Pharmacology Biochemistry & Behavior, Vol 24, pp 931–939, 1986 © Ankho International Inc Printed in the U S A 0091-3057/86 $3 00 + 00

Morphine-Stimulated Feeding: Analysis of Macronutrient Selection and Paraventricular Nucleus Lesions

GAIL SHOR-POSNER,[1] ANTHONY P. AZAR, ROLAND FILART, DONNA TEMPEL AND SARAH F. LEIBOWITZ

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

Received 19 February 1985

SHOR-POSNER, G , A P AZAR, R FILART, D TEMPEL AND S F LEIBOWITZ *Morphine-stimulated feeding Analysis of macronutrient selection and paraventricular nucleus lesions* PHARMACOL BIOCHEM BEHAV 24(4) 931–939, 1986 —The hypothalamic paraventricular nucleus (PVN) has been found to be sensitive to the feeding stimulatory effects of opiates The present experiments investigated the effect of systemic morphine (2 mg/kg) on macronutrient selection in freely-feeding and food-restricted rats and assessed the impact of PVN electrolytic and 6-hydroxydopamine lesions on the rats' ability to respond to peripheral morphine injection In satiated rats, maintained ad lib on pure macronutrient diets, morphine increased food intake This effect was associated with a preferential increase in protein ingestion, carbohydrate consumption, compared with fat and protein intake, was least affected. In food-restricted rats, permitted to eat for 6 hr, morphine instead produced a particular preference for fat, with no significant enhancement of total calorie intake. While PVN 6-hydroxydopamine lesions, which depleted PVN catecholamine levels by 70%, failed to alter morphine-stimulated feeding, electrolytic lesions of the PVN significantly attenuated this response, particularly protein and fat ingestion This suggests that opiate-induced feeding may, *in part*, be mediated through the PVN, which is known to have an important function in the control of food ingestion

Hypothalamus Paraventricular nucleus Feeding behavior Lesions Macronutrient selection Morphine

CONSIDERABLE evidence for the involvement of opiates in the regulation of feeding has accumulated over the past several years. Injection of opiate agonists or peptides has been shown to increase food intake [2, 9, 18, 21, 28, 31, 33, 38, 47], in contrast to reports of reduced consumption in food-deprived rats following the administration of opiate antagonists [10, 11, 12, 14, 27, 33, 39].

Recent investigations have revealed a stimulatory effect of medial hypothalamic injection of opiates on food intake [9, 19, 21, 31, 45, 48, 51] supporting the concept that specific brain opiate systems are involved in feeding behavior. In particular, the hypothalamic paraventricular nucleus (PVN) has specifically been found to be sensitive to both the feeding-stimulatory effects of morphine, as well as other opiate agonists, and also to the suppressive effects of opiate antagonists [8, 19, 21, 31, 45, 51].

The main objective of the present study was to determine, through PVN lesions, whether this nucleus is essential for morphine-induced feeding. An additional objective was to examine the effect of peripherally administered morphine on diet selection in freely-feeding satiated animals. In previous studies, morphine has been found to modify the selection of macronutrients in food-restricted rats, causing a particular enhancement of fat ingestion [29,30]. This evidence has suggested that, in addition to regulating total caloric intake, the opiates may be involved in the control of nutrient selection. The present study examined this question further, in both satiated vs. food-deprived animals and in intact vs PVN-lesioned animals The results obtained have been presented in a preliminary form at the Satellite Symposium of the Society for Neuroscience meeting, 1984 [43]

METHOD

Animals

Adult male Sprague-Dawley rats (n=104) weighing 350–400 g at the start of the experiment, were used They were housed individually and tested in their home cages. The animals were kept on a constant light-dark cycle, with the 12 hr light phase beginning at 0700 hr, followed by a 12 hr dark phase

Diet

All rats, unless otherwise indicated, had food and water

[1]Requests for reprints should be addressed to Dr Gail Shor-Posner

available ad lib and were allowed to select their food from separate, pure macronutrient diets of protein, carbohydrate and fat The carbohydrate ration was composed of 37% sucrose, 28% dextrin (ICN Pharmaceuticals), 28% cornstarch, 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 protein ration was composed of casein (National Casein Co) plus 4% minerals, 2 97% vitamins and 0.03% cysteine The fat component consisted of lard (Armour) mixed with 8% minerals, 5 92% vitamins and 0.08% cysteine 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 simultaneously in 3 glass food cups that were held against the side of the cage by a bolted metal spring This method generally prevented food spillage The placement of the food cups within the cage was changed daily to prevent position preference, and fresh diet was provided every other day

Surgery

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 inter-aural 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 lesion electrode Daily measurements of food intake and diet selection were recorded during the first 2 weeks following surgery Body weights were taken twice a week

An additional group of rats was studied in Experiment 3, to determine the effect of neurotoxin-induced catecholamine depletion on feeding elicited by morphine Each rat was stereotaxically implanted under pentobarbital anesthesia, and neurochemically specific lesions were made by using the CA 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-gauge hypodermic tubing with a wire insert for the top It was slowly infused over a 4 min period at a rate of 0.1 μl/15 sec To allow for dispersion of the 6-OHDA, the injector was then allowed to remain in the brain for 2 minutes before being removed An equal volume of the saline-ascorbic acid vehicle was injected into rats of the control group. The stereotaxic coordinates used for all injections 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.

Test Procedures

The effect of morphine on total food intake and diet selection was investigated in the following experiments. A relatively low dose (2 mg/kg) of morphine sulfate (Merck) was dissolved in physiological saline immediately before the start of the test and injected intraperitoneally, in a volume of 0.5 cc The saline vehicle for morphine served as the control injection and was administered in counterbalanced order with morphine and on separate days No tolerance to the appetite-stimulatory effect of morphine was observed in repeated tests with this mild dose, and, unless otherwise noted, food intake scores reported in the Results section represent the average of 2–4 test scores

EXPERIMENT 1 MORPHINE EFFECTS IN FREELY-FEEDING AND FOOD-RESTRICTED RATS

In this experiment, the effect of morphine on feeding during the daytime and at the onset of the dark period was determined in rats maintained ad lib on pure macronutrient diets Following a 3 week adaptation period to the diets and laboratory conditions, freely-feeding rats were tested either at 1200 hr (n=9) or 1800 hr (n=9) During a 1-hr pretest period, the rats were given fresh food to insure maximal satiation At the end of this pretest hour, the animals were injected with drug or vehicle (2–4 tests) and then immediately given measured food Food intake and diet selection were measured for 2 hr

To determine the effect of morphine on diet preference in food-restricted rats, 2 additional groups of animals were adapted to the diets, and then placed on a restricted feeding schedule. On this schedule, food was made available for 6 hr, either during the light phase (1230–1830 hr) or the dark phase (1900–0100 hr). Following an additional 2-week adaptation period to food-restriction, the light-fed rats (n=12) at 1230 hr and the dark-fed rats (n=9) at 1900 hr were tested once each with morphine and vehicle and food intake measurements taken after the first and sixth hour.

EXPERIMENT 2 MORPHINE EFFECTS IN RATS WITH PVN ELECTROLYTIC LESIONS

Thirteen lesion rats, with bilateral damage to the PVN, and 11 sham-operated rats were studied in this experiment. These rats were given at least 2 weeks of post-operative recovery before testing was begun Tests were conducted during the daytime (1200–1600 hr), following a 1-hr satiation period with fresh diet At the end of this pre-test hour, the rats were given either morphine or saline vehicle (2–4 tests) and food intake measurements taken 3 hr later

EXPERIMENT 3 MORPHINE EFFECTS IN RATS WITH PVN 6-OHDA LESIONS

Six-hydroxydopamine-injected rats, with marked CA depletion in the PVN (n=21), and ascorbic-acid control rats (n=20) were tested in this experiment. Drug testing was begun after a 3 week post-operative period. Animals were first satiated on fresh diet during a pre-treatment hour and then injected with drug or saline vehicle (2–4 tests) during the daytime (1200–1400 hr) Food intake measurements were taken 2 hr later

Histological Analysis

Animals with electrolytic PVN lesions were sacrificed under pentobarbital anesthesia They were perfused through the heart with isotonic saline, followed by a buffered solution of 10% Formalin The brain was removed, frozen sections (50 μ) were cut, and alternate sections stained with cresyl violet The area of totally ablated tissue was determined using the Konig and Klippel atlas [16] as a guide. Only

TABLE 1

MORPHINE-ELICITED FEEDING (Kcal) IN FOOD-RESTRICTED RATS

		Total	Protein	Fat	Carbohydrate
First Hour					
Light-fed	Saline	35 5 ± 3 0	4 8 ± 0 5	21 9 ± 2 4	8 8 ± 1 1
6 hr (n=12)	Morphine	30 6 ± 5 7	5 6 ± 1 3	22 4 ± 5 2	2 6 ± 1 0
Dark-fed	Saline	20 7 ± 2 6	4 4 ± 0 7	13 4 ± 2 7	2 9 ± 0 6
6 hr (n=9)	Morphine	33 3 ± 3 9*	7 9 ± 1 3	25 0 ± 4 3*	0 4 ± 0 0*
Six Hours					
Light-fed	Saline	108 8 ± 5 0	14 9 ± 1 4	65 6 ± 4 1	28.3 ± 3.1
(n=12)	Morphine	80 9 ± 3 8†	11 9 ± 0 7	59 5 ± 3 3	11 0 ± 2 7*
Dark-fed	Saline	82 9 ± 6 4	23 3 ± 2 9	46 4 ± 5 4	13 2 ± 2 0
(n=9)	Morphine	91 7 ± 3 8	21 5 ± 3 4	66 5 ± 3 5†	3 7 ± 0 9†

Statistical comparisons between morphine and saline scores (means ± standard error given) revealed significant differences at *$p<0.05$, †$p<0.01$

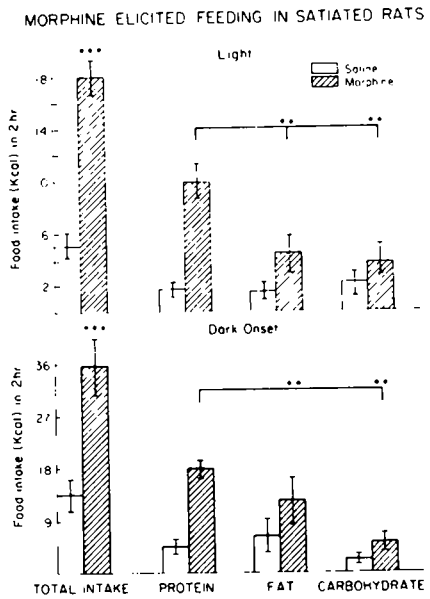

FIG 1 Compared with saline baseline, morphine injected peripherally, elicited a dramatic increase in feeding in intact animals (***$p<0.001$) Analyses of difference scores (drug minus saline) indicated that this enhancement was associated with a specific increase in protein ingestion, relative to fat and carbohydrate (**$p<0.01$) in rats tested during the light period or at the onset of the dark period

animals with bilateral lesions confirmed by histology to be within the PVN are included in the Results described below There were 13 such rats and 11 sham-operated controls Animals with misplaced lesions (n=7) were eliminated from analysis.

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 [6]. 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 scale of 1–5 (1 indicating near total lack of fluorescence and 5 indicating high normal fluorescence), modified after Fuxe [7]. Each area was separately rated by making side-by-side comparisons between ascorbic acid and 6-OHDA injected rats. 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 included in the Results described below exhibited marked CA depletion in the PVN (fluorescence rating "2"). Animals that sustained only minimal depletion (n=14), were eliminated from analysis

Statistical Analysis

Results were evaluated by 2-way analysis of variance (ANOVA) Analysis of macronutrient selection was determined by taking difference scores (drug minus baseline feeding), and then evaluated using single-factor analysis of variance for repeated measures, followed by appropriate post-hoc tests for individual mean comparisons [52]. A two-tailed Student's t-test for dependent samples was used to compare relative intakes (percentage scores) in freely-feeding intact animals (Experiment 1), independent samples were used in the comparison of lesion and control group intakes (Experiment 2), and for histochemical ratings between ascorbic acid and 6-OHDA animals.

RESULTS

Effect of Morphine in Freely-Feeding Animals

Morphine (2 mg/kg), injected peripherally into intact,

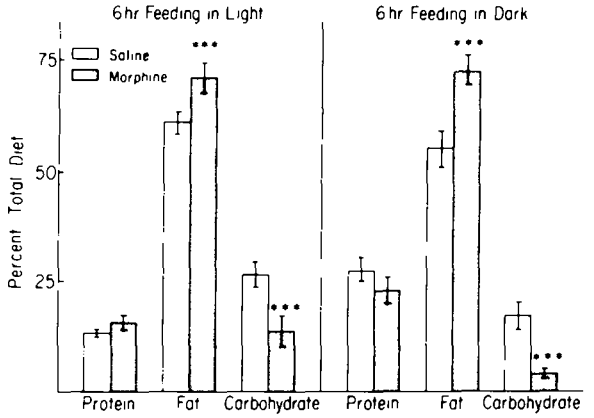

FIG 2 Effect of peripherally injected morphine on nutrient selection in food-restricted rats A significant increase (***$p<0.01$) in the percentage of total calories consumed as fat was demonstrated in both light- and dark-fed animals In contrast, the percent of carbohydrate ingested was significantly reduced (***$p<0.01$) during both time periods

fully satiated rats, had a significant stimulatory effect on food consumption. As shown in Fig. 1, morphine dramatically increased feeding during the light period, $F(1,8)=63.8$, $p<0.001$, as well as at the onset of the dark, $F(1,8)=52.37$, $p<0.001$ As indicated in Fig 1, morphine had a differential effect on diet selection, for light, $F(2,16)=5.79$, $p<0.01$· for dark onset, $F(3,24)=23.31$, $p<0.001$. Analyses of difference scores (drug minus saline feeding) revealed that these significant diet effects were associated with a preferential increase in consumption of protein, relative to fat and carbohydrate, in the daytime, $F(1,16)=11.2$, $p<0.01$, and at the beginning of the dark period, $F(1,16)=16.9$, $p<0.01$. The consumption of carbohydrate, compared with the ingestion of fat and protein, was least affected ($p<0.05$) in these tests Representation of these Kcal scores in terms of the relative intake of protein, fat and carbohydrate, i.e , the *percentage* of total calories consumed of each macronutrient, clearly indicates the effect of morphine on macronutrient selection Whereas the percentage of carbohydrate and fat ingestion was not significantly altered following morphine administration, the percent protein intake increased from 32.6% to 58.4% ($p<0.05$) during the daytime and from 39.6% to 57.2% ($p<0.10$), at the onset of the dark period

Effect of Morphine in Food-Restricted Rats

As shown in Table 1, for the one and six-hr food intake measures, peripherally injected morphine (2 mg/kg) produced only a transient potentiation in dark-fed rats, $F(1,8)=7.9$, $p<0.02$, and over the 6-hr feeding period, failed to enhance feeding in food-restricted animals This is not unexpected in light of the high saline baseline feeding scores exhibited by these animals. In fact, the light-fed animals actually showed a significant decrease in food intake in the 6-hr measurement, $F(1,11)=30.16$, $p<0.001$ In contrast to the tests with freely-feeding satiated rats (Fig 1), protein ingestion was not specifically enhanced in food-restricted rats The major stimulatory effect of morphine, as indicated in Table 1, appeared to be in terms of fat ingestion This effect was particularly apparent in animals fed in the dark, at one hour, $F(1,16)=8.3$, $p<0.05$, and across the 6-hr feeding period, $F(1,16)=22.0$, $p<0.01$ Carbohydrate, in contrast, was dramatically and significantly decreased by morphine injection during the 6-hr feeding period in both the light—($F(1,22)=8.77$, $p<0.05$ and dark—fed animals, $F(1,16)=11.5$, $p<0.01$

Representation of these Kcal scores in terms of the percentage of total calories consumed of each macronutrient, shows more clearly the effect of morphine on fat ingestion Calculating these scores ("Percent Total Diet") as shown in Fig 2, we observe a significant morphine-induced increase in percent fat intake in both the light, $F(1,22)=14.9$, $p<0.01$, and dark-fed animals, $F(1,16)=22.4$, $p<0.01$, in contrast to a signficant decrease in percent carbohydrate intake in both groups, light, $F(1,22)=23.0$, $p<0.01$, dark, $F(1,16)=12.2$, $p<0.01$

Effect of Morphine in Rats with PVN Electrolytic Lesions

Feeding is known to be elicited by injections of morphine directly into the PVN [19, 31, 51]. In this experiment, we assessed the impact of bilateral PVN electrolytic lesions on feeding induced by peripheral injections of morphine (2 mg/kg) Figure 3 shows representative bilateral lesions of these animals In all animals (n=13), the PVN was found to be completely damaged As shown in previous studies [23, 40, 42], analyses of 24-hr food intake and body weight gain during the first week following surgery showed that the bilateral PVN lesions caused a reliable increase in 24 hr food intake (+62% $p<0.01$), which led to a significantly greater body weight gain, attributed primarily to an increase in carbohydrate ingestion (+102% $p<0.01$) This effect on macronutrient selection was observed throughout all postoperative weeks

As indicated in Fig 4, sham-operated animals, just like intact rats in Experiment 1, exhibited a robust morphine-induced feeding response, $F(1,10)=45.4$, $p<0.001$ A differential effect on diet selection, $F(2,20)=13.8$, $p<0.001$, was similarly associated with a preferential increase in consumption of protein, relative to fat and carbohydrate consumption, $F(1,20)=27.99$, $p<0.01$ Moreover, the percent protein intake increased from 30.4% to 53.7% ($p<0.01$) following morphine administration, while the percentage of carbohydrate and fat was decreased in these animals Electrolytic lesion of the PVN significantly attenuated, but did not abolish, morphine-induced feeding, $F(1,12)=24.29$, $p<0.001$ Similar to sham animals, morphine stimulated feeding in lesion rats was associated with a significant drug × diet interaction, $F(2,24)=5.1$, $p<0.01$ In contrast to the protein enhancement observed in sham animals, however, morphine-stimulated feeding in lesion rats was attributed to an increase in the consumption of both carbohydrate and protein compared to significantly less consumption of fat, $F(1,24)=10.0$, $p<0.05$ Furthermore, analyses of difference scores (drug minus saline feeding) between the lesion and sham animals revealed significant differences between the groups, $F(1,22)=4.77$, $p<0.04$, as well as their diet interactions, $F(2,44)=3.6$, $p<0.04$ As shown in Fig 4, control animals consumed significantly more Kcal (11.7±1.8) following morphine administration than did lesion rats (6.9±1.8 Kcal), indicating a 41% decrease in responsiveness ($p<0.05$) Similarly, morphine's stimulatory effect on protein intake was significantly reduced by 48% ($p<0.01$) in lesion rats, relative to sham animals (3.7±0.6 vs 7.1±0.7) In addition, discrete electrolytic lesions reduced by 91% ($p<0.05$) the

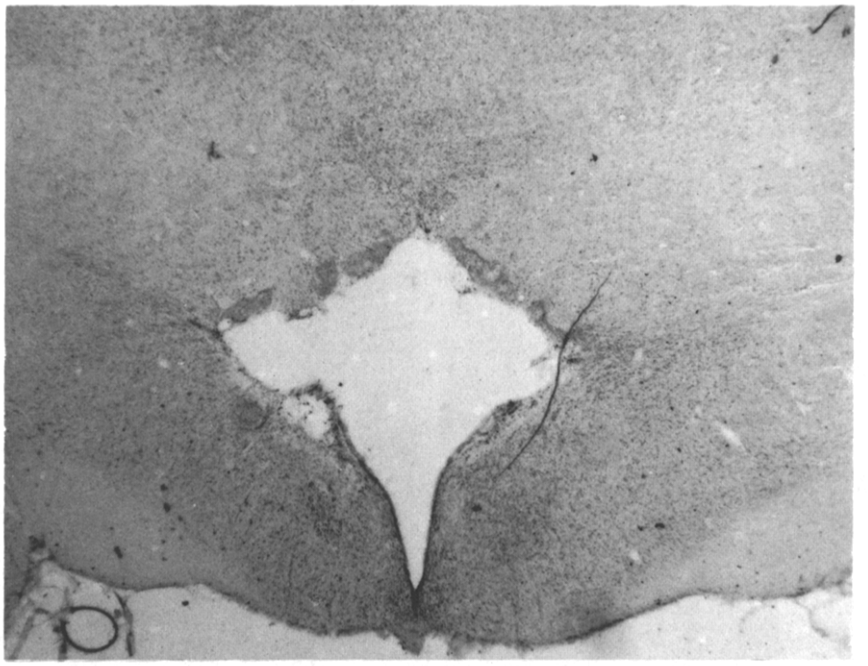

FIG 3 Photomicrograph of frontal, cresyl violet-stained section of the rat brain showing representative bilateral lesion in the area of the paraventricular nucleus (PVN)

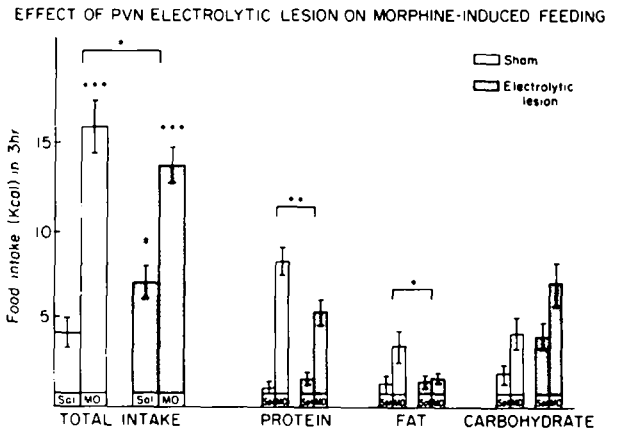

FIG 4. Morphine elicited a significant increase in total food intake (***$p<0.001$) in sham and lesion rats (despite a higher baseline feeding †$p<0.05$) Values above the standard error bar reflect the differences between drug and saline intake scores Compared to sham animals, PVN lesion rats, demonstrated a smaller enhancement (*$p<0.05$) in total intake that was attributed to smaller protein (**$p<0.01$) and fat ingestion (*$p<0.05$)

small stimulatory effect on fat intake observed in satiated, sham-operated animals

Effect of Morphine in PVN 6-OHDA-Lesioned Rats

To evaluate the importance of endogenous hypothalamic norepinephrine in feeding elicited by peripheral morphine, we examined the impact of PVN 6-OHDA lesions on morphine's stimulatory effect. Histochemical analysis of the ascorbic-acid and 6-OHDA rats failed to reveal any change in extra-hypothalamic catecholamine (CA) fluorescence, observed in the striatum, stria terminalis, caudate and median eminence. Within the hypothalamus, as shown in the photomicrographs of Fig. 5, the damage to CA innervation was largely confined to the medial aspect of this structure and was clearly greatest in the PVN (Figs. 5a, 5b). In this nucleus, an average rating of 4 1±0 1 was obtained for the intact animals as compared to 1.9±0.1 for the 6-OHDA rats ($p<0.01$). Similar damage from the lesion extended into the medial preoptic area and the anterior portion of the dorsomedial nucleus. However, CA innervation of the middle and caudal dorsomedial nucleus (Fig. 5c reflecting average group rating of 4.1±0.1 for ascorbic acid animals, versus Fig 5d, reflecting average 6-OHDA group rating of 3.6±0.1), the lateral hypothalamus just lateral to the PVN, the zona incerta and the more caudal perifornical region (Fig 5e, average ascorbic-acid group rating of 4.1±0.1 versus Fig. 5f, average 6-OHDA group rating 4.0±0.0), appeared essentially intact. Biochemical analysis of this 6-OHDA lesion [44] has shown a significant decrease in NE content within the PVN (−70%, $p<0.01$).

Similar to the intact animals in Fig. 1 and the sham rats in Fig 4, morphine injected into the ascorbic-acid vehicle rats (Fig. 6) significantly increased total caloric intake above the saline baseline level, $F(1,19)=174.7$, $p<0.001$, and this robust stimulatory effect was associated with an alteration in diet selection, $F(2,38)=4\ 28$, $p<0\ 02$. Analyses of difference scores (drug minus saline feeding) revealed this alteration was attributed to a preferential increase in protein consumption, $F(1,38)=11.3$, $p<0.01$. As shown in Fig. 6, PVN injection of 6-OHDA had no apparent impact on this pattern of food ingestion Rats with reduced CA innervation to the

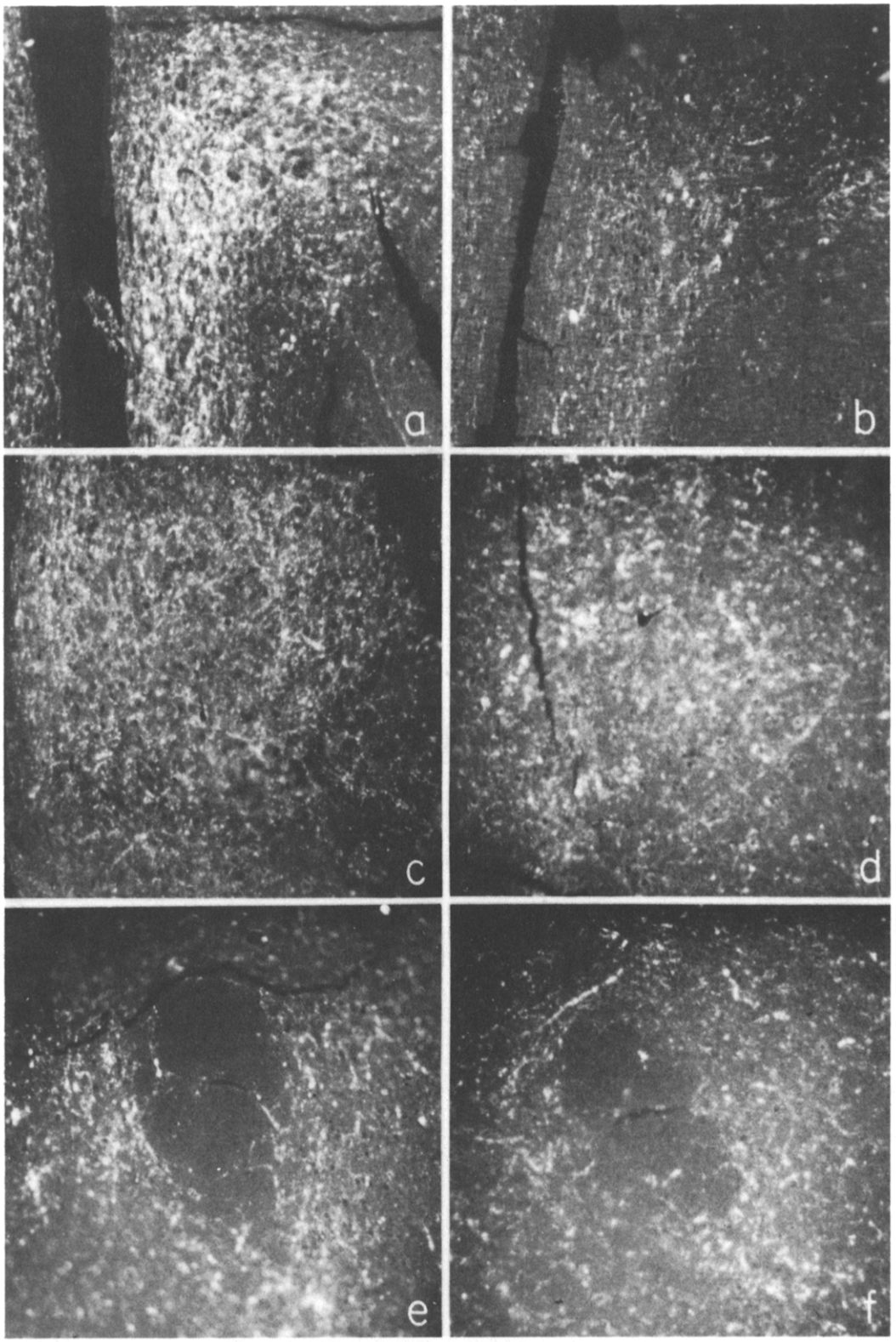

FIG 5 Fluorescence photomicrographs showing catecholamine varicosities in the hypothalamus (a) Normal CA innervation of the PVN of a vehicle-injected rat (b) Marked reduction in a rat after 6-OHDA was injected in the PVN Catecholamine innervation remained essentially intact in other brain areas such as the caudal dorsomedial nucleus (compare normal (c) and (d) 6-OHDA rat), and the caudal perifornical area as shown in a normal (e) and 6-OHDA injected animal (f)

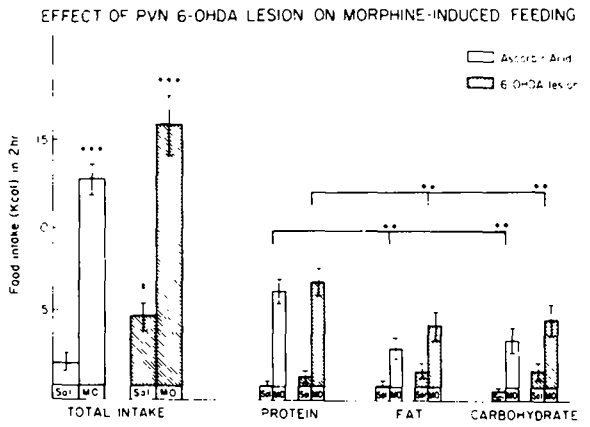

FIG 6 Compared with ascorbic-acid control animals, 6-OHDA lesion rats, with a higher baseline feeding ($\ddagger p<0.05$), demonstrated a similar pattern in nutrient selection and total intake following morphine injection A robust enhancement of feeding ($^{***}p<0.001$), associated primarily with increased protein ingestion, relative to carbohydrate and fat ($^{**}p<0.01$) was observed in both groups Values above the standard error bars reflect the difference between drug and saline intake scores

PVN exhibited a comparable morphine-stimulated increase in total diet, $F(1,20)=58.13$, $p<0.001$. A significant drug × diet interaction, $F(2,40)=3.22$, $p<0.05$, revealed in these animals, a similar, preferential increase in protein consumption, $F(1,40)=5.32$, $p<0.01$

DISCUSSION

The results of this study indicate that a low dose of morphine (2 mg/kg), peripherally injected into freely-feeding fully satiated animals, produces a large and reliable increase in feeding behavior, confirming the findings of previous studies [2, 12, 37]. Higher doses of morphine (>5 mg/kg) have been shown to be less effective and may actually decrease food intake, possibly due to sedation [5, 32, 35, 37]. In food-restricted animals, we failed to observe this stimulatory effect of morphine, which is in agreement with previous reports in food-restricted [28,29] and food-deprived animals [32,37]. While this failure of response may reflect some deprivation-induced alteration in endogenous neurochemicals, which in turn may affect sensitivity to opiates, a more parsimonious explanation is that it simply reflects the difference in baselines, where lower baseline feeding reveals the effect more readily than the higher baseline conditions

Regarding the stimulatory effect of morphine on diet preference, we have found morphine to have specific effects on the animals' macronutrient selection. Peripherally injected morphine preferentially increases protein consumption in satiated rats This is, to date, the only study to our knowledge in which a low dose of morphine that stimulates feeding has been tested in freely-feeding rats maintained on a self-selection paradigm. The robust protein enhancement we observed in these satiated rats is clearly different from the pattern of nutrient selection observed in food-restricted rats. In dark-fed restricted animals, we observed an enhancement of fat intake (Kcal), and in both dark- and light-fed restricted groups, an increase in percent fat intake (% of total calories consumed as fat) was revealed. This modification in nutrient selection shown by our restricted rats confirms earlier findings of Marks-Kaufman [29] and Marks-Kaufman and Kanarek [30], demonstrating a morphine-induced enhancement of fat consumption, with no significant increase in total intake, under similar conditions. In a recent study with chronic injection of very high doses of morphine (20 and 40 mg/kg) in freely-feeding animals, neither an increase in total food intake nor any preferential enhancement of protein selection was observed [34]. Instead, the percentage of calories consumed as fat was increased. These contrasting results may be due to at least three factors: differences in drug dose, chronic versus acute treatment, and differences in baseline feeding conditions In the Ottaviani and Riley study [34], animals ate very little protein (2.5%) in contrast to the freely-feeding rats in the present study, who consumed approximately 35% of their baseline intake as protein. Moreover, the high dose used in the Ottaviani and Riley study may have produced a taste aversion to protein (Riley, personal communication) With a low dose of acute morphine, the potentiation of protein is clearly demonstrated in animals under freely-feeding conditions This is quite different from the effect of morphine on carbohydrate selection, which is *least* affected in all feeding conditions and, in some cases, may actually be suppressed, as demonstrated in the present study and in previous reports using restricted feeding schedules or high doses of the drug [29,30].

The significant effect of morphine on food consumption may be of central origin. Recent studies have revealed a stimulatory effect of opiate drugs on feeding when administered directly into the medial hypothalamus [9, 19, 21, 31, 45, 47, 51] In particular, the PVN has specifically been found to be sensitive to local injection of morphine [19, 31, 51], as well as to other opiate agonists [21, 31, 41, 45], implicating this nucleus in opiate control of feeding behavior. In support of this suggestion is the finding that electrolytic PVN lesions in the present study significantly attenuated morphine-induced feeding. This attenuation in total food intake amounted to approximately a 40% reduction in morphine's overall effectiveness. In particular, discrete electrolytic lesions greatly reduced morphine-potentiated protein ingestion (−48%) and in addition, failed to elicit any increase in fat consumption. This is of particular interest in light of recent findings from this laboratory that have revealed an opiate-stimulatory effect on macronutrient selection after central drug administration In these studies, satiated rats injected with opiate agonists directly into the PVN, exhibit a robust stimulation of protein and fat consumption (Leibowitz and Daniel, unpublished data).

Thus, the PVN appears to play a role in mediating this opiate-elicited feeding response However, the partial attenuation indicates further, that other brain areas, in addition to the PVN, may also be involved. One possible brain site may be the dorsomedial nucleus, where electrolytic lesions attenuate anorexia induced by peripheral naloxone [3] In conflict with this are data demonstrating that naloxone has no effect on feeding when injected directly into this nucleus [51]. Another possibility is the ventromedial hypothalamus, reported to be responsive to morphine stimulation [47,48] Further studies of this nucleus, however, suggest that its apparent sensitivity to morphine may result from drug diffusion to the PVN, a considerably more sensitive site [51]. A third possibility is the perifornical lateral hypothalamus, which also appears to be very sensitive to morphine stimulation [51]. This is of interest in view of the finding that catecholamine stimulation in this area has a preferential effect on

protein intake [22], which may be related to morphine's stimulatory influence on protein ingestion Morphine at higher doses potentiates the turnover of norepinephrine and dopamine in the perifornical hypothalamus [13]

The diminished morphine-induced feeding response observed in animals with electrolytic PVN lesions, may reflect damage to NE pathways that function in feeding regulation Considerable evidence provides support for an interaction between noradrenergic and opiate systems in the potentiation of food intake. For example, both opiate and α-2-noradrenergic stimulation are effective within the same brain area, namely the PVN [20, 21, 31, 51], their eating stimulatory effect is blocked by antagonists of α-2-noradrenergic receptor sites [21, 31, 48], and peripheral injection of morphine enhances NE turnover in the PVN [13] Nevertheless, we found no change in responsiveness to morphine in animals that received direct 6-OHDA lesions These lesions significantly reduced PVN NE by 70%, suggesting that endogenous NE may not be crucial to this feeding effect However, until a complete lesion is produced, it is possible that the remaining 30% of endogenous NE in 6-OHDA animals may have been sufficient to mediate the morphine feeding response

It is clear from this work and others [29, 30, 34], that in addition to increasing food intake, the opiate system has a specific effect on macronutrient selection The noradrenergic system of the PVN has also been implicated in the modulation of appetite regulation, producing a specific, albeit different, effect on nutrient selection In contrast to the morphine-stimulated, protein ingestion exhibited in this study, noradrenergic stimulation has been shown to increase the selection of carbohydrate [25,49], while actually inhibiting consumption of protein Recent findings suggest that both these systems may become activated during the early dark hours, when food-seeking behavior and ingestion are most pronounced in the rat At this critical time, a sharp increase in α-2 receptor sites within the PVN is revealed [24], as well as an increased sensitivity to α-noradrenergic receptor stimulation [4] Similarly, a circadian variation in brain opioid levels has been reported [15,50], and morphine-elicited feeding, like noradrenergic feeding, is strongest at the start of the dark period [4] The involvement of an opiate-noradrenergic association during this critical period is further implicated by the finding that both NE and morphine feeding are critically dependent upon circulating corticosterone [4, 26, 35] and that blood levels of this glucocorticoid peak just at the onset of the dark period [17] Preliminary behavioral studies indicate that freely-feeding rats maintained on a self-selection paradigm exhibit specific patterns in nutrient selection during the early part of the night [46] In the first few hours of the dark period, rats are observed to consume meals composed primarily of carbohydrate, followed by a shift towards protein The possibility exists that this pattern of macronutrient meals may in part be determined by a change in hypothalamic neurochemistry, with a noradrenergic activation followed by opiate activation It is important to note that the complex regulation of nutrient selection may include additional neurochemical mechanisms, such as a serotonin-nutrient feedback system [1,53], as well as the involvement of brain reward processes that function in feeding behavior

ACKNOWLEDGEMENTS

This research was supported by USPHS grant MH 22879 and by funds from the Whitehall Foundation We gratefully acknowledge the excellent technical assistance of Messrs Dan Dwyer and Eric Gunther

REFERENCES

1 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
2 Ayhan, I H and A Randrup Behavioral and pharmacological studies on morphine-induced excitation of rats possible relation to brain catecholamines *Pharmacology* **29**: 317–328, 1973
3 Bellinger, L L , L L Bernardis and F E Williams Naloxone suppression of food and water intake and cholecystokinin reduction of feeding is attenuated in weanling rats with dorsomedial hypothalamic lesions *Physiol Behav* **31**: 839–846, 1983
4 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
5 Cooper, S J Behaviorally-specific hyperdipsia in the non-deprived rat following acute morphine treatment *Neuropharmacology* **20**: 469–472, 1981
6 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
7 Fuxe, K Cellular localization of monoamines in the median eminence and the infundibular stem of some mammals *Z Zellforsch* **61**: 710–724, 1964
8 Gosnell, B G , J E Morley and A S Levine Localization of naloxone-sensitive brain areas in relation to food intake *Soc Neurosci Abstr* **10**: 306, 1984
9 Grandison, L and A Guidotti Stimulation of food intake by muscimol and beta endorphin *Neuropharmacology* **16**: 533–536, 1977
10 Holtzman, S G Behavioral effects of separate and combined administration of naloxone and d-amphetamine *J Pharmacol Exp Ther* **189**: 51–60, 1974
11 Hynes, M A , M Gallagher and K V Yacos Systems and intraventricular naloxone administration effects on food and water intake *Behav Neural Biol* **32**: 334–342, 1981
12 Jalowiec, J E , J Panksepp, A Zolovick, N Najam and B Herman Opioid modulation of ingestive behavior *Pharmacol Biochem Behav* **15**: 477–484, 1981
13 Jhanwar-Uniyal, M , J S Woods, B E Levin and S F Leibowitz Cannula mapping and biochemical studies of hypothalamic opiate and noradrenergic systems in relation to eating behavior *Proc Eastern Psychol Assoc* **55**: 106, 1984
14 Jones, J G and J A Richter The site of action of naloxone in suppressing food and water intake in rats *Life Sci* **28**: 2055–2064, 1981
15 Kerdelhue, B , M Karteszi, C Pasqualini, A Reinberg, E Mezey and M Palkovits Circadian variations in B-endorphin concentrations in pituitary and in some brain nuclei of the adult male rat *Brain Res* **261**: 243–248, 1983
16 Konig, J F R and R A Klippel *The Rat Brain, A Stereotaxic Atlas* Baltimore Williams and Wilkins, 1963
17 Krieger, D T and H Hauser Comparison of synchronization of circadian corticosteroid rhythms by photoperiod and food *Proc Natl Acad Sci USA* **75**: 1577–1581, 1978
18 Kumar, R , E Mitchell and I D Stolerman Disturbed patterns of behavior in morphine tolerant and abstinent rats *Br J Pharmacol* **42**: 473–484, 1971

19 Lanthier, D., B G Stanley and S F Leibowitz Feeding elicited by central morphine injection: sites of action in the brain *Appetite* **6**: 296, 1985
20 Leibowitz, S. F. Paraventricular nucleus. A primary site mediating adrenergic stimulation of feeding and drinking *Pharmacol Biochem Behav* **8**: 163–175, 1978
21 Leibowitz, S F and L Hor. Endorphinergic and α-noradrenergic systems in the paraventricular nucleus: Effects on eating behavior *Peptides* **3**: 421–428, 1982
22. 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 *Proc Eastern Psychol Assoc* **53**: 136, 1982
23 Leibowitz, S F , H J Hammer and K Chang Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat *Physiol Behav* **27**: 1031–1040, 1981.
24 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
25 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
26 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
27 Lowy, M T and G K W Yim The anorexic effect of naltrexone is independent of its suppressant effect on water intake *Neuropharmacology* **20**: 883–886, 1981
28 Lowy, M T and G K W Yim Stimulation of food intake following opiate agonists in rats but not hamsters *Psychopharmacology (Berlin)* **81**: 28–32, 1983
29 Marks-Kaufman, R Increased fat consumption induced by morphine administration in rats *Pharmacol Biochem Behav* **16**: 949–955, 1982
30 Marks-Kaufman, R and R B Kanarek Morphine selectively influences macronutrient intake in the rat *Pharmacol Biochem Behav* **12**: 427–430, 1980
31 McLean, S and B G Hoebel Feeding induced by opiates into the paraventricular hypothalamus *Peptides* **4**: 287–292, 1983
32 Morley, J E , A S Levine, M Grace and J Kneip An investigation of the role of kappa opiate receptors in the initiation of feeding *Life Sci* **31**: 2617–2626, 1982
33 Morley, J E , A S Levine, G K W Yim and M T Lowy Opioid modulation of appetite *Neurosci Biobehav Rev* **7**: 281–305, 1983
34 Ottaviani, R and A L Riley Effect of chronic morphine on the self-selection of macronutrients in the rat *Nutr Behav* **2**: 27–36, 1984
35 Riley, A M , K Ortuno, K Hoffman, M Simon and M Heft The role of endorphins in regulatory eating and drinking narcotic-induced hyperphagia and hyperdipsia *Soc Neurosci Abstr* **6**: 783, 1980
36 Roland, C. R , P Bhakthavatsalam and S F Leibowitz Interaction between corticosterone and α-2-noradrenergic system of the paraventricular nucleus in relation to feeding behavior *Neuroendocrinology*, in press
37 Sanger, D J. and P S McCarthy Differential effects of morphine on food and water intake in food deprived and freely-feeding rats *Psychopharmacology (Berlin)* **72**: 103–106, 1980
38 Sanger, D J and P S McCarthy Increased food and water intake produced in rats by opiate receptor agonists *Psychopharmacology (Berlin)* **74**: 217–220, 1981
39 Sanger, D J , P S McCarthy and G Metcalf The effects of opiate antagonists on food intake are stereospecific *Neuropharmacology* **20**: 45–47, 1981
40 Sclafani, A and P F Aravich Macronutrient self-selection in three forms of hypothalamic obesity *Am J Physiol* **244**: R686–R694, 1983
41 Scott, P , K Jawaharial and B G Hoebel Feeding induced with kappa and mu opiate agonist injected in the region of the PVN in rats *Proc Eastern Psychol Assoc* **55**: 30, 1984
42 Shor-Posner, G , A P Azar, Insinga, S and S F Leibowitz. Deficits in the control of food intake after paraventricular nucleus lesions *Physiol Behav* **35**: 883–890, 1985
43 Shor-Posner, G , A P Azar, R Filart and S F Leibowitz Morphine induced feeding in freely-feeding animals impact of paraventricular nucleus lesions *Appetite* **6**: 212–213, 1985
44 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*, in press, 1986
45 Stanley, B G , D Lanthier and S F Leibowitz Feeding elicited by the opiate peptide D-Ala-2-Met-Enkephalinamide sites of action in the brain *Soc Neurosci Abstr* **10**: 1103, 1984
46 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
47 Tepperman, F S and M Hirst Concerning the specificity of the hypothalamic opiate receptor responsible for food intake in the rat *Pharmacol Biochem Behav* **17**: 1141–1144, 1982
48 Tepperman, F S , M. Hirst and W Gowdey A probable role for norepinephrine in feeding after hypothalamic injection of morphine *Pharmacol Biochem Behav* **15**: 555–558, 1981
49 Tretter, J R and S F Leibowitz Specific interest in carbohydrate consumption after norepinephrine (NE) injection into the paraventricular nucleus *Soc Neurosci Abstr* **6**: 532, 1980
50 Wesche, D L and R. C A Frederickson Diurnal differences in opioid peptide levels correlated with nociceptive sensitivity *Life Sci* **24**: 1861–1868, 1979
51 Woods, J S and S F Leibowitz Hypothalamic sites sensitive to morphine and naloxone effects on feeding behavior *Pharmacol Biochem Behav* **23**: 431–438, 1985
52 Winer, B J *Statistical Principles in Experimental Design*, 2nd ed New York McGraw-Hill, 1971, pp 261–271
53 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