Pharmacology Biochemistry & Behavior, Vol. 32, pp. 101–110. © Pergamon Press plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Hypothalamic Norepinephrine Synthesis Rate in Overfed, Underfed and Fasted Mice[1]

BARBARA J. MARRIAGE AND JANICE L. JOHNSTON[1]

Department of Foods and Nutrition, The University of Alberta Edmonton, Alberta, Canada T6G 2M8

Received 30 November 1987

MARRIAGE, B. J. AND J. L. JOHNSTON. *Hypothalamic norepinephrine synthesis rate in overfed, underfed and fasted mice.* PHARMACOL BIOCHEM BEHAV 32(1) 101–110, 1989.—Hypothalamic norepinephrine (NE) synthesis rate during acute (1-day and 3-day) and chronic (11-day) overfeeding (14 kcal/day) and underfeeding (7 kcal/day) and 24 hr food deprivation was determined in four-week-old female mice. Mice were fed ad lib quantity or 51% of ad lib in a meal-feeding paradigm, a 60% fat diet providing constant protein intake across groups. NE synthesis rate, during the thermic effect of a meal, was calculated from the rate of NE accumulation after monamine oxidase inhibition by pargyline and clorgyline. Acute and chronic underfeeding versus overfeeding had no effect on NE synthesis rate in the hypothalamus or in the rest of brain, or chronically in hypothalamic nuclei. In mice deprived of food for 24 hr, NE synthesis rate in the paraventricular nucleus only was five-fold higher than in fed mice. Thus, NE synthesis rate within the hypothalamus appears to be more related to short-term food intake regulation than to the thermic effect of eating or body fat content.

Overfeeding Underfeeding Fasting Norepinephrine Hypothalamus Hypothalamic nuclei

A number of complex mechanisms have been proposed for the regulation of food intake and energy expenditure, several of which have implicated the activity of catecholamine-containing neurons, specifically in the hypothalamus (35). The demonstration that intracerebroventricular injection of norepinephrine (NE) induces feeding, and that moderate depletion of brain NE causes a reduction in ad lib food intake in the rat, suggests that brain NE has a role in the facilitory control of feeding (28). Different regions of the hypothalamus are specialized for different functions, and the paraventricular nucleus (PVN) is the area within the brain where NE injections produce maximal stimulation of feeding (16).

If increased brain noradrenergic activity facilitates feeding, and decreased noradrenergic activity inhibits feeding, the activity of the hypothalamic noradrenergic system and consequent NE turnover should tend to vary as a function of food deprivation and feeding such that food-deprived animals have a relatively high NE turnover. Studies of the effect of food deprivation on noradrenergic activity have yielded conflicting results (11, 21, 33). NE concentration has been reported to be decreased (11,33) or unchanged (21) in the hypothalamus or in hypothalamic areas of 48-hr food-deprived rats. When turnover is assessed, 22-hr food deprivation has been reported to increase (8), or have no effect (27) on NE synthesis rate.

It is possible that the involvement of NE in the regulation of food intake and energy expenditure is site specific, and that in examining the whole hypothalamus, site specific responses are masked. For example, the involvement of hypothalamic noradrenergic activity in diet-induced thermogenesis is suggested by the evidence that electrical stimulation of the ventromedial nucleus (VMH) (30) and intraventricular injections of NE (4) cause lipolysis in the brown adipose tissue (BAT) of rats, as detected by a rise in plasma glycerol concentration and plasma free fatty acids (4,30). However, when noradrenergic activity in specific hypothalamic areas such as the VMH is examined as a possible site for the stimulation of diet-induced thermogenesis, no increase in NE turnover is seen in the VMH of rats chronically overfed for three months, but the turnover of NE in the PVN and dorsomedial nucleus (DM) is higher than in ad lib-fed counterparts (18). Thus, a clear demonstration of the role of NE in the hypothalamus or in hypothalamic nuclei in physiologically relevant conditions of hunger, and acute and chronic overfeeding is lacking.

Thus, the objectives of this study were to investigate the relationships between energy intake, body fat content, food deprivation and NE synthesis rate in the hypothalamus or hypothalamic nuclei of mice. First, the effects of short-term underfeeding and overfeeding on hypothalamic NE synthesis rate in meal-fed mice were examined. Second, the effects of

[1]This research was supported by Natural Sciences and Engineering Research Council of Canada, and the Central Research Fund, The University of Alberta.

[2]Requests for reprints should be addressed to Janice L. Johnston.

TABLE 1
DIET COMPOSITION (g/100 g)

Ingredients	Control	High fat Control	High fat Overfed	High fat Underfed
Casein	23.0	34.3	27.7	52.8
Cellulose	4.0	5.5	5.5	5.4
Corn oil	5.0	16.4	16.4	16.2
Lard	5.0	16.4	16.4	16.2
Glucose	29.2	10.6	13.7	1.5
Starch	29.2	10.6	13.7	1.5
Mineral mix*	3.5	4.8	4.9	4.8
Vitamin mix†	1.0	1.4	1.4	1.4
Choline bitartrate	0.2	0.3	0.3	0.3
Protein (%)‡	20.0	24.3	20.0	37.8
Carbohydrate (%)	60.0	15.7	20.0	2.2
Fat (%)	20.0	60.0	60.0	60.0
Energy density§	15.5 kJ/g	20.6 kJ/g	20.6 kJ/g	20.6 kJ/g

*AIN-76 mineral mixture (2) (ICN Nutritional Biochemicals, Cleveland, OH).

†AIN-76 vitamin mixture (2) (United States Biochemical Corp., Cleveland, OH).

‡% of metabolizable energy.

§Based on 16.8, 16.8 and 37.8 kJ metabolizable energy for protein from casein, carbohydrate from glucose and starch, and fat from corn oil and lard, respectively.

chronic underfeeding and overfeeding (resulting in stable differences in body fat content) in meal-fed mice on NE synthesis rate in the hypothalamus and hypothalamic nuclei were investigated. Third, the effect of food deprivation on NE synthesis rate in specific hypothalamic nuclei was investigated.

METHOD

Animals and Diets

Four-week-old female mice (C57BL/6J, +/+, Jackson Laboratories, Bar Harbor, ME) were used in all experiments. The mice were housed individually in hanging wire-mesh cages in a temperature-controlled room (25±2°C), lights on 0900 to 2100 hr. They had free access to water throughout the experiments. Mice were weighed daily between 0900 and 1000 hr. In all experiments, mice were adapted for one week to a purified diet (Table 1: control), which contained 20% of metabolizable energy as protein, 60% as carbohydrate and 20% as fat. In Experiments 1 and 2, the mice were adapted to meal feeding the control diet in an amount equivalent to ad lib intake (3 g/day), divided into six equal meals delivered by a small animal programmed feeder (Hofer, Switzerland). In Experiments 1, 2, 3, and 4, after one week of adaptation, all mice were either underfed or overfed a high fat diet. In addition, in Experiment 1, a control group of mice was fed the high fat control diet (Table 1: high fat control). In Experiment 5, and for the NE accumulation determination, mice were also fed the high fat control diet (Table 1). All diets provided equal quantities of protein to minimize the variation in protein available for growth, and to limit variability of tyrosine available from dietary sources (9,14). The diets, except for protein and carbohydrate, had equal nutrient densities (Table 1). Mice in the overfed group (Table 1: high fat overfed) received an energy level of 59.6 kJ (14.2 kcal/day) with 0.7 g protein. This amount is equivalent to the ad lib intake of protein and energy on this high fat diet (J. L. Johnston, unpublished observation) and equals 2.9 g of diet/day. The high fat diet was chosen to promote vigorous overfeeding (6). Mice in the underfed group (Table 1: high fat underfed) received an energy level of 30.5 kJ (7.3 kcal/day) with 0.7 g protein. This is equivalent to 51% of the energy intake of the overfed group and equals 1.5 g of diet/day. Mice in the control group in Experiment 1 received 46.9 kJ (11.2 kcal/day) with 0.7 g protein, of the high fat control diet (Table 1). This amount is equivalent to the ad lib energy intake of mice fed the basal high carbohydrate control diet and equals 2.3 g of diet/day.

Experiment Protocol

Experiment 1. Mice in the underfed, control, and overfed groups received for three days their daily intake in six equal meals delivered by a programmed feeder starting at 2300 hr. The last meal was presented at 0900 hr on the fourth experiment day to the undisturbed animals, the overfed mice receiving 0.48 g (2.37 kcal), the controls receiving 0.38 g (1.81 kcal) and the underfed receiving 0.25 g (1.22 kcal). All mice had consumed their entire last meal by 0920 hr. Mice were killed beginning at 0920 hr for determination of NE synthesis rate which was determined from the rate of accumulation of NE after inhibition of NE catabolism by the monoamine oxidase inhibitors pargyline hydrochloride (Sigma Chemical Co., St. Louis, MO) (75 mg/kg) and clorgyline hydrochloride (generous gift of May and Baker Ltd., Dagenham, England) (10 mg/kg). Mice were killed by decapitation at 0, 20, and 40 min after a single IP injection of these inhibitors mixed in physiological saline (10 ml/kg) beginning at 0920 hr. These MAO inhibitors were chosen because two catalytically distinguishable MAO activities have been found to be present in the mouse brain (7). MAO-A activity is sensitive to inhibition by low concentrations of clorgyline, whereas MAO-B is relatively sensitive to pargyline (7). The hypothalamus was dissected along natural lines of demarcation by taking the anterior commissure as the horizontal reference and the line between the posterior hypothalamus and the mammillary bodies as the caudal limit (12). The hypothalamus and the rest of the brain were immediately removed, weighed, wrapped in aluminum foil, frozen on dry ice and held at −40°C for subsequent determination of NE within one month. The carcasses (minus the brain) were frozen until body fat content could be determined.

Experiment 2. Mice were underfed or overfed the high fat diets for one day to eliminate the confounding effect of weight changes, resulting from under- and overfeeding for three days. The six meals per day paradigm was repeated as in Experiment 1. At the end of the one-day experiment, mice were killed and the hypothalamus and the rest of the brain were dissected for subsequent determination of NE as in Experiment 1.

Experiment 3. Mice were underfed or overfed the high fat diet for eleven days to produce chronic changes in body weight and body fat content. Mice received the underfeeding and overfeeding diets as one meal per day presented at 1600 hr until the last day of the experiment when the mice re-

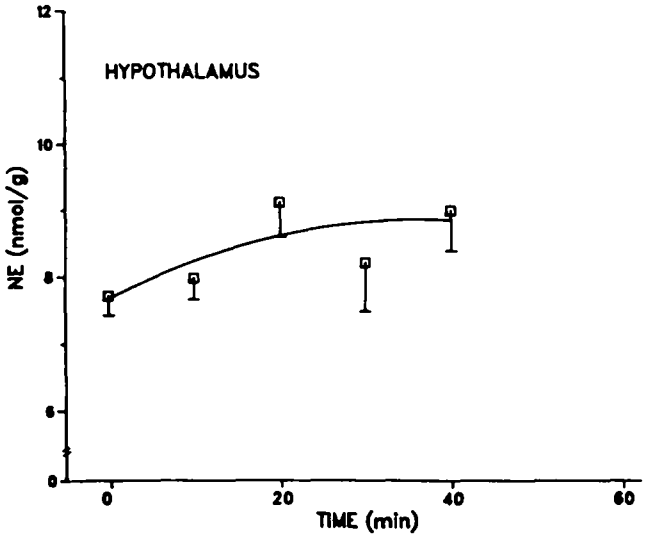


FIG. 1. Accumulation of NE in the hypothalamus after administration of pargyline and clorgyline measured at 0, 10, 20, 30 and 40 min after one week adaptation to a high fat diet to determine the time course for the best linear fit of NE accumulation. Each point represents the mean±SEM of 3–6 mice. The equation for the polynomial fit is $y = -0.894\ (10^{-3})\ x^2 + 0.651\ (10^{-1})\ x + 7.675$, $SE_p = 1.185$.

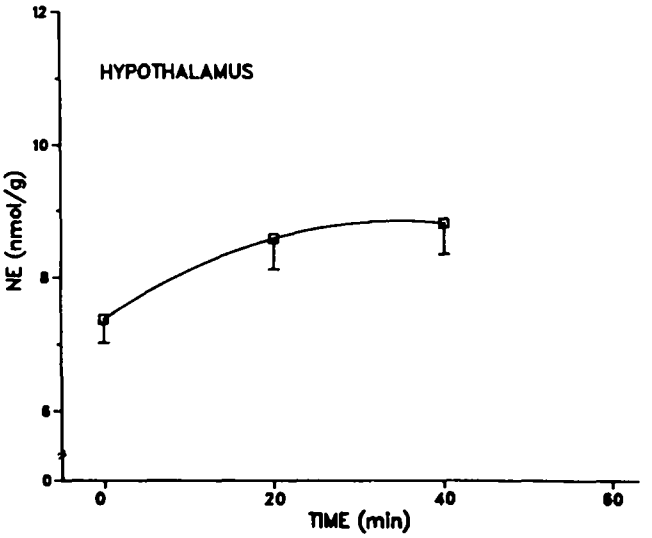


FIG. 2. Accumulation of NE in the hypothalamus after administration of pargyline and clorgyline measured at 0, 20 and 40 min after one week of adaptation to a high fat diet. The data of the two NE accumulation experiments were pooled to determine the time course where accumulation was most linear. Each point represents the mean±SEM of 11 mice. The equation for the polynomial fit is $y = -1.209\ (10^{-3})\ x^2 + 0.846\ (10^{-1})\ x + 7.375$, $SE_p = 1.305$.

ceived their daily intake as two meals: one meal equivalent to five-sixths of daily intake presented at 1600 hr and one meal equivalent to one-sixth of the daily intake presented at 0900 hr on the day of killing. Mice were killed beginning at 0920 hr for determination of NE synthesis rate as in Experiment 1. Thy hypothalamus and the rest of the brain were dissected for subsequent determination of NE as in Experiment 1. The carcasses (minus the brain) were frozen until body fat content could be determined.

Experiment 4. Mice were underfed and overfed the high fat diets for eleven days following the same feeding paradigm as in Experiment 3. Mice were killed by decapitation at 0 and 40 min after a single IP injection of pargyline and clorgyline mixed in physiological saline as described in Experiment 1. Whole brains were removed, wrapped in aluminum foil, frozen on dry ice and held at −70°C for processing within one month. For the microdissection of hypothalamic nuclei, the frozen brain was mounted on a specimen holder using an embedding medium (Tissue-Tek, O.C.T. Compound, Miles Scientific, Naperville, IL) and then placed in a histostat (Model 855, Riechert-Jung Scientific Instruments, Belleville, Ont.) maintained at −5 to −8°C. Frozen sections measuring 300 μm were prepared, using the orientations of the stereotaxic atlas of Slotnick and Leonard (32). The frozen sections were mounted on glass microscope slides by partially thawing the section on the slide, followed by immediate refreezing on dry ice. The slides were placed on a cold plate (Stir-Kool, Model SK12, Thermo Electric Co. Inc., Saddle Brook, NJ) and the suprachiasmatic nucleus (SCH), anterior hypothalamus (ANT), paraventricular nucleus (PVN), ventromedial nucleus (VMH), dorsomedial nucleus (DMN), lateral hypothalamus (LH), and the arcuate nucleus (ARC) were removed using a stainless steel needle with an inside diameter of 430 μm (NIH Style Neuropunch, Fine Science Tools, Vancouver, B.C.). The atlas of Slotnick and Leonard (32) was used as a guide to locate the nuclei. The removal of the appropriate nuclei was confirmed after microdissection by visual inspection of each slide after partial thawing. Upon removal of the nuclei, the tissue was immediately placed in 60 μl of 0.1 N perchloric acid and stored at −70°C for subsequent NE analysis.

Experiment 5. The mice were fed for seven days ad lib a high fat diet (Table 1: high fat control). Half of the mice continued to be fed ad lib and half were deprived for 24 hr before killing. Mice were killed beginning at 0920 hr at 0 and 40 min after a single IP injection of pargyline and clorgyline. Brains were removed, and hypothalamic nuclei were microdissected as in Experiment 4 and frozen at −70°C for subsequent NE determination.

NE accumulation determination. In all experiments, NE accumulation was measured over at least 40 min. Two subsequent experiments were conducted to determine the time course for the best linear fit of NE accumulation following catabolism inhibition.

NE accumulation was measured at 0, 10, 20, 30 and 40 min in the hypothalamus after one week of adaptation to the high fat diet (Table 1: high fat control). NE accumulation appeared to peak at 20 min, but not definitively so (Fig. 1). The experiment was repeated with mice killed at 0, 20 and 40 min, and the data of the two experiments were pooled at 0, 20 and 40 min. The resulting data best fit a polynomial equation, i.e., the accumulation was not linear beyond 20 min (Fig. 2). Therefore, statistical comparisons between slopes of lines were made where accumulation was most linear, i.e., over 0 to 20 min in the short-term feeding experiments (Experiments 1 and 2). In the chronic feeding experiments, the accumulation was linear for at least 40 min. Thus, in Experiments 3 and 4, and for comparison of the nuclei in Experi-

TABLE 2

EFFECT OF SHORT-TERM (3-DAY—EXPERIMENT 1, OR 1-DAY—EXPERIMENT 2) UNDERFEEDING OR OVERFEEDING ON NE SYNTHESIS RATE IN MOUSE BRAIN

		Hypothalamus				Rest of the Brain		
Group	Duration	$[NE]_o$* nmol/g	$[NE]_{20\ min}$ nmol/g	Turnover Time (T)† hr	Rate of Synthesis (K)‡ nmol/g/hr ± SEK	$[NE]_o$ nmol/g	Turnover Time (T) hr	Rate of Synthesis (K) nmol/g/hr ± SEK
Underfeeding	3-day	7.46 ± 0.79§[a]	8.14 ± 0.48	3.67	2.03 ± 2.77	0.47 ± 0.02	−5.27	−0.09 ± 0.01
Control	3-day	6.95 ± 0.68[b]	7.77 ± 0.76	3.58	1.94 ± 1.67	0.46 ± 0.02	−4.18	−0.11 ± 0.02
Overfeeding	3-day	6.05 ± 0.50[c]	7.37 ± 0.21	1.53	3.96 ± 1.62	0.49 ± 0.02	−4.36	−0.11 ± 0.02
Underfeeding	1-day	8.23 ± 0.28	9.54 ± 0.29	2.09	3.95 ± 1.21	0.60 ± 0.02	−2.34	−0.26 ± 0.08
Overfeeding	1-day	8.06 ± 0.49	9.85 ± 0.28	1.50	5.38 ± 1.70	0.59 ± 0.02	−2.89	−0.20 ± 0.07

*Values are means ± SEM, n=6. Rate of synthesis calculated from 6 mice per time point. Comparisons were made between treatments within each experiment.

†Time required for NE flow through the pool to equal pool size is equivalent to the time when $[NE]_o$ is doubled. From the equation of the line $y = a + bx$; $2a = a + bx$; $T = a/b$ or $T = [NE]_o/K$.

‡$K = k[NE]_o$ where $k = 1/T$.

§Means within each experiment with different superscripts are significantly different ($p<0.05$).

ment 5, the rate of NE synthesis and comparison of the slopes of the lines were determined from the increase over 40 min, measured over at least 2 time points. The data for all time points are shown in the figures.

Analytical Methods

NE in the whole hypothalamus was isolated by alumina extraction from the tissue homogenate supernatant under alkaline conditions by a previously reported method (3). For NE determination in the individual hypothalamic nuclei, frozen tissue in 60 μl 0.1 M perchloric acid was thawed and 10 μl 0.2 M ethylenediamine tetraacetic acid, 5 μl 1.0 M sodium bisulfite and 30 μl 0.0007 M 3,4-dihydroxybenzylamine, an internal standard, were added. The samples were sonicated (Model 300, Sonic Dismembrator, Artek Systems Corp., Farmingdale, NY) for 20 sec, and microcentrifuged at 9000 rpm for two min, and the supernatant was injected onto a liquid chromatographic system for detection.

TABLE 3

EFFECT OF CHRONIC (11-DAY) UNDERFEEDING AND OVERFEEDING OF NE SYNTHESIS RATE IN MOUSE BRAIN

	Hypothalamus			Rest of the Brain		
Group	$[NE]_0$* nmol/g	Turnover Time (T)† hr	Rate of Synthesis (K)‡ nmol/g/hr ± SEK	$[NE]_0$ nmol/g	Turnover Time (T) hr	Rate of Synthesis (K) nmol/g/hr ± SEK
Underfeeding	7.65 ± 0.45	2.22	3.35 ± 1.20	0.60 ± 0.03	−6.20	−0.10 ± 0.06
Overfeeding	7.28 ± 0.19	1.39	5.25 ± 0.78	0.54 ± 0.03	−8.77	−0.06 ± 0.05

*Values are means ± SEM, n=6. Rate of synthesis calculated from 6 mice per time point. Values are derived from data shown in Fig. 3.

†See Table 2 footnote.

‡$K = k[NE]_0$ where $k = 1/T$.

Detection of NE was achieved by high performance liquid chromatography (HPLC) (Model 2000, Varian Canada Inc., Georgetown, Ont.) with electrochemical detection (EC) (Model LC4, Bioanalytical Systems Inc., West Lafayette, IN) using a mobile phase of 0.075 M monochloroacetic acid with 250 mg/l sodium octyl sulfate as an ion-pairing reagent. The mobile phase was delivered at room temperature at a flow rate of 1.7 ml/min across a reversed-phase analytical column. The limit of detection in hypothalamic nuclei was 0.10 ng of NE. The between-run coefficient of variation on freshly prepared processed standard relative to internal standard was 3.6%. The protein content of the hypothalamic nuclei was determined by a modified Lowry procedure (24) on the resuspended pellet of the centrifuged homogenate. Values are expressed as pg of amine per μg of protein.

Body fat composition was determined after removing food residue from the stomach. The carcass (minus the brain) was homogenized (Polytron™, Brinkman Instruments, Westbury, NY) in an equal weight of water. Body fat was determined from dried aliquots of homogenized carcasses by ether extraction on a Goldfinch apparatus and quantitated gravimetrically (1).

Statistical Analysis

NE synthesis rate was calculated by linear regression of NE concentration versus the time periods specified, and the slopes of the regression lines were compared using the variance estimated for the difference between slopes (10). Treatment differences for NE concentration at time 0, weight change and body fat content were determined using Student's *t*-test (34). All values are expressed as mean ±SEM.

RESULTS

Experiment 1

The mice which were overfed the high fat diet for three days gained more weight (1.8±0.2 g) than either the control mice (0.4±0.1 g) or the underfed mice (−1.2±0.2 g), all $p<0.001$. This resulted in a higher body fat content in the overfed mice than in the underfed mice (22.5±0.8% vs. 15.3±0.7%, $p<0.001$), but no difference between either of them and the control group (19.6±1.2%). Since the aim of this experiment was to investigate the effects of short-term overfeeding on brain NE as distinct from the effects of a high body fat content, this difference in weight gain and body fat became a confounding variable.

Hypothalamic NE concentration was lower in the overfed than in the control or the underfed mice (Table 2). However, the rate of NE synthesis in the hypothalamus or in the rest of the brain was not significantly different between the underfed, control and overfed mice (Table 2). The apparently negative rates of synthesis in the rest of brain reflect low rates of synthesis, plus random error in the large mass of the rest of the brain.

Experiment 2

Change in weight did not differ between the mice underfed or overfed the high fat diet for one day only (+0.3±0.1 g vs. +0.6±0.1 g in the underfed and overfed mice, respectively). Thus, the confounding effect of alterations in body weight and hence body fat content with overfeeding that occurred with the three-day experiment was eliminated.

NE concentration and the rate of synthesis did not differ between underfed and overfed mice in the hypothalamus or in the rest of the brain (Table 2).

Experiment 3

Chronic overfeeding of the high fat diet for eleven days resulted in a higher body fat content (18.7±0.8% vs. 11.9±0.8%, $p<0.001$) and a greater weight gain (2.9±0.3 g vs. −1.3±0.2, $p<0.001$) than in the underfed mice. The difference in body fat content is mainly attributable to weight and fat loss in the underfed group. During the 7-day adaptation period, the mice who subsequently were randomly assigned to the overfed group gained 0.38±0.2 g/day and those who entered the underfed group gained 0.35±0.2 g/day. During the overfeeding period the overfed mice gained 0.29±0.18 g/day and the underfed mice lost 0.37±0.18 g/day. Thus the rate of gain slowed slightly for the overfed group but the mice were also approaching maturation and growing at a slower rate. However, body fat content in the chronically overfed mice did not differ from that of mice acutely overfed in Experiment 1.

Brain NE concentration in the hypothalamus and in the rest of the brain did not differ between the chronically underfed and overfed mice (Table 3, Fig. 3).

Experiment 4

Chronic overfeeding of the high fat diet for eleven days produced a higher body fat content (18.3±0.9% vs. 11.6±0.6, $p<0.001$) and a greater weight gain (3.2±0.2 g vs.

TABLE 4

COORDINATES USED FOR THE REMOVAL OF SPECIFIC HYPOTHALAMIC NUCLEI FROM UNDERFED, OVERFED AND FOOD-DEPRIVED MICE AND THE PROTEIN CONTENT OF EACH NUCLEUS

Hypothalamic Area	Punches/ Brain	Coordinates	Protein Content* g
Suprachiasmatic nucleus	2	F −0.3 to F −0.9	16.98 ± 1.08
Anterior hypothalamus	6	F −0.3 to F −1.2	36.23 ± 1.58
Paraventricular nucleus	4	F −0.6 to F −1.2	28.67 ± 1.55
Ventromedial nucleus	8	F −1.2 to F −2.4	39.93 ± 1.24
Dorsomedial nucleus	6	F −1.2 to F −2.1	29.50 ± 1.18
Lateral hypothalamus	8	F −1.2 to F −2.4	40.34 ± 1.50
Arcuate nucleus	4	F −1.5 to F −2.7	19.75 ± 0.93

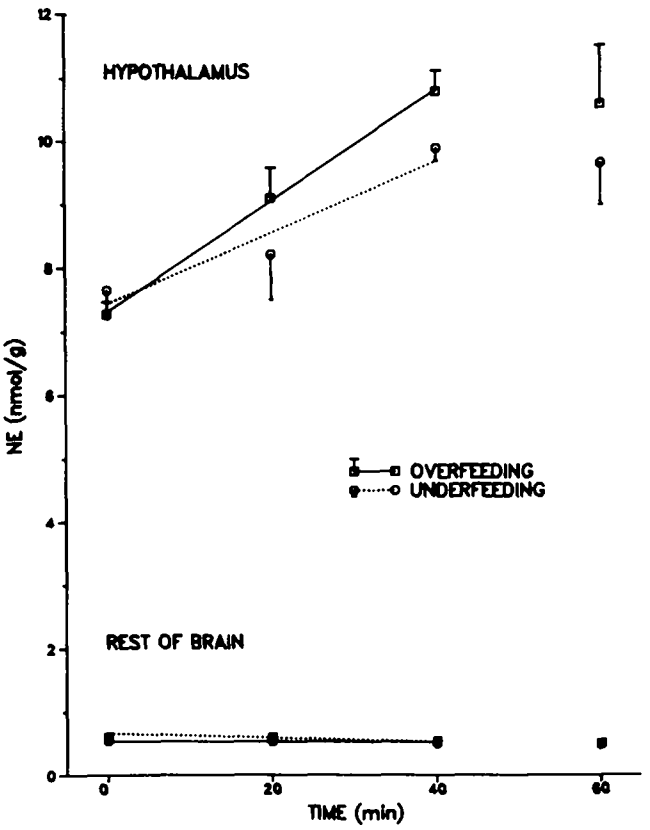
*Values are means ± SEM, n=18–24. For the punches, all nuclei were removed using a 430 μm internal diameter needle. Coordinates are from the atlas of Slotnick and Leonard (32).

TABLE 5

EFFECT OF CHRONIC (11-DAY) UNDERFEEDING AND OVERFEEDING ON NE TURNOVER IN HYPOTHALAMIC NUCLEI IN MICE (EXPERIMENT 4)

Nucleus	Group U=Underfed O=Overfed	$[NE]_o$* pg/μg Protein	$[NE]_{40\ min}$ pg/μg Protein	Turnover Time (T)† hr	Rate of Synthesis (K)‡ pg/μg Protein/hr ± SEK
Suprachiasmatic	U	12.41 ± 0.30	18.81 ± 2.71	1.28	9.70 ± 4.05
nucleus	O	13.01 ± 1.01	19.17 ± 1.37	1.39	9.33 ± 2.77
Anterior	U	12.37 ± 1.86	17.14 ± 0.96	1.71	7.24 ± 2.97
hypothalamus	O	11.69 ± 1.10	19.40 ± 1.19	1.00	11.69 ± 2.47
Paraventricular	U	16.51 ± 2.12	18.86 ± 1.20	4.65	3.55 ± 4.24
nucleus	O	13.24 ± 2.36	17.33 ± 2.37	2.14	6.20 ± 4.74
Ventromedial	U	13.41 ± 1.53§[a]	17.00 ± 1.86	2.47	5.44 ± 2.79
nucleus	O	19.79 ± 1.47[b]	28.55 ± 2.71	1.49	13.29 ± 4.05
Dorsomedial	U	23.65 ± 1.99	25.06 ± 3.00	11.00	2.15 ± 5.79
nucleus	O	25.56 ± 2.12	27.44 ± 2.40	8.24	2.86 ± 4.99
Lateral	U	12.43 ± 1.13	15.67 ± 1.04	2.54	4.90 ± 2.33
hypothalamus	O	12.81 ± 0.51	18.21 ± 2.43	1.39	9.19 ± 4.08
Arcuate	U	17.08 ± 2.55	23.61 ± 4.32	1.72	9.91 ± 6.16
nucleus	O	16.98 ± 2.28	23.53 ± 3.56	1.71	9.93 ± 5.82

*Values are means ± SEM, n=6. Rate of synthesis calculated from 6 mice per time point.
†Time required for NE flow through the pool to equal pool size is equivalent to the time when $[NE]_o$ is doubled.
‡$K = k[NE]_o$, where $k = 1/T$.
§Means in the group with different superscripts are significantly different ($p<0.05$).



-1.4 ± 0.2 g, $p<0.001$) in the overfed mice similar to Experiment 3.

Sections used for the removal of specific hypothalamic areas and their protein content are presented in Table 4.

The VMH was the only hypothalamic area studied that showed a significant difference in NE concentration between underfed and overfed mice, with chronically overfed mice having a 32.2% higher NE concentration (Table 5). Rate of NE synthesis did not differ between underfed and overfed mice in any of the nuclei studied (Table 5).

Experiment 5

When mice were ad lib fed or deprived of food for 24 hr to assess potential alterations in NE synthesis rate within specific hypothalamic sites in relation to the feeding status of the mice, concentration of NE between ad lib and food-deprived mice did not differ significantly in any of the hypothalamic areas studied (Table 6). However, in the food-deprived mice, rate of NE synthesis in the PVN and at no other site was five-fold higher than in the fed mice (Table 6).

FIG. 3. Accumulation of NE in the hypothalamus and the rest of the brain after administration of pargyline and clorgyline in chronically (11-day) underfed and overfed mice (Experiment 3). Each point represents mean±SEM of 6 mice. Equations for least squares fit for the hypothalamus were $y = 7.65 + 3.35x$, $SE_b = 1.20$, $r = 0.64$, and $y = 7.28 + 5.25x$, $SE_b = 0.78$, $r = 0.89$, and for the rest of the brain were $y = 0.60 - 0.10x$, $SE_b = 0.06$, $r = 0.40$ and $y = 0.54 - 0.06x$, $SE_b = 0.05$, $r = 0.35$ in underfed and overfed mice respectively.

TABLE 6

EFFECT OF 24-HR FOOD DEPRIVATION ON NE TURNOVER IN HYPOTHALAMIC NUCLEI IN MICE (EXPERIMENT 5)

Nucleus	Group D=Deprived F=Fed	$[NE]_0$* pg/μg Protein	$[NE]_{40\ min}$ pg/μg Protein	Turnover Time (T) hr	Rate of Synthesis (K) pg/μg Protein/hr ± SEK
Suprachiasmatic	D	15.68 ± 1.56	19.23 ± 3.34	3.79	4.14 ± 4.93
nucleus	F	13.34 ± 1.90	21.05 ± 3.05	1.14	11.68 ± 3.75
Anterior	D	12.20 ± 0.92	20.35 ± 2.81	0.99	12.36 ± 4.49
hypothalamus	F	12.26 ± 0.96	20.95 ± 1.54	0.93	13.17 ± 2.76
Paraventricular	D	19.41 ± 2.12	38.20 ± 3.63	0.68	28.47 ± 6.76†[a]
nucleus	F	20.54 ± 2.36	24.35 ± 1.32	3.55	5.79 ± 3.93[b]
Ventromedial	D	12.81 ± 1.83	22.61 ± 2.00	0.86	14.85 ± 4.69
nucleus	F	13.55 ± 1.01	26.43 ± 3.35	0.69	19.53 ± 3.31
Dorsomedial	D	27.12 ± 2.41	33.02 ± 2.38	3.04	8.93 ± 3.90
nucleus	F	28.99 ± 2.24	31.67 ± 3.06	7.14	4.06 ± 3.75
Lateral	D	11.07 ± 1.82	13.11 ± 2.17	3.57	3.10 ± 4.30
hypothalamus	F	9.53 ± 0.64	16.70 ± 0.93	0.88	10.87 ± 1.71
Arcuate	D	14.67 ± 1.93	22.13 ± 2.90	1.24	11.84 ± 5.53
nucleus	F	15.05 ± 1.64	23.82 ± 3.60	1.40	10.73 ± 3.86

*Values are means ± SEM, n=6. Rate of synthesis calculated from 6 mice per time point. See Table 5 for footnotes.
†Means in the group with different superscripts are significantly different ($p<0.05$).

DISCUSSION

The results of this study indicate that hypothalamic NE synthesis rate is more related to short-term food intake regulation than to the thermic or thermogenic effects of overeating. In addition, the effects of food intake and deprivation appear to be site-specific in the hypothalamus. Specifically, mice deprived of food for 24 hr had a five-fold higher NE synthesis rate in the PVN than in the fed mice. Altering the body fat content of normal mice by chronically underfeeding or overfeeding did not alter brain NE synthesis rate, neither in the whole hypothalamus nor in hypothalamic nuclei of fed mice.

These results are important for several reasons. First, they separate the effects of relative overfeeding and underfeeding from food deprivation on NE synthesis rate. Second, they help to clarify the causal nature of the reduced brain NE synthesis rate seen in the obese genotype of this strain of mouse. Genetically obese (ob/ob) mice, the obese genotype of this strain of mice, have a lower whole brain NE synthesis rate than their lean litter mates at 6 weeks of age (15). However, the ob/ob mice are fatter and normally eat more than their lean litter mates at this age. Thus, it had not been possible to determine if the reduction in brain NE synthesis was a cause of their obesity or a consequence of overeating or of a high body fat content. The present findings indicate that neither overfeeding per se, nor body fat content per se, affect brain NE synthesis, thus ruling out these explanations for the reduced brain NE synthesis in the obese mice. It remains possible then that the low brain NE metabolism of the ob/ob mice is a cause of their obesity, or is at least an association with the obese phenotype.

In relation to relative overfeeding and underfeeding, the rate of NE sythesis in the hypothalamus was not significantly different between underfed and overfed mice in either the acute (1-day, 3-day) or chronic (11-day) feeding studies. All animals in the acute and chronic under- and overfeeding studies were in the fed state, having each received a meal [0.25 g (1.2 kcal) for underfed; 0.48 g (2.4 kcal) for overfed mice] 20 min prior to the time zero injection and killing. All food in the meal was consumed prior to injection. Thus, NE synthesis rate was measured at a time when the thermic effect of underfeeding and overfeeding should be maximally apparent. The likelihood that these mice were experiencing the thermic effect is supported by our observation that when additional mice were fed a meal [0.50 g (2.8 kcal)] after a 24-hr fast, oxygen consumption increased 17% above fasting level at 20 min after presentation of the meal (J. L. Johnston and R. Graval, unpublished observation). Thus, NE synthesis rate in the whole hypothalamus or in specific hypothalamic nuclei was not differentially affected during the thermic effect of the previous meal, in either acutely or chronically underfed or overfed mice.

In the 24-hr food-deprived mice, the rate of NE synthesis in the PVN and at no other site was five-fold higher than in the fed mice. This is the first report of increased NE synthesis rate in the PVN of fasted mice. However, Jhanwar-Uniyal *et al.* (13) using α-methyl tyrosine to deplete endogenous NE 3 hr prior to decapitation and hence estimate NE turnover, similarly found an increase in NE turnover exclusively in the PVN following 48-hr food deprivation in rats (13). Since NE administration into the PVN induces hyperphagia and body weight gain (17), these data support a physiologcial role of NE specifically in the PVN in short-term food intake regulation.

In the present study, altering body fat content of normal mice did not alter brain NE synthesis rate. The difference in body fat content in the present study is mainly attributable to weight and fat loss in the underfed group. Body fat content in chronically overfed mice did not differ from that of mice which were acutely overfed, possibly because although ad lib quantity was fed, the food was given once daily and therefore the mice were not truly ad lib fed. However, the rate of weight gain in the overfed mice during this period of maturation was slowed only slightly below that in the prior one-week adaptation period. In the chronic overfeeding experiment (Experiment 4), the concentration of NE in the VMH was significantly higher in the overfed than in the underfed animals, but no difference in NE synthesis rate was observed. Although there was no difference in NE synthesis rate at the level of the whole hypothalamus or in specific nuclei, the increased concentration of NE in the VMH of chronically overfed mice suggests NE metabolism may be altered and warrants further investigation.

NE synthesis rate in the present study was determined from the rate of accumulation of NE after inhibition of NE catabolism by the MAO inhibitors, pargyline and clorgyline. The use of NE accumulation as an indicator of brain NE synthesis relies on the assumption that pargyline and clorgyline completely block MAO activity. Pargyline at a high dose (75 mg/kg), as used in this study, is an irreversible inhibitor of both MAO-A and MAO-B activity, resulting in a 98% inhibition of MAO activity within 5 min of injection, lasting 3 hr in rats (25). A 93% inhibition of brain MAO-A activity occurs 1 min after a low dose (10 mg/kg) of clorgyline in mice and lasts 1 hr (19). Although MAO activity was not measured in the present study, it was assumed that the combination of pargyline and clorgyline blocked MAO activities similarly in these mice. It is possible that this method of measuring rate of synthesis may tend to underestimate the true rate of synthesis, because accumulation of NE may diminish its own synthesis by end-product inhibition of tyrosine hydroxylase (22). This appeared to occur in the acute feeding studies beginning at 20 min after injection, because the rate of accumulation of NE was not linear beyond 20 min. In the chronic feeding experiments, the NE accumulation was linear for at least 40 min, suggesting the possibility of developmental changes in NE metabolism. It is possible, during the 11-day period of over- and underfeeding, that some maturation of NE synthetic or catabolic enzymes occurred. This is supported by the observation in 12-day-old rats that NE concentration in the hypothalamus is very low (33.6 nmole/g) and gradually increases reaching a NE concentration of 99.0 nmol/g at 75 days of age (23). The curvilinear accumulation after 20 min in the acute feeding experiments, and after 40 min in the chronic feeding experiments was somewhat unexpected due to the previously observed 3-hr duration of MAO inhibition in the whole brains of rats (25). It appears that the rate of accumulation is much faster in the hypothalamus than it is in the whole brain, and hence feedback inhibition by this rapidly accumulating NE may be apparent much sooner in the hypothalamus than in the whole brain. Li *et al.* (19) have shown a linear accumulation of NE in whole mouse brain for 30 min after a combination of MAO inhibition by pargyline and clorgyline, plus catechol-O-methyltransferase inhibition by tropolone. This resulted in an NE synthesis rate of 944 pmol/g/hr in whole brain over the linear 30 min, with the accumulation becoming curvilinear after 1 hr (19). Johnston *et al.* (15) observed a linear NE accumulation until 0.75 hr using pargyline to block NE

catabolism, resulting in a NE synthesis rate of 1046 pmol/g/hr in whole mouse brain (15). Our values ranged from 2030 pmol/g/hr to 5380 pmol/g/hr in the whole hypothalamus, values comparable to those previously reported (2074 pmol/g/hr) determined from the rate of decline of NE over 3 hr following α-methyl-para-tyrosine administration (20).

NE concentration in the whole hypothalamus at time zero of both underfed and overfed mice in the acute and chronic feeding studies averaged 7.46±0.77 nmol/g and was in close agreement with other reported values of 9.51±0.33 nmol/g observed by Lorden *et al.* (20). The concentrations of NE in the specific hypothalamic nuclei found in the present study were also similar to those reported by Oltmans (26). Differences in NE concentration in the whole hypothalamus did not parallel differences in NE synthesis rate: NE concentration was significantly higher in mice underfed for 3 days than in their overfed counterparts, but the rate of NE synthesis tended to be higher in the overfed group. This observation of a higher NE concentration in underfed than in overfed mice is similar to that in adult male rats semistarved for three weeks, in which NE concentration was significantly higher in the mediobasal hypothalamus, and 3-methoxy-4-hydroxyphenylglycol concentration was lower (suggesting lower NE turnover) than in controls when measured serially at 8 time points over a 24 hr period (29).

The NE concentration in the VMH was 32.2% higher in chronically overfed mice, but the NE synthesis rate was not significantly different between the underfed and overfed mice. Levin *et al.* (18) found no difference in the NE concentration or turnover in the VMH of rats which were chronically overfed for three months, compared to ad lib fed controls, but observed a higher turnover of NE in the PVN and the dorsomedial nucleus of the overfed rats (18). It is difficult to compare these results to the present study, due to the differences in species, the type of diets fed, the duration of overfeeding, and the time of killing. Levin *et al.* fed control animals a 4.5% fat diet, ad lib, whereas the overfed animals received a 16% fat diet, producing a 70% greater weight gain in the overfed rats (18). As well, they did not indicate whether the rats were killed at the end of the dark or light period, whereas the mice in our study were killed at the end of the dark period, in the fed state. Since prior food deprivation affects PVN-NE synthesis rate, a difference in study design may explain this discrepancy.

In our study, the chronically overfed mice did not differ in body fat content from the acutely overfed mice. Hence, the resulting intake did not produce obese mice, but clearly resulted in the overfed mice being much fatter than the underfed mice. A longer experiment allowing a more pronounced gain in body fat content of the overfed mice would be desirable to test if specific brain areas such as the VMH are a stimulus for diet-induced thermogenesis in chronically overfed mice.

It is concluded that hypothalamic NE synthesis rate is more related to short-term food intake regulation than to the thermic effects of eating. The effects of food intake and deprivation appear to be site-specific in the hypothalamus, with NE synthesis rate being higher in the PVN of 24 hr food-deprived mice than in fed mice. This observation is consistent with previous pharmacological evidence that an increase in noradrenergic activity in the PVN may be a stimulus for food ingestion. Altering the body fat content of normal mice by chronic over- and underfeeding did not alter brain NE synthesis rate in the whole hypothalamus or in specific hypothalamic nuclei of fed mice. This finding indicates that the reduced brain NE synthesis rate previously seen in the obese genotype of this strain of mice may cause their obesity, or at least an association with the obese phenotype and not a consequence of their elevated body fat or overeating.

REFERENCES

1. Association of Official Analytical Chemists (AOAC) Official methods of analysis. 13th ed. Washington: AOAC; 1980.
2. Bieri, J. G.; Stoewsand, G. S.; Briggs, G. M.; Phillips, R. W.; Woodard, J. C.; Knapka, J. J. Report of the American institute of nutrition ad hoc committee on standards for nutritional studies. J. Nutr. 107:1340–1348; 1976.
3. Bioanalytical System Inc. Tissue catecholamines: simultaneous assay for norepinephrine, epinephrine and dopamine. LCEC Application Note 12, 1980.
4. Barbosa, M. C.; Migliorini, R. H. Free fatty acid mobilization in rats following intracerebroventricular norepinephrine. Am. J. Physiol. 242:E248–E252; 1982.
5. Brodie, B. B.; Costa, E.; Dlabec, A.; Neff, N. N.; Snookler, H. H. Application of steady-state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. J. Pharmacol. Exp. Ther. 154:493–498; 1966.
6. Faust, I. M. Signals from adipose tissue. In: Cioffi, L. A.; James, W. P. T., Van Itallie, T. B., eds. The body weight regulatory system: Normal and disturbed mechanisms. New York: Raven Press; 1981:36–43.
7. Fowler, C. J.; Ross, S. B. Selective inhibitors of monoamine oxidase A and B: biochemical, pharmacological and clinical properties. Med. Res. Rev. 4:323–358; 1984.
8. Friedman, E.; Starr, N.; Gershon, S. Catecholamine synthesis and the regulation of food intake in the rat. Life Sci. 12:317–326; 1973.
9. Gibson, C. J.: Wurtman, R. J. Physiological control of brain catechol synthesis by brain tyrosine concentration. Biochem. Pharmacol. 26:1137–1142; 1978.
10. Gill, J. L. Design and analysis of experiments in animal and medical sciences. vol. 1. Ames, IA: Iowa State University Press; 1978:95–107.
11. Glick, S. D.; Waters, D. H.; Milloy, S. Depletion of hypothalamic norepinephrine by food deprivation and interaction with D-amphetamine. Res. Commun. Chem. Pathol. Pharmacol. 6:775–778; 1973.
12. Iverson, L. L.; Glowinski, J. Catecholamine regional metabolism in the rat brain. J. Neurochem. 13:655–669; 1966.
13. Jhanwar-Uniyal, M.; Darwish, M.; Levin, B. E.; Leibowitz, S. F. Alterations in catecholamine levels and turnover in discrete brain area after food deprivation. Pharmacol. Biochem. Behav. 26:271–275; 1987.
14. Johnson, D. J.; Anderson, G. H. Prediction of plasma acid concentration from diet amino acid content. Am. J. Physiol. 243:R99–R103; 1982.
15. Johnston, J. L.; Romsos, D. R.; Bergen, W. G. Reduced brain norepinephrine metabolism in obese (ob/ob) mice is not normalized by tyrosine supplementation. J. Nutr. 116:435–445; 1986.
16. Leibowitz, S. F. Paraventricular nucleus: A primary site mediating adrenergic stimulation of feeding and drinking. Pharmacol. Biochem. Behav. 8:163–175; 1978.

17. Leibowitz, S. F.; Roossin, P.; Rosenn, M. Chronic norepinephrine injection into the paraventricular nucleus produces hyperphagia and increased body weight in the rat. Pharmacol. Biochem. Behav. 21:801–808; 1984.
18. Levin, B. E.; Triscari, J.; Sullivan, A. The effect of diet and chronic obesity on brain catecholamine turnover in the rat. Pharmacol. Biochem. Behav. 24:299–304; 1986.
19. Li, P. P.; Warsh, J. J.; Godse, D. D. Formation and clearance of norepinephrine glycol metabolites in mouse brain. J. Neurochem. 43:1525–1433; 1984.
20. Lorden, J. F.; Oltmans, G. S.; Margules, D. L. Central catecholamine turnover in genetically obese mice (ob/ob). Brain Res. 117:357–361; 1976.
21. Loullis, C. C.; Felten, D. L.; Shea, P. A. HPLC determinations of biogenic amines in discrete brain areas in food-deprived rats. Pharmacol. Biochem. Behav. 11:89–93; 1979.
22. Lovenberg, W.; Victor, S. J. Regulation of tryptophan and tyrosine hydroxylase. Life Sci. 14:2337–2353; 1974.
23. Macho, L.; Kvetnansky, R.; Culman, J.; Fickova, M. Neurotransmitter levels in the hypothalamus during postnatal development of rats. Exp. Clin. Endocrinol. 88:142–150; 1986.
24. Markwell, M. K; Haas, S. M.; Bierber, L. L.; Tolbert, N. E. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. 87:206–210; 1978.
25. Meek, J. L.; Neff, N. H. The rate of formation of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate in brain as an estimate of the rate of formation of norepinephrine. J. Pharmacol. Exp. Ther. 184:570–575; 1973.
26. Oltmans, G. S. Norepinephrine and dopamine levels in hypothalamic nuclei of the genetically obese mice (ob/ob). Brain Res. 173:369–373; 1983.
27. Pirke, K. M.; Spyra, B. Catecholamine turnover in the brain and the regulation of lutenizing hormone and corticosterone in starved male rats. Acta Endocrinol. 100:168–172; 1980.
28. Rossi, J., III; Zolovick, A. J.; Davis, R. F.; Pansksepp, J. The role of norepinephrine in feeding behavior. Neurosci. Biobehav. Rev. 6:195–204; 1982.
29. Schweiger, U.; Warnhoff, M.; Pirke, K. M. Norepinephrine turnover in the hypothalamus of adult male rats: alteration of circadian patterns by semistarvation. J. Neurochem. 45:706–709; 1985.
30. Shimazu, T.; Takahashi, A. Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose. Nature 284:62–63; 1980.
31. Shor-Posner, G.; Azar, A. P.; Insinga, S.; Leibowitz, F. S. Deficits in the control of food intake after hypothalamic paraventricular nucleus lesions. Physiol. Behav. 33:883–890; 1985.
32. Slotnick, B. M.; Leonard, C. M. A stereotoxic atlas of the albino mouse forebrain. DHEW Publication No. (ADM). Rockville, MD: U.S. Department of Health, Education and Welfare: 1975:75–100.
33. Stachowiak, M.; Białowas, J.; Jurkowski, M. Catecholamines in some hypothalamic and telencephalic nuclei of food-deprived rats. Acta Neurobiol. Exp. 38:157–165; 1978.
34. Steel, R. G. D.; Torrie, J. H. Principles and procedures of statistics: A biometrical approach. 2nd ed. New York: McGraw-Hill; 1983:106–107.
35. Van Itallie, T. B.; Gale, S. K.; Kissileff, H. R. Control of food intake in the regulation of depot fat: an overview. In: Katzen, H. M.; Mahler, R. J., ed. Advances in modern nutrition. London: Hemisphere Press; 1978:427–492.