Pharmacology Biochemistry & Behavior, Vol. 23, pp. 541–550, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Norepinephrine, Clonidine, and Tricyclic Antidepressants Selectively Stimulate Carbohydrate Ingestion Through Noradrenergic System of the Paraventricular Nucleus

SARAH F. LEIBOWITZ, OWEN BROWN, JOHN R. TRETTER AND ANNETTE KIRSCHGESSNER

The Rockefeller University, 1230 York Avenue, New York, NY 10021

Received 18 June 1984

LEIBOWITZ, S. F., O. BROWN, J. R. TRETTER AND A. KIRSCHGESSNER. *Norepinephrine, clonidine, and tricyclic antidepressants selectively stimulate carbohydrate ingestion through noradrenergic system of the paraventricular nucleus.* PHARMACOL BIOCHEM BEHAV 23(4) 541–550, 1985.—Using a self-selection feeding procedure, the present experiments examined the impact of central and peripheral injection of the α-adrenergic agonist clonidine (CLON) and the tricyclic antidepressant drugs amitriptyline (AMIT) and chlorimipramine (CIMIP) on nutrient selection in the adult male rat. In tests with mixed diets or with separate sources of the 3 macronutrients (carbohydrate, protein, and fat) simultaneously available, the following results were obtained: (1) Peripheral and paraventricular nucleus (PVN) injection of CLON stimulated total food intake and preferentially increased ingestion of carbohydrate. Little or no change in protein or fat intake was observed. This pattern of response is similar to that observed with norepinephrine. (2) PVN injection of AMIT and peripheral injection of CIMIP also selectively enhanced carbohydrate intake. (3) These drug effects on carbohydrate selection occurred under a variety of conditions, including with mixed diets and pure dietary nutrients; under ad lib and restricted feeding conditions; in short (1 hr) as well as long (6 hr) test intervals; and in the absence or presence of a change in total calorie intake. Based on this and other evidence, it is proposed that noradrenergic neurons innervating the PVN in the rat play a role in regulating carbohydrate selection, and that this neurochemical system mediates the stimulating action of CLON and antidepressants on carbohydrate ingestion.

Paraventricular nucleus Norepinephrine Antidepressants Clonidine Feeding behavior Carbohydrate ingestion Nutrients α-Adrenergic

EVIDENCE accumulated over the past several years has supported the hypothesis that noradrenergic receptor mechanisms located in the hypothalamic paraventricular nucleus (PVN) have a physiological role in the control of eating behavior [24]. Norepinephrine (NE) injected directly into this nucleus, in near physiological doses, stimulates food intake in the satiated rat [22,23], and this effect is blocked by selective α-adrenergic receptor antagonists [24] and also by discrete electrolytic lesions of the PVN [31]. Studies with the α-adrenergic agonist clonidine (CLON) have demonstrated a similar eating response; this drug is effective when peripherally administered [10,51], as well as when administered into the lateral ventricles [49], in the anterior hypothalamic area [8], or directly into the PVN [40]. The tricyclic antidepressants have also been found to enhance food intake in the rat, specifically after injection into the PVN [27]. This response, to such drugs as desipramine, protriptyline, and amitriptyline, is selectively blocked by α-adrenergic receptor antagonists and is also antagonized by drugs which inhibit the synthesis of endogenous NE. This evidence has led to the hypothesis that these antidepressant agents, which are known to block the neuronal reuptake of NE, are eliciting eating by enhancing the synaptic availability of endogenous NE specifically in the PVN [27].

Until recently, most studies of the neuropharmacology of eating behavior have utilized in their experiments a single, nutritionally complete diet, and have focused their attention on measurements of *total* food intake, with little concern for the nutritional composition of the diet. In nature, most foods are a complex mixture of their essential nutrients, and thus, their variety and diversity require that physiological mechanisms exist for regulation of nutrient selection (e.g., carbohydrate, protein, and fat), as well as for control of total caloric intake. There is evidence demonstrating that rats are capable of selecting nutritionally balanced diets from different, simultaneously available food sources, even pure nutrients [6, 45, 48]. There is further evidence that nutrient selection is altered by many different factors, including conditions of the external environment (e.g., temperature, time of day, food deprivation), internal environ-

TABLE 1
DIETS USED IN PHARMACOLOGICAL TESTS WITH SELF-SELECTION FEEDING PARADIGM

	Lab Chow Diets				Nutrients + Corn Oil		Pure Nutrients		
	Chow Pellet	Sucrose Pellet	Sucrose Chow[1]	Fat Chow[1]	Carbohydrate[2]	Protein[2]	Carbohydrate[3]	Protein[3]	Fat[3]
Caloric density[4] (Kcal/g)	3.5	4.0	3.7	5.3	4.2	4.2	3.7	3.7	7.7
Protein (%)	24	0	16	16	0	83	0	93	0
Carbohydrate (%)	53	100	69	36	83	0	93	0	0
Fat (%)	5	0	3	36	10	10	0	0	86
Vitamins, Minerals, Fiber (%)	18	0	12	12	7	7	7	7	14

[1] Sugar and fat chow diets consist of 67% lab chow power mixed with 33% sucrose or 33% corn oil, respectively.
[2] Carbohydrate diet consists of dextrin mixed with 10% corn oil; protein diet consists of casein mixed with 10% corn oil.
[3] Carbohydrate diet consists predominantly of 37% sucrose, 28% dextrin, and 28% corn starch; protein diet consists predominantly of casein; and fat diet consists predominantly of lard.
[4] Calculation of caloric density is based on caloric coefficients of 4 Kcal/g for protein and carbohydrate and 9 Kcal/g for fat.

ment (e.g., adrenalectomy, thryoidectomy, and pregnancy), behavioral activity (e.g., exercise), age, sex, and precise nature of macronutrient constituents (e.g., [2, 6, 13–15, 45, 48]). Recently, it has been discovered that peripheral pharmacological manipulations alter macronutrient selection, leading to the hypothesis that specific brain neurotransmitters affected by the drugs may have a function in balancing the proportion of carbohydrate, protein, and fat consumed by an animal (e.g., [3, 7, 16, 30, 36, 38, 44, 56, 57]).

In light of this evidence, we have conducted a series of experiments to determine whether hypothalamic catecholamine systems, known to modulate total energy intake in the rat, have a particular function in controlling or balancing the ingestion of specific macronutrients. In these studies, we have made simultaneously available to the subjects a variety of diets that permit them to reveal their natural food preferences. Using this self-selection feeding paradigm, we have examined the impact of *central* pharmacological manipulations on nutrient selection, specifically PVN injection of NE [35,54]. The results of this study demonstrated that NE administration, which is known to stimulate total food intake, increases the rats' preference for a specific dietary nutrient, namely, carbohydrate, with protein and fat intake minimally affected. This preference was observed in the case of non-sweet, as well as sweet, carbohydrate.

To examine further this phenomenon of noradrenergic-induced carbohydrate selection, the present study investigated the effects of the α-adrenergic agonist CLON and the tricyclic antidepressants, in addition to norepinephrine. As described above, these drugs are known to stimulate food intake when injected into the PVN, and the effect of the antidepressants is believed to be mediated through the release of endogenous NE. In the experiments of this study, these drugs were administered peripherally, as well as directly into the PVN of brain-cannulated rats. Like exogenous NE, CLON and the antidepressants amitriptyline (AMIT) and chlorimipramine (CIMIP) were each found to have a specific stimulatory effect on the selection of carbohydrate, suggesting that endogenous noradrenergic neurons of the PVN may provide the physiological substrate for mediating these drug effects. It is proposed that endogenous NE, specifically within the PVN, has a function in regulating the ingestion of carbohydrate and that the specific selection of this high-energy nutrient may represent an adaptive response to particular conditions requiring rapid energy expenditure.

METHOD

Animals

The animals were 65 male albino Sprague-Dawley rats weighing 350–400 g at the start of the experiment. They were housed individually and tested in their home cages. They were kept on a constant light-dark cycle, with the 12-hr light phase beginning at 6:00 a.m. followed by a 12-hr dark phase.

Surgery

Those rats used in experiments requiring drug injections directly into the PVN were stereotaxically implanted, under pentobarbital anesthesia, with a chronic unilateral cannula. These cannulas (made from 26-gauge hypodermic needles), which had a screw-on protective cap and an inner stylette [19], were aimed at the PVN using the following coordinates: with the nose bar raised 3.1 mm above the center of the aural bars, the anterior-posterior position was 0.2 mm caudal to bregma, 0.4 mm lateral to midline, and the depth was 8.2 mm below skull surface. The cannulas were fixed in place on the top of the skull with acrylic cement and stainless steel hooks penetrating the bone. These coordinates have routinely been found to direct the cannula tip toward the dorsal portion of the PVN [22,23]. To confirm this placement for the present experiment, 20% of the cannulated rats were sacrificed, their brains removed, and 50-μ frozen sections were cut and stained with cresyl violet. Analysis of these brain sections showed that all rats had their cannula tips

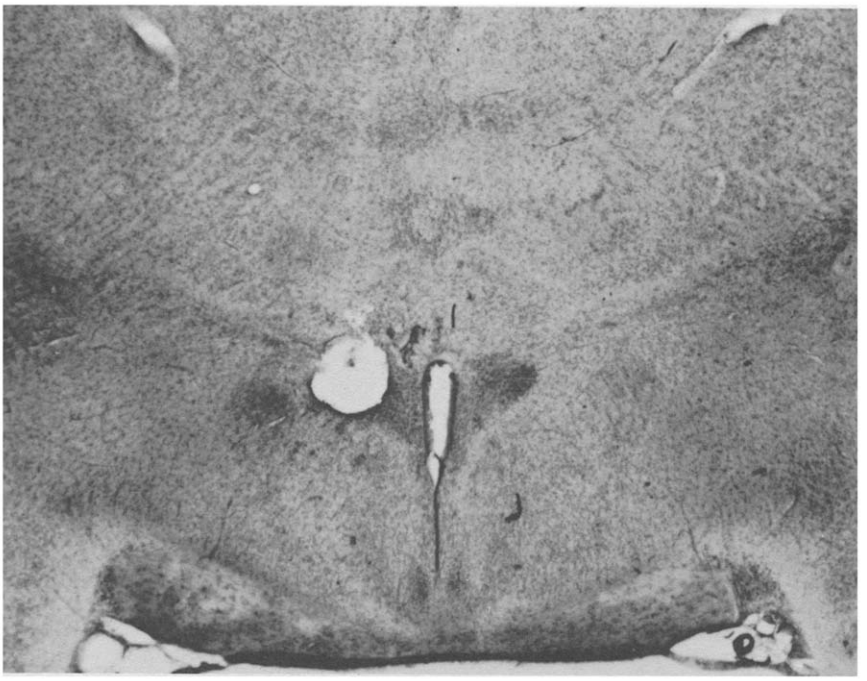

FIG. 1. Photomicrograph of a cresyl-violet stained section of the rat brain showing the damage produced at a typical injection site (arrow) in the dorsolateral portion of the PVN.

either directly within the PVN (Fig. 1) or within 0.3 mm of its dorsal surface.

Diets

Four sets of diets were used in these experiments (see Table 1). Each set consisted of either 2 or 3 separate diets which were simultaneously available to the rats. Experiment 1 utilized two sets of diets containing Purina rodent lab chow. The first set was composed of two diets, namely, lab chow pellets and pure Domino sugar cubes (referred to as "sucrose pellets"). The second set consisted of ground lab chow (67% by weight) mixed either with additional sucrose (33%) or Mazola corn oil (33%). These two diets are referred to as "sucrose chow" and "fat chow," respectively. Experiment 2 used one set of two isocaloric diets, namely, casein (National Casein Co.) and dextrin (ICN Pharmaceuticals), each mixed with 10% corn oil (Mazola), 4% minerals (USP XIV Salt Mixture, ICN Pharmaceuticals), and 3% vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals). Experiment 3 made available separate sources of each of the 3 macronutrients, namely, carbohydrate, protein, and fat. The carbohydrate ration was composed of 37% sucrose, 28% dextrin, 28% corn starch, 4% minerals, and 3% vitamins. The protein ration was composed of casein plus 4% minerals and 3% vitamins. The fat component consisted of lard mixed with 8% minerals and 6% vitamins. Calculation of caloric density was based on caloric coefficients of 3.7 Kcal/g for protein and carbohydrate and 7.7 Kcal/g for fat. All diets were provided in glass food cups braced with a metal bar against the side of the cage to prevent spillage. When spillage did occur, food lost under the cage was collected and added to the unconsumed total. The placement of the food cups with the cage was changed daily to prevent position preferences. Fresh diet was provided daily.

Drug Injections

The drugs tested in this study were: *l*-norepinephrine-*d*-bitartrate (Sigma); clonidine hydrochloride (Boehringer Ingelheim); amitriptyline hydrochloride (Merck Sharp and Dohme); and chlorimipramine hydrochloride (CIBA-Geigy). All drugs were dissolved in sterile physiological (0.9%) saline immediately before the start of the test. Central injections were given through the implanted PVN cannulas in a volume of 0.5 μl. Peripheral injections were given intraperitoneally in a volume of 0.5 ml. Each drug, tested at a single, moderately high dose, was injected in counterbalanced order with the saline vehicle on separate days. The rats received a total of at least 12 tests (6 vehicle and 6 drug tests), and all food intake scores presented in the Results section represent an average of these test scores. Regular and repeated testing on the same set of animals was found to be important, if not essential, for obtaining stable baseline food intake scores and reliable drug effects. Diet selection is particularly sensitive to environmental conditions; thus, it is important to maintain constant conditions to reduce, as much as possible, baseline variability that may occur from day to day.

Test Procedure

The animals were given at least 2 weeks to adapt to the dietary conditions. During this adaptation period with food and water available ad lib, body weight and nutrient intake measurements were taken daily to determine whether the rats were exhibiting normal growth patterns and balanced nutrient selection. In all but a few cases, the rats were found to gain at least 1.5 g/day and to consume at least 10–15% of their total food intake from each of the separate diets offered. After the 2 weeks of adaptation, the rats in

TABLE 2

FOOD INTAKE (Kcal ± SEM) IN 60 OR 360 MIN AFTER SALINE OR CLONIDINE INJECTION INTO THE PARAVENTRICULAR HYPOTHALAMUS

	Total Food Intake			Sucrose pellet			Lab Chow Pellet		
Test Interval	Saline	CLON	%Δ	Saline	CLON	%Δ	Saline	CLON	%Δ
0–60 min	40.5 ± 5.4	54.5 ± 4.0	+35*	11.9 ± 2.6	34.7 ± 3.3	+192§	28.7 ± 3.8	19.8 ± 3.8	−31
0–360 min	88.3 ± 3.3	110.0 ± 4.4	+25†	29.4 ± 3.7	56.8 ± 5.7	+ 93§	59.0 ± 3.4	53.4 ± 4.6	− 9
	Total Food Intake			Sucrose Chow			Fat Chow		
0–60 min	39.0 ± 2.0	41.5 ± 2.8	+ 6	19.3 ± 2.5	26.3 ± 3.2	+ 36§	19.7 ± 1.0	15.2 ± 1.5	−23§
0–360 min	76.3 ± 4.0	86.2 ± 3.3	+13†	38.6 ± 4.8	48.9 ± 5.1	+ 27†	37.7 ± 2.7	37.3 ± 3.6	− 1

Specific comparisons between saline and CLON/Kcal scores were statistically significant at: *$p < 0.05$; † $p < 0.01$; § $p < 0.001$.

Experiments 1 and 2 were placed on a 6-hr restricted feeding schedule, whereas the rats of Experiment 3 continued to have their diets available ad lib. Water was available ad lib to all rats. For the 6-hr feeding schedule, food was presented daily between 9:00 a.m. and 3:00 p.m. This schedule yielded a relatively stable baseline nutrient-selection pattern and total diet intake scores that were similar (±10%) to the rat's 24-hr food intake scores under ad lib food conditions. The purpose of this schedule was to assess drug effects on total daily food intake (namely, 6-hr feeding), as well as on shorter interval (1–2 hr) food intake. It also permitted a comparison of drug effects on food intake under restricted versus ad lib feeding conditions. Previous work has suggested that drug effects may differ depending upon the feeding regimen used [6, 36, 56].

The rats were permitted 2 additional weeks to adapt to their feeding schedule and their drug injection paradigm. All rats were tested 3 days/week (on alternate days), between 9:00 a.m. and 3:00 p.m. For the rats maintained on the 6-hr restricted feeding schedule, 3 food intake measurements were taken over the course of the 6-hr test, namely, after the first, second, and sixth hours. To maximize the drug effects over this 6-hr period, the rats received 2 drug injections during this test, at the beginning when food was first presented and halfway through the test, at the start of the fourth hour. For the rats with food available ad lib, the test started with a 1-hr adaptation interval, during which the rats were satiated on fresh diet. At the end of this pretest hour, the rats were given a single drug injection, and food intake measurements were taken at 1 hr and 2 hr after injection.

All data were analyzed by analyses of variance design, followed by appropriate *post-hoc* comparisons between Kcal scores for the different diets or drugs (Dixon and Brown, BMDP-77 Biomedical Computer Programs, P-series, 1977, and [55]).

RESULTS

Experiment 1: Effect of PVN CLON Injection on Selection of Lab Chow Diets

Earlier experiments with lab chow diets have demonstrated that NE injection into the PVN preferentially increase ingestion of diets dense in carbohydrate [47]. This occurred with two-diet tests comparing sucrose pellets (100% carbohydrate) with lab chow pellets (54% carbohydrate) and comparing sucrose chow mixtures (69% carbohydrate) with fat chow mixtures (36% carbohydrate). This preference for diets richer in carbohydrate occurred in the case of non-sweet, as well as sweet, carbohydrate sources. It is recognized that the use of such mixed diets (as opposed to separate sources of macronutrients) makes it difficult to assess changes in the amount versus ratio of nutrient selection. For purposes of comparison with these NE results, however, we designed the first experiment to examine the impact of PVN-injected CLON on nutrient intake in similar two-diet conditions. In this experiment, 2 groups of 10 PVN-cannulated rats were maintained on a 6-hr restricted feeding schedule. One group was fed lab chow pellets and sucrose pellets, and the other group was maintained on sucrose chow and fat chow (Table 1). Clonidine (20 nmoles) or saline were administered into the PVN at the start of the test and at the start of the fourth hour of food access.

Results and Discussion

As shown in Table 2, PVN injection of CLON, relative to the saline baseline, produced a significant enhancement of total food intake during the first hour after injection, as well as over the entire 6-hr feeding period. This main drug effect, which is similar in magnitude to that seen with NE tested at 40 nmoles [35,54], was observed despite already high food intake baseline scores of 40–90 Kcal. The effectiveness of CLON at the 6-hr interval, showing a significant change in total daily food ingestion, reveals that PVN noradrenergic stimulation may modulate long-term, as well as short-term, feeding patterns [11, 33, 34, 49].

Analyses of the food intake scores for the separate diets available to the rats revealed, for both sets of diets, a significant drug × diet interaction, at the 0–60 min and 0–360 min time intervals. (For the sucrose pellet versus lab chow pellet diets, $F(1,9) = 63.9$, $p<0.001$ at the 0–60 min interval and $F(1,9) = 34.8$, $p<0.001$ at the 0–360 interval. For the sucrose chow versus fat chow diets, $F(1,9) = 32.3$, $p<0.001$ and $F(1,9) = 8.80$, $p<0.01$, respectively.) In all cases, this significant interaction was attributed predominantly to a selective increase in consumption of the diet richer in carbohydrate (Table 2). At both time intervals, individual *t*-test comparisons between the saline and CLON scores revealed a significant CLON-induced potentiation (ranging from 27 to 192%) of sucrose pellet (100% car-

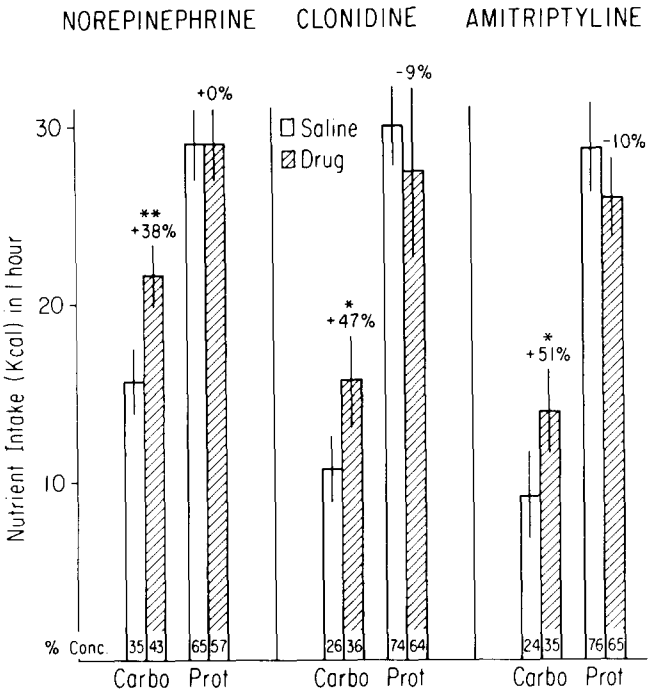

FIG. 2. Impact of PVN injection of norepinephrine (20 nmoles), clonidine (20 nmoles), and amitriptyline (50 nmoles) on nutrient (Kcal ± SEM) intake. Rats (N = 12) with cannulas aimed at the PVN were maintained on a 6-hr restricted feeding schedule. During the 6-hr period of food access, they were permitted to select from two diets, namely, a carbohydrate (CARBO) diet composed of dextrin with 10% corn oil and a protein (PROT) diet composed of casein with 10% corn oil. Scores presented are from the first hour of the 6-hr feeding period. Percent concentration (% conc.) refers to the numbers within the bars, which indicate the percent of one diet eaten relative to the total diet consumed (carbohydrate + protein). Values above the standard error bars indicate the percent difference between the drug and saline scores. Direct comparisons between these Kcal intake scores yielded statistically significant differences at $p<0.05$ (*) and $p<0.01$ (**).

bohydrate) and sucrose chow (65% carbohydrate) ingestion. No significant change in consumption of the lab chow pellet and fat chow diet was detected, except at the 0–60 min interval where fat chow intake was significantly reduced by 23%. These results are essentially identical to those produced by PVN injection of NE [35,54], suggesting that both agents may be acting through similar brain systems to control diet selection [40].

Experiment 2: Effects of PVN Injections of NE, CLON, and AMIT on Selection of Carbohydrate Versus Protein Diets

In previous two-diet tests with the pure protein nutrient casein and the carbohydrate nutrient dextrin, NE injected into the PVN has been shown to selectively enhance the rats' consumption of the carbohydrate ration [35,54]. The present experiment repeated these tests with NE and, in addition, tested the effects of CLON and AMIT for comparison with the effects of NE. A group of 12 PVN-cannulated rats were used, and the three drugs or saline were injected directly into the PVN of rats maintained on a 6-hr restricted feeding schedule. During the 6-hr feeding period, these rats were permitted to select from two diets, namely, casein mixed with 10% fat and dextrin also mixed with 10% fat (Table 1). Norepinephrine (20 nmoles), CLON (20 nmoles), AMIT (50 nmoles), or saline were administered at the start of the test and at the start of the fourth hour of food availability.

Results and Discussion

The food intake scores obtained during the first hour of this test are presented in Fig. 2. Analyses of total calorie intake revealed a small but significant (+13%, $p<0.05$) increase after NE injection. Separate analyses for CLON and AMIT revealed no reliable change. Despite this lack of effect on total food intake, all 3 drugs were found to significantly enhance selection of the carbohydrate diet, dextrin. A significant drug × diet interaction was obtained for NE ($F(1,11)=5.90$, $p<0.05$), CLON ($F(1,11)=6.25$, $p<0.05$), and AMIT ($F(1,11)=16.3$, $p<0.005$), and individual comparisons between the drug and saline Kcal scores revealed a reliable enhancement of carbohydrate intake for NE (+38%, $p<0.005$), CLON (+47%, $p<0.05$), and AMIT (+51%, $p<0.05$), with no change or a slight reduction in protein consumption. To relate the scores for ingestion of one diet to the scores for the other diet, percent concentration values were calculated according to the following formula: (Intake (Kcal) of diet 1)/(Total intake (Kcal for diet 1 + diet 2) × 100. As shown in Fig. 2, the animals, during the first hour after saline injection, selected 25–35% of their diet from the carbohydrate ration and 65–75% from the protein ration. In response to NE, CLON, and AMIT injection, the proportion of carbohydrate selected by the rats was significantly increased ($p<0.05$), by approximately 10 percentage points.

These results confirm those obtained with NE in previous studies [35,54] and are consistent with the results of the two-diet test in Experiment 1, which showed CLON to preferentially enhance selection of the diet richer in carbohydrate. Analyses of the 6-hr calorie intake scores of the present experiment revealed a similar but somewhat smaller effect of these 3 drugs on carbohydrate intake, approximately a 20% increase ($p<0.05$), with no change in protein intake.

Experiment 3: Impact of Peripheral Injection of CLON and Antidepressant Drugs on Selection of Carbohydrate, Protein and Fat

Using a two-diet, self-selection feeding paradigm, the previous experiment demonstrated that PVN injection of CLON and AMIT selectively enhances ingestion of carbohydrate. The present experiment was designed to determine, using a three-diet condition, whether these two drugs and the antidepressant CIMIP, when injected *peripherally*, produce effects similar to those obtained with PVN injection.

For this experiment, 3 diets were used to separately monitor selection of carbohydrate, protein, and fat (Table 1). As described in the Method section, the carbohydrate diet contained a mixture of sucrose, dextrin, and corn starch; the protein diet consisted predominantly of casein, and the fat diet was predominantly lard. For these tests, the animals were maintained ad lib on the 3 separate diets, and nutrient intake measurements were taken at 1 and 2 hr after drug or saline injection. (The 1-hr scores were similar to but not as consistent as the 2-hr scores; thus, only the 2-hr scores are reported here.) All drugs were administered intraperitoneally in this experiment, and each rat (total N = 23) re-

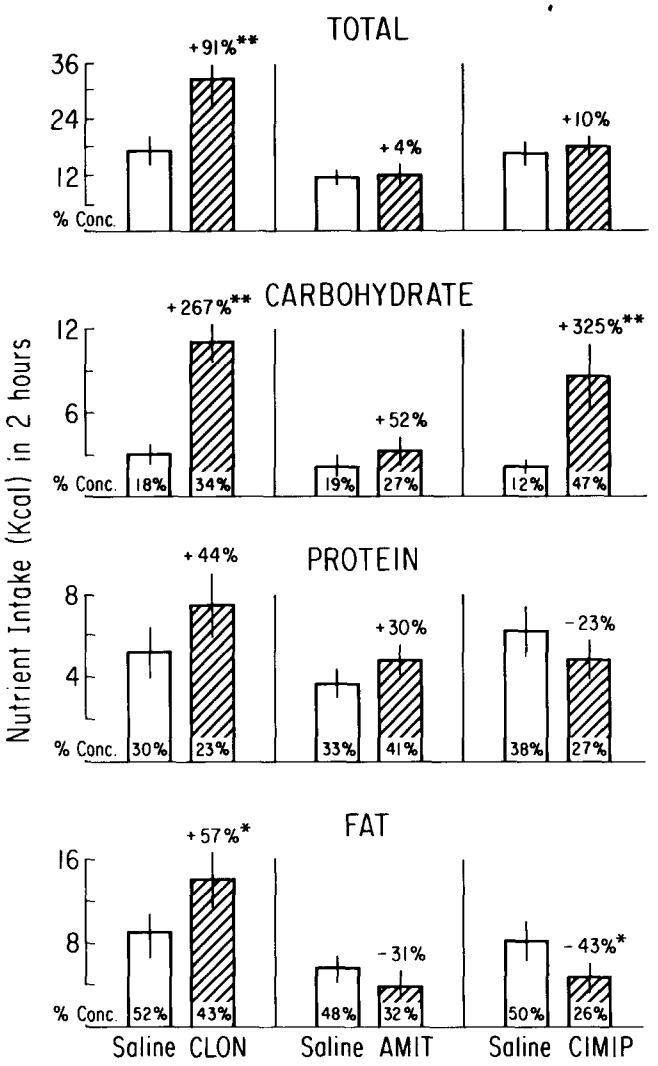

FIG. 3. Effect of peripheral injection of clonidine (CLON, 0.025 mg/kg), amitriptyline (AMIT, 1 mg/kg), and chlorimipramine (CIMIP, 4 mg/kg) on nutrient (Kcal ± SEM) intake. Rats (N = 23) were maintained ad lib on 3 separate sources of carbohydrate (sucrose, dextrin, and corn starch), protein (casein), and fat (lard). During a 2-hr test after drug or saline injection, they were permitted to select from these 3 nutrients. Percent concentration (% conc.) refers to the numbers within the bars, which indicate the percent of one nutrient eaten relative to the total diet consumed (carbohydrate + protein + fat). Values above the standard error bars reflect the percent differences between the drug and saline nutrient intake scores. Direct comparisons between these Kcal ± SEM scores yielded statistically significant differences at $p<0.05$ (*) and $p<0.01$ (**).

ceived a series of tests on two drugs, either CLON and AMIT (N = 10) or CLON and CIMIP (N = 13). These drugs or saline were injected once, at the start of the test, in the following doses: CLON, 0.025 mg/kg; AMIT, 1 mg/kg; and CIMIP, 4 mg/kg.

Results and Discussion

As shown in Fig. 3, the rats' baseline pattern of nutrient selection can be seen in the percent concentration scores (% conc.) for saline treatment. (This score indicates percent of one diet eaten relative to total diet eaten.) On the average, the rats ate approximately 16% of their diet in the form of carbohydrate, 34% as protein, and 50% as fat. These 2-hr scores are generally consistent with the scores obtained for a 24-hr period, except that the proportion of carbohydrate eaten over 24 hr increased slightly to 20% and protein decreased to 30%. Thus, each of the 3 diets was eaten in significant quantities, resulting in normal body weight gain as assessed during the initial few weeks (see Procedure section).

After peripheral injection of CLON (0.025 mg/kg), this pattern of nutrient selection shifted significantly, toward a substantial increase in consumption of the carbohydrate diet. The analysis of variance revealed a significant drug effect on total diet intake, +91%, $F(1,22)=40.8$, $p<0.001$, and a significant drug × diet interaction, $F(2,42)=3.09$, $p<0.05$. Carbohydrate intake was dramatically increased by 267% ($p<0.001$), consequently increasing the proportion of carbohydrate selected relative to the other nutrients (percent concentration) from 18% to 34% ($p<0.001$). Clonidine had little effect on protein intake, resulting in a decrease in percent protein concentration from 30% to 23% ($p>0.10$). Fat intake was significantly increased by 57% ($p<0.05$); however, this effect was considerably smaller (at $p<0.02$) than the potentiation of carbohydrate intake (+267%) and, consequently, a significant decline in percent fat concentration (from 53% to 43%, $p<0.05$) was obtained.

Similar analyses of calorie intake scores for the AMIT test revealed a significant drug × diet interaction, $F(1,18)=3.98$, $p<0.05$. However, there was no main drug effect, and comparisons between saline and AMIT scores for the 3 separate diets yielded no significant changes in nutrient selection. Although centrally injected antidepressants are effective in stimulating total food intake [27], we have tested a variety of these drugs (AMIT, CIMIP, protriptyline, desipramine, and tranylcypromine) with peripheral injection and, consistent with the literature [4,43], have failed to observe any stimulatory effect (Leibowitz, Brown and McCabe, unpublished data). In fact, with peripheral administration of these antidepressants, we have frequently observed an inhibition of feeding, particularly at doses higher than 5 mg/kg. In the present study, AMIT was tested at a dose of 1 mg/kg, to avoid the feeding inhibition at higher doses. This dose had no effect on total calorie intake and produced a small (+52%) but insignificant increase in carbohydrate intake. Out of the 10 rats tested, 5 appeared to show a more consistent and selective effect on carbohydrate ingestion, whereas the other 5 were unresponsive. Thus it remains possible that this lack of a reliable change in nutrient selection after AMIT injection may be due to the fact that this drug was tested at a sub-threshold dose.

For this reason, we tested CIMIP at a somewhat higher dose (4 mg/kg). In preliminary tests, this drug, relative to AMIT, appeared to cause less of an inhibition of total food intake, and, at 4 mg/kg, total food intake scores appeared normal. The results obtained in the present experiment (Fig. 3) also revealed no main drug effect; however, a significant drug × diet interaction was obtained, $F(2,24)=5.55$, $p<0.01$, and individual comparisons between saline and CIMIP scores revealed a significant potentiation of carbohydrate ingestion (+325%, $p<0.01$) and a significant decline in fat ingestion (−43%, $p<0.05$). This pattern of effects increased the percent concentration of carbohydrate consumed from 12% to 47% ($p<0.01$) and decreased the percent fat from 50% to 26% ($p<0.05$). Protein intake ap-

peared to be essentially unaffected by peripheral CIMIP injection. Thus, these findings basically confirm those obtained in Experiment 2 with PVN injection of the antidepressant AMIT.

GENERAL DISCUSSION

These results, obtained with the self-selection feeding paradigm, indicate that the α-adrenergic agonist CLON and the tricyclic antidepressants AMIT and CIMIP produce a large and selective increase in the ingestion of carbohydrate. This enhancing effect on carbohydrate intake can be observed under a variety of conditions: (1) with peripheral drug injection, as well as with injection into the hypothalamic PVN where exogenous NE acts to potentiate carbohydrate ingestion; (2) under both ad lib and restricted feeding conditions; (3) with mixed diets as well as with pure dietary nutrients; (4) in either the absence or the presence of a change in total calorie intake, indicating a potential dissociation of the mechanisms controlling total energy versus specific nutrient intake; and (5) in a short test, single-meal situation (1 hr) as well as over a longer period of time (6 hr) involving a sequence of meals. Similar tests conducted with other drugs known to stimulate food intake (namely, yohimbine and cyproheptadine) have revealed that these compounds, in contrast to NE, CLON, and the antidepressants, preferentially enhance ingestion of protein and fat ([52]; Leibowitz and Brown, unpublished data). This evidence indicates that at least some degree of specificity exists for the selective carbohydrate stimulation observed with noradrenergic agonists and suggests that hypothalamic (PVN) NE may play a role not only in the control of total calorie intake but also in control of a specific nutrient intake, namely, carbohydrate [35,54].

These results demonstrate that the stimulating effect of NE, CLON, and the antidepressants on carbohydrate is not restricted to a specific experimental condition but, rather, becomes apparent under a variety of circumstances. This evidence becomes important in providing a more solid foundation for generalizing our results and formulating hypotheses concerning the role of brain neurotransmitter systems in control of nutrient selection. There is relatively little information available on this issue, since only recently have investigators asked the pertinent questions and appropriately shifted their focus from total calorie intake to specific nutrient intake [2, 3, 6, 7, 13–16, 26, 36, 38, 44, 45, 48, 56, 57]. Although there exist some inconsistencies in the results obtained, perhaps due to the varied diets and feeding conditions employed [6, 36, 44, 56], specific hypotheses have emerged. Concerning the role of brain serotonin, this neurotransmitter is believed to regulate either protein intake or the ratio of protein/carbohydrate, with serotonin preferentially inhibiting carbohydrate intake [2, 3, 5, 6, 36, 56, 57]. With regard to brain catecholamines and nutrient selection, Anderson and Ashley [2, 3, 36] have proposed that these neurotransmitters are involved more generally in control of total energy consumption. This hypothesis has been based primarily on a correlational study showing a positive relationship between total food intake (as opposed to specific nutrient intake) and plasma levels of the amino acids believed to affect brain catecholamine synthesis. Unfortunately, there are insufficient pharmacological or anatomical data available to permit an adequate evaluation of this hypothesis. There are very few studies with catecholaminergic drugs and a general lack of direct and systematic analyses of specific hypotheses. The drug that has received the greatest attention is the anorectic agent amphetamine, which is believed to act through the release of brain catecholamines, perhaps within the perifornical lateral hypothalamus [20, 21, 24]. The evidence to date suggests that amphetamine may preferentially inhibit protein intake in man as well as in rats [6, 7, 30, 38], although under certain conditions fat ingestion may ultimately be affected [44]. At present, the significance of these results remains to be explained. They do not appear, however, to be directly relevant to the present study, which focuses specifically on the PVN. This nucleus does not seem to be involved in amphetamine's actions, since direct injection of amphetamine into this nucleus has little or no effect on feeding [20], and electrolytic lesions leave intact or even potentiate the anorectic effect of peripheral amphetamine [39].

In one other study with a catecholaminergic drug, Mauron *et al.* [37] tested peripherally injected CLON in weanling rats. In contrast to the present study in adult rats, these investigators found CLON to potentiate the ingestion of high protein diets as compared with low protein diets. (Percent protein concentration, however, did not appear to be affected in any of the experiments.) Based on these results, Mauron *et al.* suggested that CLON has a preferential effect on the ingestion of protein. Although there are several differences between the present study and that of Mauron *et al.*, there are two in particular which may explain our differential results. The first is the age and growth of the experimental subjects. Mauron *et al.* tested weanling rats, as opposed to the adult rats (350–400 g) used in this study. The contrasting effects of CLON on nutrient selection may be a function of age and may possibly reflect the differences in dietary needs and controlling brain mechanisms of weanling versus adult rats. Consistent with this intriguing possibility is the evidence that, in weanling rats maintained on low protein diets, CLON is totally ineffective in altering either total food intake or nutrient selection [37]. In contrast, in adult rats, CLON stimulates ingestion of a wide variety of diets ([7, 8, 10, 40, 50], and the present study), including (and particularly) sucrose pellets which contain no protein. The second main difference between these two studies is the diets used. Mauron *et al.* tested a variety of mixed diets, whereas the present study examined selection of macronutrients from separate dietary sources. The mixed diets of Mauron *et al.* permitted comparisons between high and low protein concentrations, but, unfortunately, did not allow fat intake to be separately analyzed, and, furthermore, carbohydrate was studied in only one test, a test in which CLON was found to be ineffective in altering total food intake. Thus, to determine whether weanling and adult rats actually differ in their responsiveness to CLON, it will be essential to examine CLON in weanling rats maintained on separate sources of the 3 macronutrients, to permit a direct comparison with the results of the present study. It may be noted that, in adult rats tested on lab chow powder diets mixed with sugar or fat, peripherally injected CLON has also been found to enhance preferential intake of the high carbohydrate mixture [10], similar to its effect on ingestion of the pure carbohydrate diet.

The task of determining whether specific neurotransmitter mechanisms of the brain are involved in control of nutrient selection ultimately requires that localized brain manipulations be investigated. In our initial report [35,54], we examined the impact of PVN NE injection on the ingestion of a variety of diets, using the self-selection feeding par-

adigm. The results of this investigation, which were confirmed in Experiment 2 of the present study, demonstrated a selective potentiation of carbohydrate ingestion with NE injection. This finding led to the hypothesis that noradrenergic neurons terminating in the PVN play a role in regulating carbohydrate selection [35,54]. The findings of the present study support this hypothesis by revealing a similar effect with PVN or peripheral injection of CLON or the tricyclic antidepressants. Earlier work with CLON has shown it to stimulate total food intake after injection into the PVN [40], and a similar effect observed with peripheral CLON has been found to be abolished by localized electrolytic PVN lesions [40]. Thus, it appears that the PVN might be a primary site in the mediation of CLON-induced hyperphagia, and the present results indicate that the PVN noradrenergic receptors, upon which CLON appears to act, are specifically involved in eliciting carbohydrate ingestion.

With regard to the effect of the tricyclic antidepressants, these drugs have been shown to stimulate total calorie intake after PVN injection. Pharmacological studies of this phenomenon suggest that, although these drugs may affect several monoamines, their stimulatory action on food intake involves specifically the release of endogenous NE [27]. In light of these findings, the present results, showing similar effects with the antidepressants and exogenous NE on carbohydrate intake, support the existence of an endogenous noradrenergic mechanism, within the PVN, which regulates or modulates selection of this nutrient. These findings offer a potential explanation for the clinical evidence revealing carbohydrate craving in patients treated with AMIT [42,46] or CIMIP [17]. Both of these drugs have been used in the treatment of anorexia nervosa, an eating disorder with a particular disturbance in consumption of carbohydrate [17, 42, 46]. Furthermore, a convergence of evidence obtained in the rat has recently led to the proposal that anorexia nervosa may, in part, reflect a disturbance in central noradrenergic function, specifically within the medial hypothalamus [25,26].

Based upon a convergence of physiological, neurochemical, and endocrinological evidence, we have recently proposed that NE may mediate eating behavior and specifically carbohydrate ingestion in response to a physiological need, that is, under conditions of increased energy expenditure [24,54]. Such conditions may be food deprivation, stress, activity, and cold temperature. Each of these states would be expected to diminish energy stores in the brain and body, and, through the release of brain (as well as peripheral) NE, they are predicted to initiate various adaptive responses, including ingestion of carbohydrate, a nutrient most rapidly converted to usable energy. Whereas this hypothesis requires more systematic investigation and direct analysis, the possibility of such a physiological mechanism receives indirect support from the present results obtained with CLON and with the antidepressants which enhance the synaptic availability of endogenous NE. Additional supporting evidence is obtained from a series of studies with localized hindbrain lesions [28,29]. These investigations indicate that damage to specific noradrenergic fibers innervating the PVN produce several related effects, including abolishing the antidepressant-induced eating response, suppressing spontaneous ingestion of sucrose, and inhibiting compensatory food intake which occurs in response to food deprivation.

Consistent with the above hypothesis is the evidence that these hindbrain lesions also suppress eating behavior induced by insulin and 2-deoxy-D-glucose [28]. Both of these agents have been found to have a very potent and generally selective stimulatory effect on carbohydrate ingestion in rats [10, 12, 15, 16, 46, 52] and in humans [53]. They also both affect carbohydrate metabolism, with 2-DG blocking intracellular glucose utilization and insulin enhancing tissue uptake of glucose and metabolism of glucose. It has been demonstrated that both insulin and 2-DG potentiate the release of endogenous NE in the area of the PVN [41]. Furthermore, NE injected into the PVN is an effective stimulus for the release of insulin [9], as well as for stimulating carbohydrate intake. While hypothalamic NE may not be the sole or even primary mediator of insulin- and 2-DG-inducing eating, the convergence of evidence supports the hypothesis that noradrenergic neurons innervating the medial hypothalamus are involved in modulating or coordinating the process of energy (glucose) ingestion and metabolism. Consistent with this idea is the evidence that NE-induced eating is closely associated with circulating levels of corticosterone, a hormone whose primary function is the control of blood glucose [32]. In Experiments 1 and 2 of the present investigation, NE, CLON, and AMIT were found to affect carbohydrate ingestion over a 6-hr (as well as 1-hr) interval, during which the rats were required to ingest their total daily ration. This indicates that hypothalamic (PVN) NE may interact with processes controlling long-term feeding patterns, as well as determine the size and composition of an individual meal [11, 24, 33, 34, 49].

ADDENDUM

While this manuscript was in press, a report by Storlien and his colleagues has appeared in *Pharmacology, Biochemistry and Behavior* (Vol. 22, Pages 119–125 (1985)), which describes the effects of chronic amitriptyline treatment on ingestion of a 24% sucrose solution in rats. Consistent with the present report, this study by Storlien *et al.* showed that peripherally-injected amitriptyline, while having no effect on total calorie intake, increased sucrose ingestion, and this effect was positively correlated with circulating levels of insulin. Corticosterone levels were also elevated by amitriptyline and suppressed dramatically by ingestion of carbohydrate.

ACKNOWLEDGMENTS

This research was supported by grant MH 22879 and by funds from the Whitehall Foundation. We wish to thank Amend, Boehringer Ingelheim, CIBA-Geigy, and Merck Sharp and Dohme for their generous supply of drugs used in this research. We also want to thank Mr. John Lai for his assistance in performing the computer statistical analyses.

REFERENCES

1. Anderson, G. H. Regulation of protein intake by plasma amino acids. *Adv Nutr Res* **1:** 145-166, 1977.

2. Anderson, G. H. Control of protein and energy intake: Role of plasma amino acids and brain neurotransmitters. *Can J Physiol Pharmacol* **57:** 1043-1057, 1979.

3. Ashley, D. V. M., D. V. Coscina and H. G. Anderson. Selective decrease in protein intake following brain serotonin depletion. *Life Sci* **24:** 973-984, 1979.
4. Blavet, N. and F. V. DeFeudis. Inhibition of food intake in the rat by antidepressants. *Pharmacol Res Commun* **14:** 663-669, 1982.
5. Blundell, J. E. Is there a role for serotonin (5-hydroxytryptamine) in feeding? *Int J Obesity* **1:** 15-42, 1977.
6. 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. 164-221.
7. Blundell, J. E. and P. J. Rogers. Effects of anorexic drugs on food intake, food selection and preferences and hunger motivation and subjective experiences. *Appetite* **1:** 151-165, 1980.
8. Broekkamp, C. and J. M. Van Rossum. Clonidine induced intrahypothalamic stimulation of eating in rats. *Psychopharmacologia* **25:** 162-168, 1972.
9. de Jong, A., J. H. Strubbe and A. B. Steffens. Hypothalamic influence on insulin and glucagon release in the rat. *Am J Physiol* **233:** E380-E388, 1977.
10. Fahrbach, J. E., J. R. Tretter, P. F. Aravich, J. McCabe and S. F. Leibowitz. Increased carbohydrate preference in the rat after injection of 2-deoxy-D-glucose and clonidine. *Soc Neurosci Abstr* **6:** 784, 1980.
11. Grinker, J., C. Marinescu and S. F. Leibowitz. Effects of central injections of neurotransmitters and drugs on freely-feeding rats. *Soc Neurosci Abstr* **8:** 604, 1982.
12. Jacobs, H. L. Studies on sugar preference: 1. The preference for glucose solutions and its modification by injections of insulin. *J Comp Physiol Psychol* **51:** 304-310, 1958.
13. Jacobs, H. L. and K. N. Sharma. Taste vs. calories: Sensory and metabolic signals in the control of food intake. *Ann NY Acad Sci* **157:** 1084-1125, 1969.
14. Johnson, D. J., E. T. S. Li, D. V. Coscina and G. H. Anderson. Different diurnal rhythms of protein and non-protein energy intake in rats. *Physiol Behav* **21:** 777-780, 1979.
15. Kanarek, R. B., R. Marks-Kaufman and B. J. Lipeles. Increased carbohydrate as a function of insulin administration in rats. *Physiol Behav* **25:** 779-782, 1980.
16. Kanarek, R. B., R. Marks-Kaufman, R. Ruthazer and L. Gualtieri. Increased carbohydrate consumption by rats as a function of 2-deoxy-D-glucose administration. *Pharmacol Biochem Behav* **18:** 47-50, 1983.
17. Lacey, S. H. and A. H. Crisp. Hunger, food intake and weight: The impact of clomipramine on a refeeding anorexia nervosa population. *Postgrad Med J* **56:** Suppl 1, 79-85, 1980.
18. Lat, J. Self-selection of dietary components. In: *Handbook of Physiology, Vol. 1, Alimentary Canal*, edited by C. F. Code. Washington, DC: American Physiological Society, 1976.
19. Leibowitz, S. F. Pattern of drinking and feeding produced by hypothalamic norepinephrine injection in the satiated rat. *Physiol Behav* **14:** 731-742, 1975.
20. Leibowitz, S. F. Amphetamine: Possible site and mode of action for producing anorexia in the rat. *Brain Res* **84:** 160-167, 1975.
21. Leibowitz, S. F. Catecholaminergic mechanisms of the lateral hypothalamus: Their role in the mediation of amphetamine anorexia. *Brain Res* **98:** 529-545, 1975.
22. Leibowitz, S. F. Paraventricular nucleus: A primary site mediating adrenergic stimulation of feeding and drinking. *Pharmacol Biochem Behav* **8:** 163-175, 1978.
23. Leibowitz, S. F. Adrenergic stimulation of the paraventricular nucleus and its effects on ingestive behavior as a function of the drug dose and time of injection in the light-dark cycle. *Brain Res Bull* **3:** 357-363, 1978.
24. Leibowitz, S. F. Neurochemical systems of the hypothalamus: Control of feeding and drinking behavior and water-electrolyte excretion. In: *Handbook of the Hypothalamus, Vol. 1, Part A, Behavioral Studies of the Hypothalamus*, edited by P. J. Morgane and J. Panksepp. New York: Marcel Dekker, 1980, pp. 299-437.
25. Leibowitz, S. F. Brain monoamine projections and receptor systems in relation to food intake, diet preference, meal patterns, and body weight. In: *Neuroendocrinology of Psychiatric Disorder*, edited by G. M. Brown, S. H. Koslow and S. Reichlin. New York: Raven Press, 1984, pp. 383-399.
26. Leibowitz, S. F. Noradrenergic function in the medial hypothalamus: Potential relation to anorexia nervosa and bulimia. In: *The Psychobiology of Anorexia Nervosa*, edited by K. M. Pirke and D. Ploog. Berlin: Springer Verlag, 1984, pp. 35-45.
27. Leibowitz, S. F., A. Arcomano and N. J. Hammer. Potentiation of eating associated with tricyclic antidepressant drug activation of α-adrenergic neurons in the paraventricular hypothalamus. *Progr Neuro-Psychopharmacol* **2:** 349-358, 1978.
28. Leibowitz, S. F. and L. L. Brown. Analysis of behavioral deficits produced by lesions in the dorsal and ventral midbrain tegmentum. *Physiol Behav* **25:** 829-843, 1980.
29. Leibowitz, S. F. and L. L. Brown. Histochemical and pharmacological analysis of noradrenergic projections to the paraventricular hypothalamus in relation to feeding stimulation. *Brain Res* **201:** 289-314, 1980.
30. Leibowitz, S. F., O. Brown and J. R. Tretter. Peripheral and hypothalamic injections of α-adrenergic and dopaminergic receptor drugs have specific effects on nutrient selection in rats. *Proceedings of 53rd Annual Meeting of the Eastern Psychological Association* 1982, p. 136.
31. Leibowitz, S. F., N. J. Hammer and K. Chang. Feeding behavior induced by central norepinephrine injection is attenuated by discrete lesions of the hypothalamic paraventricular nucleus. *Pharmacol Biochem Behav* **19:** 945-950, 1983.
32. Leibowitz, S. F., C. R. Roland, L. Hor and V. Squillari. Noradrenergic feeding elicited via the paraventricular nucleus is dependent upon circulating corticosterone. *Physiol Behav* **32:** 857-864, 1984.
33. Leibowitz, S. F., P. Roossin 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.
34. Lichenstein, S. S., C. Marinescu and S. F. Leibowitz. Chronic infusion of norepinephrine and clonidine into the hypothalamic paraventricular nucleus. *Brain Res Bull* **13:** 591-595, 1984.
35. Leibowitz, S. F., G. F. Weiss, F. Yee and J. B. Tretter. Noradrenergic innervation of the paraventricular nucleus: Specific role in control of carbohydrate ingestion. *Brain Res Bull* **14:** 561-567, 1985.
36. Li, E. T. S. and G. H. Anderson. 5-Hydroxytryptamine. A modulator of food composition but not quantity? *Life Sci* **34:** 2453-2460, 1984.
37. Mauron, C., J. J. Wurtman and R. J. Wurtman. Clonidine increases food and protein consumption in rats. *Life Sci* **27:** 781-791, 1980.
38. McArthur, R. A. and J. E. Blundell. Protein and carbohydrate self-selection: Modification of the effects of fenfluramine and amphetamine by age and feeding regimen. *Appetite* **4:** 113-124, 1983.
39. McCabe, J. T. and S. F. Leibowitz. Determination of the course of brainstem catecholamine fibers mediating amphetamine anorexia. *Brain Res* **311:** 211-224, 1984.
40. McCabe, J. T., M. deBellis and S. F. Leibowitz. Clonidine-induced feeding: Analysis of central sites of action and fiber projections mediating this response. *Brain Res* **309:** 85-104, 1984.
41. McCaleb, M. L., R. D. Myers and G. Singer. Hypothalamic norepinephrine in the rat during feeding and push-pull perfusion with glucose, 2-DG, or insulin. *Am J Physiol* **236:** 312-321, 1978.
42. Needleman, H. L. and D. Waber. Amitriptyline therapy in patients with anorexia nervosa. *Lancet* **2:** 580, 1976.

43. Nobrega, J. N. and D. V. Coscina. Effects of chronic antidepressant treatment on feeding behavior in rats. In: *The Neural Basis of Feeding and Reward*, edited by B. G. Hoebel and D. Novin. Maine: Haer Institute, 1982, pp. 525-534.
44. Orthen-Gambill, N. and R. B. Kanarek. Differential effects of amphetamine and fenfluramine on dietary self-selection in rats. *Pharmacol Biochm Behav* **16:** 303-309, 1982.
45. Overmann, S. R. Dietary self-selection by animals. *Psychol Bull* **83:** 218-235, 1976.
46. Paykel, E. S., P. S. Muelter and P. M. de la Vergue. Amitriptyline, weight gain and carbohydrate craving: A side effect. *Br J Psychiatry* **123:** 501-507, 1973.
47. Richter, C. P. Increased dextrose appetite of normal rats treated with insulin. *Am J Physiol* **135:** 781-787, 1942.
48. Richter, C. P. Total self-regulatory functions in animals and human beings. *Harvey Lect* **38:** 63-103, 1943.
49. Ritter, R. C. and A. N. Epstein. Control of meal size by central noradrenergic action. *Proc Natl Acad Sci USA* **72:** 3740-3743, 1975.
50. Ritter, S., C. D. Wise and L. Stein. Neurochemical regulation of feeding in the rat: Facilitation by α-noradrenergic, but not dopaminergic, receptor stimulants. *J Comp Physiol Psychol* **88:** 778-784, 1975.
51. Schlemmer, R. F., Jr., R. C. Casper, N. Narasimhachari and J. M. Davis. Clonidine-induced hyperphagia and weight gain in monkeys. *Psychopharmacology (Berlin)* **61:** 233-234, 1979.
52. Shor-Posner, G., A. Azar and S. F. Leibowitz. Electrolytic paraventricular nucleus (PVN) lesions and feeding behavior: Relation to food restriction, drugs and corticosterone. *Soc Neurosci Abstr* **10:** 302, 1984.
53. Thompson, D. A. and R. G. Campbell. Hunger in humans induced by 2-deoxy-D-glucose: Glucoprivic control of taste preference and food intake. *Science* **198:** 1065-1068, 1977.
54. Tretter, J. B. and S. L. Leibowitz. Specific increase in carbohydrate consumption after norepinephrine (NE) injection into the paraventricular nucleus (PVN). *Soc Neurosci Abstr* **6:** 532, 1980.
55. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1962.
56. Wurtman, J. J. and R. J. Wurtman. Fenfluramine and fluoxetine spare protein consumption while suppressing caloric intake by rats. *Science* **198:** 1178-1180, 1977.
57. Wurtman, J. J. and R. J. Wurtman. Drugs that enhance central serotoninergic transmission diminish elective carbohydrate consumption by rats. *Life Sci* **24:** 895-904, 1979.