, as before, and lesions produced by using the neurotoxin, 6-hydroxydopamine hydrobromide (6-OHDA, Sigma Chemical). 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 (free base)/1.5 μl/side was delivered bilaterally into the PVN, through a cannula made from 25-guage hypodermic tubing with a wire insert for the top. It was infused over a 4 min period at a rate of 0.1 μl/15 sec. To allow time for dispersion of the 6-OHDA, the injector was kept in the brain for an additional 2 minutes before being removed. An equal volume of the saline-ascorbic acid vehicle was injected into control rats. The stereotaxic coordinates used for all injections were: −0.1 mm relative to bregma, ±0.4 mm lateral to the midline, and 6.4 mm beneath the surface of the skull, with the top of the incisor bar 3.1 mm above the center of the interaural line. These coordinates were employed based on pilot studies indicating that they yielded the most extensive depletion of catecholamine throughout the entire PVN.

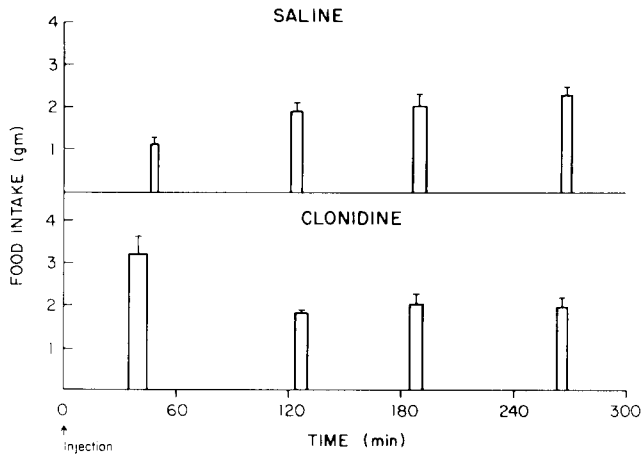

FIG. 1. Comparison of the temporal effects of central administration of saline and clonidine (25 nmoles) 1 hr before the start of the dark cycle. The first four meals (4–5 hr postinjection) are shown. Animals (n=5) were maintained under freely-feeding conditions and data collected using computerized meal pattern apparatus. See Table 1 for data.

Procedure

Experiment 1. In this experiment, the effect of clonidine hydrochloride (CLON, Boehringer Ingelheim) on the microstructure of feeding patterns was determined in freely-feeding rats (n=5) maintained on a sweetened powder diet (80% Purina lab chow mixed with 20% sucrose). Following recovery from surgery and adaptation to the housing chambers, drug tests were conducted approximately 5 days/week (generally Monday through Saturday). Injections of either CLON (25 nmoles) or saline were administered alternately, each day, approximately 1 hr before the start of the dark period (16:00 hr). Over a three-week period, each rat received 7–8 paired days of saline versus drug tests, from which approximately 4–5 days of scorable data were available and averaged for saline and for drug. Data records had to be excluded occasionally due to malfunction of the lick-detector apparatus, computer breakdown or food spillage.

The microstructure of feeding was analyzed by means of data collected via a PDP 8 computer connected to solid-food eatometers, which are constructed from standard glass feeding jars and attached to a detection circuit [32]. This established and automated procedure enables us to monitor and continuously record spontaneous consumption of solid food without disturbing the animals. A full description of this apparataus and the method of data collection has been provided elsewhere [32]. Briefly, the computer was programmed to record every lick ("bite") of food, the initiation and termination of all meals, and the total number of licks occurring in each meal. As described in a previous meal-pattern study [30] a meal was defined for data collection purposes as a minimum of 10 licks occurring within 10 sec and separated from other meals (intermeal interval, IMI) by at least one minute of no feeding. This conservative criterion was employed to prevent the exclusion of potentially valuable data. After data inspection, a meal was characterized as a minimum intake of 0.4 g and separated from other meals by

TABLE 1

EFFECTS ON MEAL PATTERNS OF PVN INJECTION OF SALINE OR CLONIDINE (CLON)

Meal	Drug	IMI (min)	Size (g)	Duration (min)	Rate (g/min)
1	Saline	46.4 ± 12.4	1.11 ± 0.2	3.9 ± 0.5	0.29 ± 0.02
	CLON	34.5 ± 13.5	3.22 ± 0.4*	10.3 ± 1.8*	0.33 ± 0.03
2	Saline	70.5 ± 12.1	1.89 ± 0.2	5.9 ± 0.5	0.34 ± 0.05
	CLON	79.0 ± 12.9	1.84 ± 0.1	6.4 ± 0.6	0.29 ± 0.02
3	Saline	59.5 ± 4.4	2.02 ± 0.3	7.0 ± 1.0	0.30 ± 0.05
	CLON	54.9 ± 7.9	2.07 ± 0.2	6.4 ± 0.7	0.32 ± 0.03
4	Saline	71.4 ± 10.4	2.27 ± 0.2	6.4 ± 0.6	0.35 ± 0.04
	CLON	72.2 ± 8.5	1.98 ± 0.2	5.8 ± 0.4	0.36 ± 0.04

Statistical comparisons between clonidine and saline scores (means ± standard error given for 5 rats) revealed significant differences at $*p<0.05$. The intermeal interval (IMI) is given for intervals preceding each meal. The IMI for meal 1 reflects the latency to eat after injection.

an intermeal interval (IMI) of 15 minutes [32], in the analysis of both drug and vehicle scores. This excluded random responses or noise but did not eliminate actual meals. In order to understand CLON's impact on spontaneous ingestion and the nature of the recovery process, we examined in detail the first 4 meals of each test, which generally occurred within 4–5 hr after CLON or saline injection.

Experiment 2. To determine the impact of PVN damage on CLON's stimulatory effect on food intake and diet selection, rats (n=5) with discrete PVN electrolytic lesions, as compared to Sham-operated rats (n=5), were maintained on three separate sources of pure macronutrients, protein, carbohydrate and fat for approximately 2 weeks prior to surgery. The protein ration (3.7 kcal/g) was composed of casein (National Casein Co.) mixed with 4% minerals (USP XIV Salt Mixture, ICN Pharmaceuticals), 2.97% vitamins (Vitamin Diet Fortification, ICN) and 0.03% cysteine (L-Cysteine hydrochloride, ICN). The carbohydrate ration (3.7 kcal/g) was composed of 37% sucrose, 28% dextrin (ICN), 28% cornstarch, plus 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. These pure nutrient diets which have been routinely used in our diet selection studies [17, 28–30], are mixed with vitamins, minerals and cysteine, in proportion to caloric values, to ensure normal growth and body weight gain.

The rats in this experiment were given at least 2 weeks of postoperative recovery before drug testing was begun. Tests were conducted during the daytime (1200–1600 hr) and were preceded by a 1-hr pretest with fresh food to insure maximal satiation. At the end of this pretest hour, the animals were injected intraperitoneally, in counterbalanced order and on separate days with either physiological saline (0.5 cc) or CLON (0.5 mg/kg) dissolved in physiological saline and then immediately given measured food. Food intake and diet selection were measured 3 hr later, and all food intake scores reported in the Results section represent the average of several (2–4) test scores.

Experiment 3. All animals were maintained ad lib on the three pure macronutrient diets for approximately 2 weeks prior to surgery. Following a 3 week postoperative period, tests with systemic injections of CLON (0.05 mg/kg) versus saline were conducted in 6-OHDA-injected rats with CA depletion in the PVN (n=17) and in ascorbic acid control rats (n=11). The drug tests were identical to those described above in Experiment 2.

Histological Analysis

After completing their drug tests, animals with electrolytic PVN lesions were sacrificed under pentobarbital anesthesia and transcardially perfused with 10% buffered formalin. The brains were removed, frozen sections (50 μ) cut, and alternate sections stained with cresyl violet. Sections were analyzed with the help of the stereotaxic atlas of König and Klippel [8].

Histochemical Analysis

All animals were confirmed for the extent of their 6-OHDA lesion through fluorescence histochemistry. Histochemical procedures to visualize brain CA fibers were carried out according to the Falck-Hillarp fluorescence technique [3] and have been described elsewhere [29]. Briefly, with this technique, 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 semiquantitative method based on a rating scale of 1–5 (1 indicating near total lack of fluorescence and 5 indicating high-normal fluorescence). Photographs of the hypothalamic paraventricular and dorsomedial nuclei and the lateral perifornical hypothalamus were taken for direct comparisons between lesion- and sham-operated animals. All animals (n=17) included in the Results section described below exhibited CA depletion in the PVN (fluorescence rating of "2").

Statistical Analysis

Statistical evaluations of the meal patterns and various diet intake comparisons were based on a two-way analysis of variance (ANOVA) for repeated measures. Significant effects were followed by specific comparisons between means using the Student's t-test [34]. Macronutrient preference (Experiment 3) was determined using single-factor ANOVA for repeated measures with comparisons of means evaluated

using the F statistic as described by Winer [34]. A two-tailed Student's t-test for independent samples was used in the comparison of the lesion and control group scores in Experiments 2 and 3.

RESULTS

Experiment 1: Effects of PVN CLON Injection on Meal Patterns

Figure 1 demonstrates the temporal effects of hypothalamic CLON injection (25 nmoles) on food intake during the first 4 meals of the evening test. As illustrated in this figure and summarized in Table 1, the potentiating effect of CLON on food intake was associated with an alteration in meal size rather than a change in meal frequency. Two-way analysis of variance performed on all four meals yielded significant interaction effects on meal size and duration. The initial meal after PVN CLON injection was significantly larger ($p<0.05$), and lasted longer ($p<0.05$) than the first meal after saline injection. This facilitory effect of CLON was essentially confined to the first meal after injection, followed by a gradual trend towards recovery over the next three meals. There appeared to be no difference in the latency to meal onset, nor in the rate of eating between the two treatments. Analyses of subsequent meals (2–4) revealed no significant change in meal size, meal frequency or IMI with drug treatment. In addition, 24-hr food intake measures showed no significant effect of CLON (25.2±1.3 g) compared with saline (24.7±0.6 g).

Experiment 2: Effect of Peripheral CLON in Rats With PVN Electrolytic Lesions

In this experiment, we assessed the impact of bilateral PVN electrolytic lesions on food intake and macronutrient selection elicited by intraperitoneal injection of the α-adrenergic agonist CLON. As illustrated in our previous studies [10,28], all of the animals (n=5) had the tip of their cannulas located in the vicinity of the PVN, generally along the dorsal aspects of the nucleus. As shown in Fig. 2, CLON (0.05 mg/kg) injected into satiated, sham-operated rats significantly increased total caloric intake above the saline control levels +304%, $F(1,4)=57.19$, $p<0.002$. As described previously [16], this strong stimulatory effect was associated with a selective increase in consumption of carbohydrate ($p<0.025$). Ingestion of fat appeared somewhat enhanced, but this effect was not statistically reliable due to a large variability in the response. Intake of protein was also not significantly altered.

The impact of bilateral PVN electrolytic lesions on feeding induced by peripheral CLON administration was considered in the next analyses. As indicated by the total intake scores in Fig. 2, the saline baseline for PVN-lesioned rats appeared increased relative to the Sham rats' saline baseline; this enhancement, however, was not statistically significant, due to the large variability and relatively small group size.

In contrast to the CLON-stimulated feeding effect observed in Sham rats, PVN-electrolytic lesion rats failed to display any enhanced feeding in response to peripheral injection of CLON (Fig. 2). The potentiated food intake and carbohydrate selection induced by CLON in Sham rats was apparently abolished in PVN-lesioned rats, as there was no increase in feeding with CLON over saline baseline, $F(1,4)=0.95$, $p<0.385$. It should be noted that a high baseline does not prevent PVN-lesioned rats from responding to other drugs, e.g., 2-deoxy-D-glucose, with an enhancement in total food consumption and increase in carbohydrate intake [28].

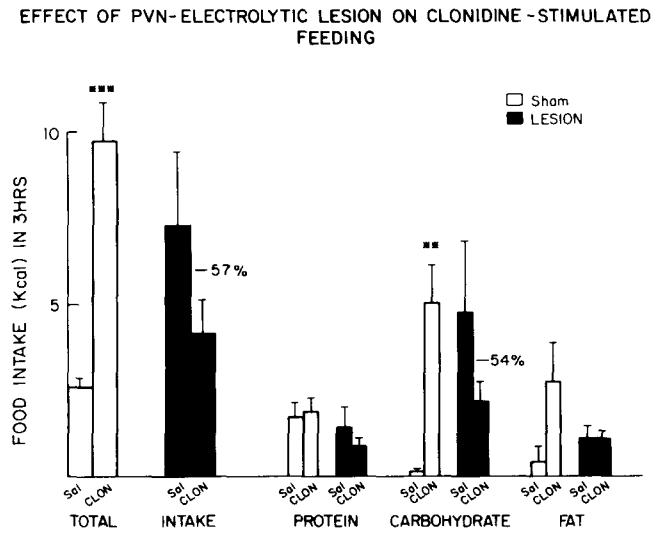

FIG. 2. In Sham-operated rats (n=5), peripheral clonidine (CLON, 0.05 mg/kg) elicited a significant increase in total food intake (***$p<0.002$) associated with a specific enhancement of carbohydrate ingestion (**$p<0.025$). PVN-lesion rats (n=5), in contrast, failed to show any enhanced feeding in response to peripheral clonidine (0.05 mg/kg) administration.

Experiment 3: Effect of Peripheral CLON Injection in Rats With PVN 6-OHDA Lesions

To determine the importance of noradrenergic innervation to the PVN in permitting animals to respond to peripheral CLON, we injected the neurotoxin 6-OHDA into this nucleus and tested its effect on the animals' CLON-induced feeding response. Histochemical analyses of this lesion, as compared to ascorbic acid-injected animals, failed to reveal any change in extrahypothalamic CA fluorescence as observed in the striatum, stria terminalis, caudate and median eminence. Within the hypothalamus, as shown in the photomicrographs of Fig. 3, the damage to CA innervation was largely confined to the medial aspect of this structure and was clearly greatest in the PVN. This may be observed by comparing an ascorbic acid-injected rat (3a) with a 6-OHDA-injected animal (3b). Damage from the lesion extended into the medial preoptic area and the anterior portion of the dorsomedial nucleus, but normally dense CA varicosities were observed in the middle and caudal dorsomedial nucleus of 6-OHDA injected rats (3d as compared to ascorbic acid control in 3c), the lateral hypothalamus just lateral to the PVN, the zona incerta and the more caudal perifornical region (3e ascorbic acid versus 3f 6-OHDA).

Biochemical analysis of the 6-OHDA lesion [29] has substantiated the relatively localized nature of this neurotoxin lesion. Specifically, injection of 6-OHDA directly into the PVN has been shown to produce a reliable decrease in NE content within this nucleus (−75%) in addition to smaller reductions of PVN dopamine (−56%) and epinephrine −47%). Other hypothalamic areas, including the nearby medial preoptic area, dorsomedial nucleus and perifornical hypothalamus, retained close to normal dopamine and epinephrine levels. In contrast to the strong depletion of NE in

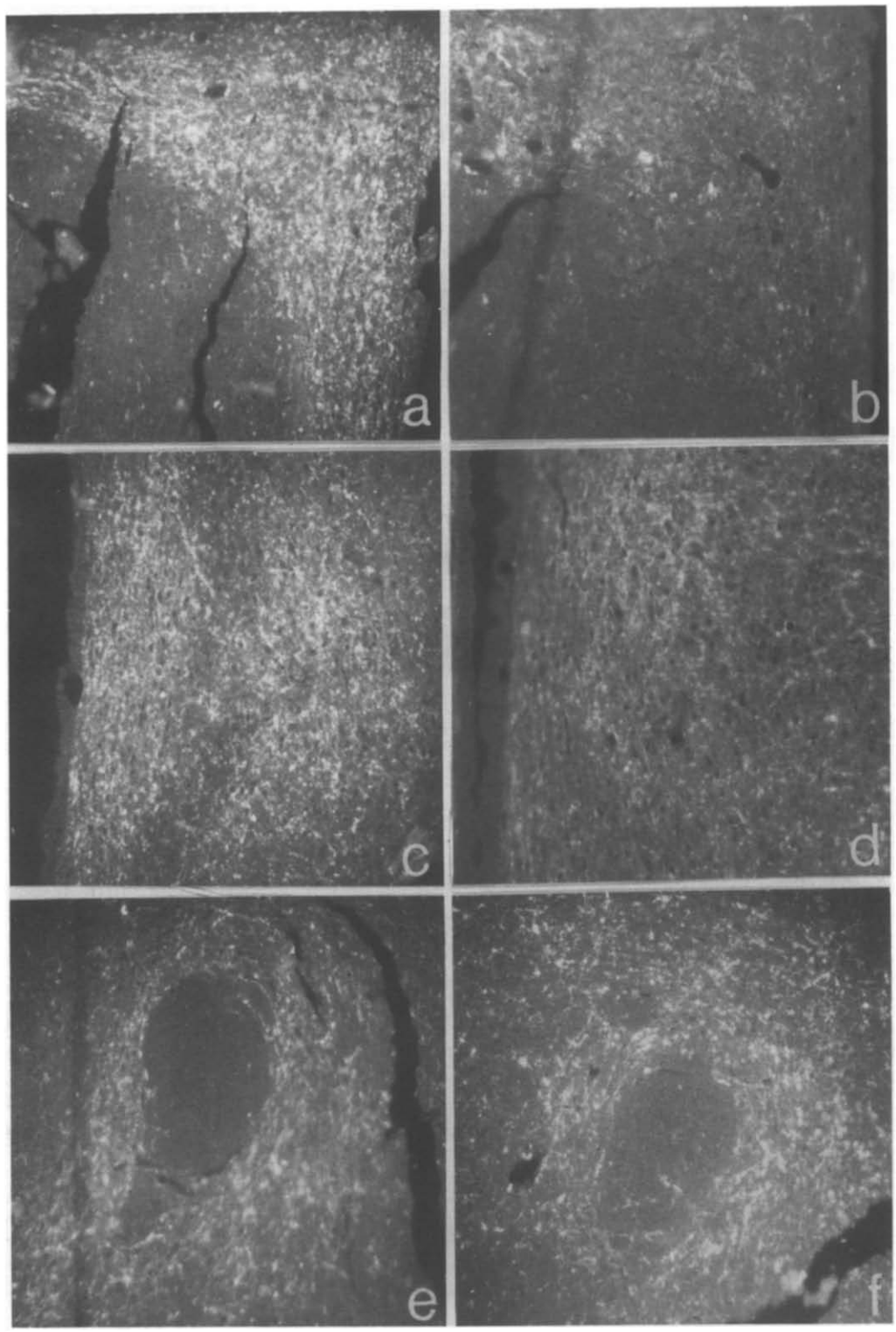

FIG. 3. Fluorescence photomicrographs showing catecholamine varicosities in the hypothalamus. (a) Normal CA innervation of the PVN of a vehicle-injected rat. (b) Marked reduction in a rat after 6-OHDA was injected in the PVN. Catecholamine innervation remained essentially intact in other brain areas such as the caudal dorsomedial nucleus [compare normal (c) and (d) 6-OHDA rat], and the caudal perifornical area as shown in the normal (e) and 6-OHDA injected animal (f).

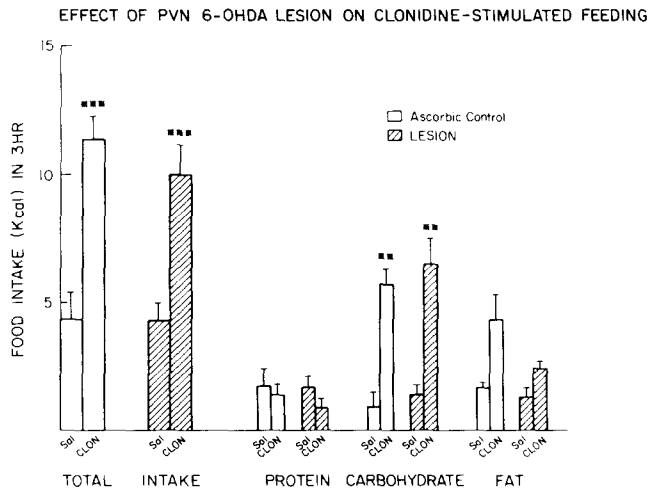

FIG. 4. Ascorbic acid control animals (n=11) and PVN 6-OHDA lesion rats (n=17), demonstrated a similar pattern in nutrient selection and total intake following systemic clonidine (0.5 mg/kg) injection. A robust enhancement of feeding (***$p<0.001$) associated primarily with increased carbohydrate ingestion, relative to protein and fat (**$p<0.01$), was observed in both groups.

the PVN, a more moderate and significantly smaller ($p<0.01$) decline of NE content (−37% to 50%) was observed in surrounding hypothalamic areas.

Similar to the Sham rats illustrated in Fig. 2, the ascorbic acid vehicle control rats (Fig. 4) significantly increased their total caloric intake, above the saline baseline level, after CLON injection, $F(1,10)=109.99$, $p<0.001$. This CLON-induced stimulatory effect was once again accompanied by a significant alteration in diet selection, namely, a selective increase in carbohydrate intake, $F(1,20)=9.9$, $p<0.01$, and only a small increase in fat consumption and no enhancement of protein ingestion. Injection of 6-OHDA into the PVN had no apparent impact on this drug-induced pattern of food selection (Fig. 4). Rats with reduced CA innervation to the PVN displayed a comparable CLON-stimulated increase in total diet, $F(1,16)=23.02$, $p<0.001$. Similar to the ascorbic acid rats, 6-OHDA-injected rats exhibited a significant drug × diet interaction ($p<0.001$), along with a preferential increase in carboyhydrate consumption, relative to protein and fat intake, $F(1,32)=41.5$, $p<0.01$.

DISCUSSION

The results of these experiments, which extend and confirm earlier studies demonstrating PVN sensitivity to noradrenergic stimulation [11], strengthen the proposal that NE and CLON stimulate feeding through similar mechanisms [19,33]. The increase in food intake elicited by PVN injections of CLON, as indicated by meal pattern analyses, is accomplished primarily through an increase in meal size and feeding duration. The onset of feeding and the frequency of meals consumed, however, is not affected. Injection of NE directly into the PVN also produces an increase in meal size and duration with no change in meal initiation or frequency [15, 23, 30]. This has led to the proposal that endogenous NE acts to elicit feeding through an inhibition of satiety rather than through stimulation of hunger. The finding that CLON exerts effects on spontaneously motivated feeding similar to those of NE supports the idea that these agonists have a common site of action.

An additional manner in which CLON may affect feeding patterns is through its influence on the animals' selection of specific nutrients. Our investigation, which reveals a CLON-induced preference for carbohydrate in animals provided with pure macronutrient diets, confirms the recent findings of Leibowitz *et al.* [16], who have demonstrated a similar diet preference following PVN as well as peripheral CLON administration. This selective carbohydrate effect is also observed with PVN administration of NE [16,17] and has been shown after PVN and peripheral injection of tricyclic antidepressant drugs, which are believed to act via the release of hypothalamic NE [13,16]. These findings provide further support for the suggestion that CLON and NE may be acting through the same central mechanisms. Our results, however, contrast with an earlier report of protein-potentiation following peripheral injection of CLON [21]. This difference in results may be due to the fact that Mauron *et al.* [21] used food-deprived, weanling rats as opposed to the satiated adult rats in the present study. They also analyzed mixed diets, which in contrast to the pure diets, did not permit a separate evaluation of the three nutrients.

After destruction of the PVN, a primary site in the mediation of CLON-induced carbohydrate hyperphagia appears to be lost. The present findings indicate that electrolytic lesions of the PVN abolish the feeding stimulated by peripheral injection of CLON. This is consistent with the findings of McCabe *et al.* [19], who demonstrated that lesions of this nucleus, as opposed to other hypothalamic sites, significantly attenuated CLON-stimulated feeding in rats maintained on a single mixed diet. This effect is also similar to the disruption of NE-induced feeding displayed in animals with hypothalamic knife cuts or lesion of the PVN as opposed to other hypothalamic areas [14,33]. Together, these findings provide strong support for the proposal that the paraventricular hypothalamus is involved in the mediation of hyperphagia produced by noradrenergic stimulation.

To futher examine the synaptic mechanisms involved in PVN α-noradrenergic feeding, the effects of neurotoxin-induced CA destruction on CLON-stimulated intake were investigated. The results indicated that CLON-stimulated eating remains intact in animals with reduced CA innervation to the PVN. In spite of a 75% loss of NE in the PVN, the feeding response produced by peripheral CLON administration was the same in the 6-OHDA lesioned rats and the ascorbic acid control rats. Although one may suggest that the remaining 25% of the NE-containing neurons provide a sufficient substrate for CLON's potential action on presynaptic α_2-receptors, the present findings, in light of other results, argue for a postsynaptic site of action in the mediation of the α_2-noradrenergic feeding response. This proposal is supported by the results of Goldman *et al.* [4], which demonstrate that prior injection of the CA synthesis inhibitor, α-methyl-p-tyrosine, fails to disturb the effectiveness of CLON or NE in eliciting eating. It is also in agreement with a variety of other pharmacological, biochemical and lesion studies [6, 12, 13, 19], which provide evidence that postsynaptic receptor sites in the PVN mediate CLON as well as NE's action on feeding. Taken together, these investigations support the functional importance of CLON's postsynaptic effects that have been emphasized in other recent studies describing CLON's effect on exploration, blood pressure control and catecholamine metabolism [2, 5, 22, 31].

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