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Chronic Norepinephrine Injection into the Hypothalamic Paraventricular Nucleus Produces Hyperphagia and Increased Body Weight in the Rat[1]

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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**(5) 801–808, 1984.—A single injection of norepinephrine (NE) into the paraventricular nucleus (PVN) is known to elicit a feeding response in the satiated rat. Through repeated NE injections, the present study set out to determine whether chronic noradrenergic stimulation of the PVN is effective in producing changes in total daily food intake, as well as in body weight gain. The results indicate that repeated injections of NE (20 nmoles/injection given 4 times/day) cause a stimulation of eating with each injection and consequently produce a significant increase in total daily food intake. This stimulatory effect on feeding behavior occurs under food-restricted conditions, where food is available only at times (in the daytime) when NE is injected, and also under food-satiated conditions where food is available essentially ad lib. This hyperphagia results in a gradual increase in body weight which develops over the course of a 5-day sequence of repeated NE injections. There is some evidence to suggest that the overeating produced by NE throughout the day may be attributed specifically to an increase in meal size rather than to a change in meal frequency. This evidence suggests that medial hypothalamic NE, particularly within the PVN, may play a role in long-term feeding behavior and body weight regulation.

Feeding behavior Hypothalamus Body weight Norepinephrine Chronic drug injections Paraventricular nucleus

IT has been demonstrated by numerous investigators that a single injection of norepinephrine (NE) into the hypothalamus of different mammalian species, including rats, monkeys, and ruminants, causes an increase in feeding behavior [6, 10, 14] through α-adrenergic receptor mediation [1, 9, 36]. These receptors appear to be most concentrated within the hypothalamic paraventricular nucleus (PVN), where NE is uniquely potent in stimulating feeding [11, 12, 25] and where electrolytic lesions are effective in attenuating or abolishing the eating response produced by intraventricular NE [21]. The significance of this noradrenergic feeding phenomenon, to peripheral or central regulatory mechanisms controlling food ingestion and body weight, remains to be determined. There is some evidence to suggest that hypothalamic NE may be physiologically active in the modulation of normal feeding behavior. For example, it has been demonstrated that eating exhibited spontaneously by a rat is associated with an increase in the efflux of ^{14}C-labeled NE, specifically within the paraventricular and ventromedial hypothalamic area [24]. Furthermore, there are several studies which indicate that eating induced by the glucoprivic compound 2-deoxy-D-glucose may be dependent upon hypothalamic noradrenergic innervation [27, 28, 32]. Moreover, drugs which act through the release of endogenous NE are found to stimulate eating when injected into the PVN [17,18] and to require the integrity of PVN noradrenergic innervation [15,16].

To date, essentially all pharmacological analyses of noradrenergic stimulation of eating have utilized an "acute" injection procedure, whereby a single drug injection is given followed by a short-term feeding test (1 hour). The limitations of this testing paradigm are obvious, and it becomes apparent that in order to establish the nature of NE's effect on normal feeding behavior, it will be necessary to determine what impact a chronic change in NE stimulation may have on spontaneous feeding patterns and ultimately on body weight regulation. The present report examines this question by testing the effect of repeated manual injections of NE (up to 7/day) administered directly into the PVN. The results, presented previously in preliminary form [33], indicate that chronic NE administration is effective in stimulating multiple eating bouts throughout the day, and that the hyperphagia occurring over 5 consecutive days of NE results in a significant increase in body weight.

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METHOD

Subjects

Male albino Sprague-Dawley rats (N=39) were used. They were housed individually and kept on a 12:12 light-dark cycle with lights on at 6:00 a.m. The animals were maintained and tested, in their home cages, on one of two diets plus tap water. The diets were Purina lab chow pellets or a highly palatable "milk-mash" diet (presented in a dish) consisting of Purina lab chow powder mixed with undiluted sweetened condensed milk. The initial body weight of the animals used in these experiments was kept within a narrow range, between 375 and 400 g.

Surgery

All animals were stereotaxically implanted under pentobarbital anesthesia with a chronic unilateral cannula (23-gauge hypodermic needle with a screw-on protective cap and inner stylette). All cannulas were aimed at the PVN, using the coordinates of 0.1 mm caudal to Bregma, 0.3 mm lateral to the midline, and 8.2 mm ventral to the skull surface, with the incisor bar positioned 3.5 mm above the interaural bars. These coordinates were found in previous mapping studies [9] to direct the cannula to the rostro-dorsal tip of the PVN and to yield the strongest eating response after injection of NE. Routine histological analysis of approximately 30% of the animals tested in the present experiments confirmed the placement of the cannula tip to lie within 0.3 mm of the PVN.

General Test Procedure

The rats were given 2 weeks of postoperative recovery before testing was started. During this period, they were frequently handled and mock-injected to adapt them to the injection procedure. Before being placed in the experiment, each rat was screened for its responsiveness to a single PVN injection of NE (*l*-norepinephrine-*d*-bitartrate), on the same diet (either lab chow pellets or milk-mash) that was subsequently to be used in the main experiment. This screening test was conducted in the morning every 2 or 3 days. During a 1-hr pretest period, the rats were first given fresh food and water, to initiate spontaneous eating and produce maximal satiation. Subsequently, the rats were injected with sterile physiological saline (0.5 μl) and then immediately given measured food. Measurements were taken 60 min later, at which time the rats received a single injection of NE (20 nmoles in 0.5 μl of saline), followed by another 1-hr test period. Each animal was given up to 6 of these NE screening tests and was subsequently included in the main experiment if he exhibited a consistent eating response of at least 1.5 g on pellets (compared with 0.2 g after saline) and 3.5 g on milk-mash (compared with 0.9 g after saline). These criteria eliminated approximately 30% of the subjects that were initially cannulated.

Subsequent to the NE screening tests, the responsive animals were placed on one of two feeding schedules and one of two drug injection schedules used in the main experiment. The feeding paradigms were: (1) a *food-restricted schedule*, where food was available during the day but absent at night, and (2) a *foot-satiated schedule*, where food was available both day and night. For both schedules, testing occurred between 9:00 a.m. and 4:00 p.m. Measured food was available during only 4 of these 7 hours, presented during alternate hours starting at 9:00 a.m., 11:00 a.m., 1:00 p.m., and 3:00 p.m. At 4:00 p.m., the food-restricted rats had their food removed until the start of the next day's test, whereas the food-satiated rats were permitted to retain their food and have it available ad lib throughout the night.

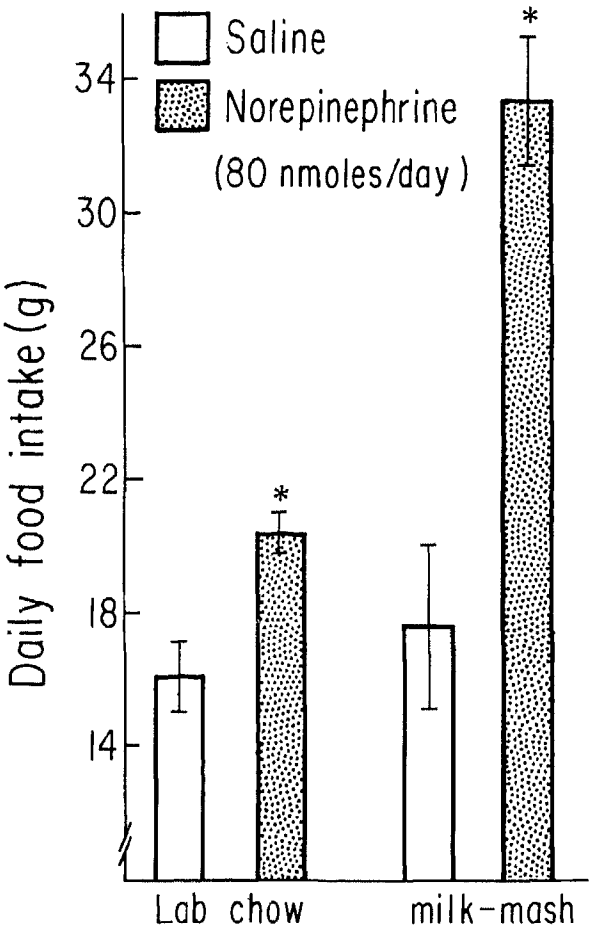

FIG. 1. The effect of repeated norepinephrine (NE) injections into the PVN on total daily food intake of rats (N=8) maintained on lab chow pellets or milk-mash. For this experiment, NE (20 nmoles/injection) was administered 4 times/day to rats maintained on a food-restricted schedule and treated according to an alternate 1-day injection schedule. See text for description of the protocol and analysis of the results. Asterisk (*) reflects a statistically reliable effect (at $p<0.001$) of NE on food intake, relative to the saline baseline.

The purpose of this daytime schedule of alternate 4-hr feeding periods was to maximize the impact of the repeated administration of NE. For all animals (with the exception of one group), the injections of saline or NE (20 nmoles/injection) were given manually 4 times/day. (In pilot tests, more frequent daytime injections were not found to be any more effective.) Since the feeding response to NE is known to occur during the first hour after injection [8], these 4 injections were scheduled to occur at the start of each of the 4 hourly test intervals, which were then followed by a second hour when no food was available. On a given day, the same substance (either saline or NE) was administered throughout the day. With regard to the sequence of administering saline or NE on subsequent days, however, two drug injection schedules were examined: (1) an *alternate 1-day schedule*,

where saline or NE was injected on alternate days according to an ABAB design, and (2) a *consecutive 5-day schedule*, where NE or its vehicle was administered over a sequence of 5 consecutive days, followed by 2 days of no injections and then another 5-day sequence of the alternate substance. A total of 4 such drug sequences were included in each experiment, 2 with saline and 2 with NE. The experiments using the alternate 1-day injection schedule generally had between 10 and 15 test days, half saline and half NE.

There was one exception to the typical paradigm of administering the drugs 4 times/day. In one set of rats (used in Experiment 2), saline or NE was injected 7 times between 9:00 a.m. and 4:00 p.m., at the start of each hour. For this group, food was continuously available throughout the 7-hr period, and measurements were taken hourly.

Water was available ad lib throughout all tests. Water intake, however, was not recorded. Body weight measurements were taken twice daily, in the morning (at 9:00 a.m. just before the start of the test) and in the afternoon (at 4:00 p.m. just after the last food measurement).

The data were analyzed using a Student's *t*-test for dependent and independent means and, in some cases, a two-factor analysis of variance with repeated measures on both factors.

RESULTS

Experiment 1

The initial experiment compared the relative effectiveness of the two diets, lab chow pellets and milk-mash, in revealing overeating with repeated PVN injections of NE. The animals in this experiment (N=8) were tested on both diets in counterbalanced order. They were maintained on a food-restricted feeding schedule and an alternate 1-day injection schedule. The impact of 4 injections/day of NE on the animals' total daily food consumption (with food available only 4 hr each day) is shown in Fig. 1. After injection of saline, the animals consumed approximately 16 g of lab chow and 18 g of milk-mash. On lab chow, the animals exhibited a 27% increase in food intake ($p<0.001$ relative to saline baseline score) under the influence of 80 nmoles of NE (20 nmoles/injection) administered on alternate days. On milk-mash, the animals exhibited an 89% increase ($p<0.001$ relative to saline baseline) in consumption after NE, an effect which was significantly larger (at $p<0.05$) than the NE-induced increase on lab chow pellets. This difference between the two diets is in agreement with other evidence revealing considerably larger effects with single drug injections in animals tested on milk-mash [17,18].

The main finding of this experiment is that NE, in addition to eliciting a single meal when injected once/day, is effective in eliciting multiple meals with repeated injections and ultimately affecting total daily food intake (on the restricted feeding schedule employed). Previous studies have tested only single injections of NE and, while observing an immediate increase in feeding, have failed to detect any alteration in total daily food intake (e.g., [6]). Analysis of the 4 hourly intervals that followed the injections revealed that NE reliably stimulated eating (at $p<0.05$) during each of the 4 test hours. Whereas no significant difference could be detected between the effectiveness of NE in these 4 hours, there was a consistent tendency towards a larger increase during the first interval (e.g., a 34% increase on lab chow) compared with the increase during the subsequent 3 hours (a 20 to 30% increase on lab chow).

Body weight measurements taken twice daily in these animals revealed an expected increase in daily body-weight gain after injection of NE. This increase closely reflected the amount of food consumed during the course of the day, if water ingestion was also taken into account. On dry lab chow pellets, other tests have indicated that rats generally ingest (in grams) at least as much water as they do food. Thus, after saline injection, the animals gained 29 g during the course of the day (16 g of food plus the remainder of water), and after NE injection they gained 43 g (21 g of food plus the remainder of water). This difference between body weight gain after NE versus saline injection was reliable at $p<0.001$. On the wet milk-mash diet, the rats have been found to drink considerably less water, and thus the daily body weight gain (19 g after saline versus 36 g after NE, $p<0.01$) more closely reflected the food intake (Fig. 1). It should be noted that hypothalamic injection of NE is known to cause a strong suppression of water ingestion [14]. This effect becomes more relevant in the case of the 5-day drug injection schedule, where body weight gain is measured across 5 consecutive days of chronic NE injection (Experiments 3 and 5).

Experiment 2

The animals used in this experiment (N=6) were tested on milk-mash and were given 7 injections of either saline or NE each day. They were maintained on a specific food-restricted schedule, where food was available every hour between 9:00 a.m. and 4:00 p.m., as opposed to every other hour as in the case of the 4 injection/day schedule used in the other experiments (see the Method section). The animals received saline or NE at the start of each hour, according to an alternate 1-day injection schedule. The results yielded by this test are presented in Table 1. Consistent with Experiment 1, total daily food intake and body weight gain were significantly increased by NE administered 7 times/day (20 nmoles/injection or a total of 140 nmoles/day). Further analyses of the data revealed an important additional effect, namely, that NE apparently has greater impact on the size of the eating bouts that occur during the course of the 7 hours than it does on the number of eating bouts during this period. Although a complete meal pattern analysis was not performed in this experiment, observations of the animals indicated that they generally ate their food in discrete meals (lasting 10 to 20 min) and that, in a given hour, either no meal or just one meal occurred, followed by a period of rest or sleep. With this pattern of behavior, the hourly measurements of food intake were taken to reflect the number as well as size of the individual meals. As can be seen in Table 1, the animals after saline injection ate their total daily food intake (15.6 g) in an average of 2.6 meals, consuming 6.0 g/meal. Seven hourly injections of NE produced a significant increase in total daily food intake (37.7 g), which was attributed almost solely to a significant increase in meal size (to 12.2 g/meal) rather than to a change in meal frequency (3.1 meals). Thus, NE, when administered at hourly intervals, effectively stimulates eating but not after every injection. This contrasts with the 4 injection/day schedule (Experiment 1) where NE was administered every other hour and found to significantly enhance eating after each injection. It should be noted that the animals maintained on the 4 injection schedule exhibited spontaneous eating during each of the 4 hours that food was available. This would appear to suggest that NE may potentiate an on-going eating response more effectively than

TABLE 1

IMPACT OF REPEATED PVN INJECTIONS OF NOREPINEPHRINE ON EATING BEHAVIOR, MEAL PATTERNS AND BODY WEIGHT GAIN DURING 7 HOURLY DAYTIME INTERVALS

	Food Intake (g/7 hr)	Body Weight Gain (g/7 hr)	Meal Frequency (No./7 hr)	Meal Size (g/meal)
Saline	15.6 ± 1.63	10.2 ± 1.70	2.6 ± 0.33	6.0 ± 1.17
Norepinephrine	37.7 ± 1.11	33.3 ± 1.70	3.1 ± 0.29	12.2 ± 1.24
% Change	+142†	+226†	+19	+103

*See text for description of experimental protocol. Scores are expressed as means ± SEM. For comparisons between saline and norepinephrine scores: **$p<0.05$; †$p<0.001$.

initiating a new response (see Discussion). This suggestion, however, does not take into account the fact that, on the 7 injection/day schedule, NE was injected at hourly intervals, whereas on the 4 injection/day schedule, it was administered every other hour.

Experiment 3

The evidence obtained so far indicates that repeated injections of NE during the course of a day (in animals on a food-restricted schedule) effectively stimulate total daily food intake and consequently increase body weight gain during that day. The question addressed in the present experiment is whether this effect of NE may be manifested over several consecutive days of NE injections and, furthermore, whether significant body weight gain, reflecting more than just gut filling, may ultimately become apparent. The animals in this experiment (N=10) were maintained on a food-restricted schedule. They received 4 injections/day of saline or NE (20 nmoles/injection) and were carried through a 5-day sequence in which either saline or NE was injected throughout the entire sequence.

The results of this paradigm can be found in Fig. 2. The graph to the left reflects the animals' total daily food intake on the 5 consecutive days, and the graph to the right indicates body weight measurements taken at the *end* of each test day. A two-factor analysis of variance (drug × days) comparing the saline and NE curves revealed a significant stimulatory effect of NE on both food intake, $F(1,9)=15.56$, $p<0.01$, and body weight, $F(1,9)=10.39$, $p<0.05$, an effect that remained generally stable across days. The morning body weight reading taken on the first day was 501 g for the saline sequence and 502 g for the NE sequence. This indicates that these animals on the food-restricted schedule gained 28 g on the first day of saline injection, in contrast to 35 g after NE injection ($p<0.01$). This directly reflects the differential food intake exhibited by the rats on that day. The question of whether a progressive change in body weight occurred over the course of the 5-day sequence is addressed by the significant drug × days interaction that was obtained for the body weight curve, $F(4,36)=3.06$, $p<0.05$. This interaction reflects the gradual increase in body weight that the animals exhibit across the 5 days of repeated NE injections. The afternoon body weight measurements (Fig. 2) revealed a 9 g *increase* after NE and a 3 g *decrease* after saline ($p<0.01$). Morning measurements (taken from Day 1 to Day 6, i.e., the morning after the last test day) revealed a similar trend, with the animals gaining almost twice as much after NE (15.5 g) as after saline (8.2 g, $p<0.05$). This evidence

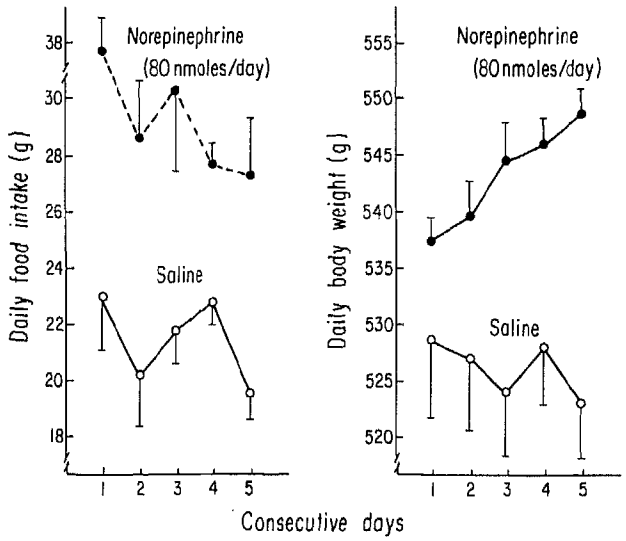

FIG. 2. The stimulatory effect of repeated norepinephrine (NE) administration into the PVN on daily food intake and body weight of rats (N=10) maintained on milk-mash diet. The animals in this experiment were maintained on a food-restricted schedule and received NE (20 nmoles/injection) 4 times/day over 5 consecutive days. See text for description of testing procedure and analysis of results.

indicates that NE has the potential for producing and sustaining an increase in body weight, at least over a 5-day period of chronic injections.

Experiment 4

The first three experiments employed a food-restricted schedule which permitted food to be available only during the daytime hours immediately following drug injection. Needless to say, this schedule is unnatural and prohibitive. Whereas it permitted us to reveal a potent stimulatory effect of chronic NE on food intake and body weight gain, the question is whether these effects may also be apparent under more natural conditions with food available ad lib. Experiments 4 and 5 were designed to test this question in rats maintained on milk-mash according to a food-satiated schedule, where food was available overnight as well as during the 4 daytime hours when the injections were given. The rats (N=8) of the present experiment were injected accord-

TABLE 2

FOOD INTAKE AND BODY WEIGHT CHANGE IN RATS THAT HAVE FOOD AVAILABLE AD LIB AND RECEIVE FOUR DAYTIME INJECTIONS OF SALINE OR NOREPINEPHRINE (4×20 nmoles) ON ALTERNATE DAYS AND NO INJECTIONS AT NIGHT*

Food Intake (g)			
Saline	12.0 ± 1.2	16.5 ± 1.1	28.4 ± 1.9
Norepinephrine	27.6 ± 1.9‡	9.5 ± 1.4‡	37.2 ± 1.8‡
Body Weight Change (g)			
Saline	+5.4 ± 1.1	−2.9 ± 1.7	+2.4 ± 1.8
Norepinephrine	+22.2 ± 2.8‡	−7.6 ± 2.0†	+14.6 ± 1.5§

*Scores are expressed as mean food intake (g ± SEM) and body weight change (g ± SEM) that occurs during the daytime, nighttime, and over 24 hours.

For comparisons between saline and norepinephrine scores: †$p<0.05$; ‡$p<0.001$; §$p<0.01$.

ing to an alternate 1-day schedule and received 4 injections of saline or NE (20 nmoles) per day on alternate hours between 9:00 a.m. and 4:00 p.m.

The results of this experiment are presented in Table 2. It can be seen that 4 daytime injections of NE reliably stimulated food intake (+130%) and body weight (311%) during the day. At night, when *no* injections were given, this effect of NE was somewhat reversed, such that the animals receiving daytime NE ate significantly less and lost more body weight than they did after daytime saline. (Both the saline and NE injection conditions revealed a night-time body weight loss, which may be attributed to increased activity and urine excretion.) Despite this reversal at night when no injections occurred, an analysis of the total 24-hour measures (day plus night) demonstrated that NE's effectiveness in stimulating food intake and body weight could still be detected as much as 18 hours after the last daytime injection. Thus, the compensatory response during the night was insufficient to overcome the impact of chronic NE during the day.

Experiment 5

The findings obtained in Experiment 4 (which employed an alternate 1-day injection schedule) demonstrate that daytime injections of NE, in animals with food available essentially ad lib, are effective in potentiating food intake and body weight at the end of the 24-hr cycle. The present experiment examined additional animals (N=7) in a similar feeding paradigm, to determine the impact of chronic NE injection over a 5-day injection sequence. The results of this series of tests are shown in Fig. 3 and Table 3. As can be seen in Fig. 3, 4 daytime injections of NE (versus saline) significantly potentiated food intake during the day, $F(1,6)=16.43$, $p<0.01$. This effect appeared relatively stable across all 5 days. At night, when no injections took place, the NE curve fell below the saline curve, although a significant difference between these curves was not revealed via an analysis of variance. As in Experiment 4, the NE stimulatory effect could still be observed in the 24-hr (day plus night) food intake measures, $F(1,6)=7.49$, $p<0.05$, and this effect remained stable across the 5 days of daytime NE injections. Table 3 presents the morning and afternoon body weights of these animals and shows that NE significantly increases daytime body weight gain (2.6 g after saline versus 11.8 g after NE, $p<0.001$). More importantly, the animals under the influence of NE showed a steady increase in body weight during the course of the 5 days, such that the morning readings from Day 1 to Day 6 revealed 17.5 g potentiation of body weight after daytime NE compared with only a 3.2 g increase after saline ($p<0.05$).

DISCUSSION

The outcome of these experiments is clear. In addition to eliciting a single eating response after acute administration, NE injected directly into the PVN of brain-cannulated animals is effective in altering daily food intake and body weight under chronic injection conditions. This stimulatory effect on feeding behavior occurs under food-restricted conditions, where food is available only at times (in the daytime) when the drug is injected, and also under food-satiated conditions where food is available essentially ad lib. This hyperphagia results in a gradual increase in body weight gain which develops during the course of a 5-day sequence of repeated NE injections. One experiment suggests that the overeating produced by NE throughout the day may be attributed specifically to an increase in meal size rather than to a change in meal frequency.

In acute injection studies, the stimulatory effect of hypothalamic NE injection on feeding behavior has been found to occur most effectively within the PVN, where NE elicits the strongest response with the shortest latency and at the lowest threshold dose [11,12]. This stimulatory response can be observed in already hungry as well as food-satiated animals [10], and is found to be mediated by α-adrenergic receptors [1, 9, 36]. A detailed behavioral analysis of the eating response elicited in satiated rats reveals that it closely resembles the eating behavior exhibited by rats under natural conditions [8]. The NE and spontaneously elicited eating bouts are similar in magnitude and duration and are associated with drinking behavior in a similar fashion. Although there is clear evidence that NE injection can elicit a vigorous eating response in a fully satiated rat, there is some evidence to indicate that NE may be more effective in potentiating an ongoing eating response in hungry rats than initiating a response in satiated rats. That is, the potentiation in hungry rats is associated with a wider sensitive zone within the medial hypothalamus [11] and is found to occur at a lower threshold dose [31].

Studies which have conducted direct tests of NE's effects on the animals' meal patterns [5,31] have demonstrated that NE has a more significant role in controlling meal size than in initiating a meal or altering meal frequency. Certain results

TABLE 3

MORNING (A.M.) AND AFTERNOON (P.M.) BODY WEIGHT MEASURES (g) TAKEN FROM RATS THAT HAVE FOOD AVAILABLE AD LIB AND RECEIVE FOUR DAYTIME INJECTIONS OF SALINE OR NOREPINEPHRINE (4×20 nmoles) OVER 5 CONSECUTIVE DAYS AND NO INJECTIONS AT NIGHT

	Saline			Norepinephrine		
Days	a.m.	p.m.	Weight Gain	a.m.	p.m.	Weight Gain
1	585	587	2	579	596	7
2	587	589	2	587	600	13
3	590	594	4	591	601	10
4	592	596	4	590	606	16
5	591	592	1	596	609	13
6	588	—	—	596	—	—
Body Weight Gain*						
a.m. (6 days)	3.2 ± 1.6			17.5 ± 3.4†		
p.m. (5 days)		4.6 ± 1.7			13.5 ± 2.4†	
Daytime (a.m.–p.m.)			2.6 ± 0.6			11.8 ± 1.5§

*Mean scores (±SEM) represent body weight gain that occurred within subjects between the first (Day 1) and last (Day 6) a.m. reading; the first (Day 1) and last (Day 5) p.m. reading; and the a.m. and p.m. readings of each of the 5 days.

For comparisons between the saline and norepinephrine scores for body weight gain: †$p<0.05$; §$p<0.001$.

obtained in the present study (Experiment 2) are in agreement with this proposal. With chronic daytime injections of NE, it was found that the number of eating bouts exhibited by the rats remained unaltered, whereas the size of each bout was increased by 100%. Results obtained by Martin and Myers [24], using the push-pull cannula to measure the efflux of ^{14}C-labeled brain NE, are consistent with these findings and provide direct evidence to support a role for medial hypothalamic NE in the maintenance of eating behavior. These investigators have detected, at the onset of a rat's spontaneous eating bout, an increase in the efflux of ^{14}C-labeled NE in the periventricular area of the hypothalamus at the level of the PVN and ventromedial nucleus. This effect was not observed prior to the onset of eating, suggesting that endogenous NE is released to control meal size rather than to initiate a new eating bout.

Implicit in any hypothesis that a neurotransmitter system is active and essential for normal control of behavior is the prediction that chronic changes or dysfunction in this system should result in specific behavioral disturbances. The present findings are at least consistent with a potential role for a PVN noradrenergic system in normal food intake regulation, which, as a consequence, may have impact on body weight regulation as well. In addition to the evidence described above, showing marked similarities between the patterns of spontaneous and NE-evoked eating behavior [8,9], there is other work which has revealed reliable feeding changes with near physiological doses of exogenous NE [12, 14, 31]. Furthermore, biochemical studies of endogenous NE turnover and receptor activity have demonstrated dramatic changes in these parameters, specifically in the PVN, that are associated with changes in the animals' hunger state [7]. The present study reveals how chronic noradrenergic activation of the PVN has the power and robustness to override normal body weight control. As indicated by the preliminary study with continuous, remotely-controlled NE infusion [22], this effect may occur over a longer period of at least 14 days and without any apparent development of tolerance. It is interesting to note that, in the monkey, chronic peripheral administration of the α-adrenergic agonist clonidine is effective in producing overeating and obesity [35], and in humans, increased body weight has been reported to be a common side effect of clonidine, which is used in the treatment of hypertension (see Physicians' Desk Reference). A variety of recent studies have identified the PVN as a potential mediator of clonidine hyperphagia. Direct PVN administration of clonidine is effective in stimulating eating [26], and discrete

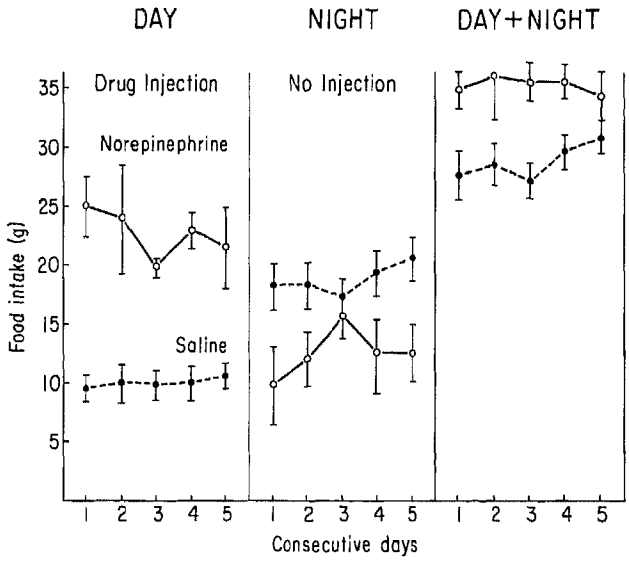

FIG. 3. The stimulatory effect of repeated norepinephrine (NE) administration into the PVN on food intake during the day, night, and day + night. The animals in this experiment (N=7) had food (milk-mash) available ad lib and received four daytime injections of NE (20 nmoles/injection) or saline over 5 consecutive days. No injections were given at night.

electrolytic lesions of this nucleus abolish the eating response produced by peripheral clonidine administration [26]. As with chronic NE ([22] and the present study), continuous infusions of clonidine into the PVN, over a 14-day period, effectively enhance daily food intake and body weight gain [22].

Relevant to these findings with chronic PVN noradrenergic stimulation is the recent finding that discrete electrolytic lesions of the PVN, which leave intact the ventromedial hypothalamic area, produce significant hyperphagia and increased body weight in both female and male rats [20]. In view of the unique sensitivity of the PVN to direct NE administration [11], this result has led to the suggestion that NE acts to elicit eating through the inhibition of "feeding suppressive" neurons located within the PVN [20,21]. The outcome of the present experiments supports this conclusion and demonstrates that chronic noradrenergic stimulation has similar consequences to those produced by irreversible destruction of the PVN through electrolytic lesions. The relationship between these phenomena will need to be clarified through more direct tests of this hypothesis. In our efforts to understand their relevance to the development of various obesity syndromes, we will need to relate the above described findings obtained in albino Sprague-Dawley rats with biochemical evidence obtained in genetically obese Zucker rats [3,23]. In two separate studies, it has been established that obese Zucker rats, in contrast to their lean littermates, exhibit a significant decline in NE levels, specifically within the PVN and median eminence as opposed to other brain areas where no change was observed. Although it is difficult to interpret this change in steady-state levels of endogenous NE in relation to dynamic processes, one reasonable proposal is that it reflects an activated PVN noradrenergic system where NE turnover has increased beyond the capacity of synthesis to replenish its presynaptic stores. This hypothesis is supported by the present findings which demonstrate overeating and increased weight gain in albino rats with increased PVN noradrenergic activation.

When interpreting these effects of chronic NE injection, it is important to bear in mind recent evidence that has linked NE-induced eating to peripheral autonomic and neuroendocrine functions. Recent anterograde and retrograde tracing studies have provided evidence for neuroanatomical projections between the PVN, median eminence, hypophysis, and autonomic nuclei of the lower brainstem [37]. These projections may provide the neural substrates for the potential interrelation between NE feeding and autonomic-neuroendocrine processes [26]. For example, it has been demonstrated that eating elicited by PVN injection of NE is blocked to total vagotomy and peripheral atropine and scopolamine injection; is significantly attenuated by selective coeliac vagotomy; and is unaffected by hepatic and gastric vagotomy [34]. In light of this evidence, it should be noted that medial hypothalamic injection of NE causes a release of insulin [4]. Further, since chronic insulin injections are known to produce an increase in daily food intake [30], it is pertinent that infusion of insulin directly into the medial hypothalamus enhances the release of endogenous NE [27]. Although a potential interrelation between glucodynamic processes and NE-induced eating has similarly been proposed for the glucoprivic agent 2-deoxy-D-glucose [27, 28, 32], direct tests defining the nature of this relationship have yet to be conducted.

In understanding how chronic disturbances of PVN noradrenergic activation may alter long-term eating patterns, we will also need to consider the convergence of evidence relating PVN NE to the ingestion, as well as metabolism, specifically of carbohydrate. Norepinephrine injected into the PVN preferentially enhances carbohydrate. Norepinephrine injected into the PVN preferentially enhances carbohydrate intake [38], and the eating potentiating effect of NE is found to be dependent on the glucocorticosteroid corticosterone [19]. Furthermore, glucose infusions directly into the duodenum, known to produce satiety, have actually been shown to *inhibit* the release of endogenous NE, specifically in the PVN [29]. This finding, of a physiological inhibition of NE release, may reflect a negative feedback mechanism for control of further food (carbohydrate) ingestion. Under conditions of chronic exogenous NE administration, this negative feedback process becomes inconsequential, and overfeeding ensues.

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