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Psychology

Control of meal size by central noradrenergic action

(feeding behavior/noradrenaline/hunger/biogenic amines/hypothalamus)

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ABSTRACT **Previous investigations of central noradrenergic effects on food intake have concentrated on the use of high doses of noradrenaline (two to 200 times brain noradrenaline content). In this work we examined the effect of low doses of noradrenaline (not exceeding brain noradrenaline content) on the parameters of spontaneous ingestive behavior. By arranging for rats to trigger remote infusions of noradrenaline into their own anterior forebrains at the beginning of spontaneously initiated meals, meal size was very reliably increased more than 200% by doses of 0.015–0.37 nmol (2.5–62 ng of noradrenaline base) (n = 12). The effect was alpha-adrenergic. It was blocked by phentolamine but not by propranolol.**

Infusions of noradrenaline at the above doses, which nearly triple meal size, did not elicit eating when made during an intermeal interval, nor did they influence the length of the intermeal interval when made 60 min after the termination of an uninfused meal.

These results show that noradrenaline increased food intake at doses less than 1% of the brain's endogenous noradrenaline content. Meal size is more susceptible to alteration by noradrenaline manipulations than is meal frequency. The brain's own noradrenergic system may function to sustain food intake once feeding is initiated. This function of brain noradrenaline may control spontaneous meal size.

During the last fourteen years, a great deal of evidence has accumulated suggesting that brain noradrenaline participates in control of food intake. The elicitation of eating by intrahypothalamic injection of noradrenaline (1–5) is the most compelling evidence for a central noradrenergic control of feeding behavior. In addition, it is now known that noradrenaline-elicited eating depends upon α receptor activation (3) and that the most sensitive sites for elicitation of eating are in the anterior hypothalamus (4). Recently, Evetts *et al.* (6) and Slangen and Miller (7) have shown that eating can be elicited by pharmacologic release of endogenously synthesized noradrenaline. While β-adrenergic agonists are not effective eliciters of feeding behavior, Leibowitz (8) and Margules (9) have described β suppressant effects on feeding, and Leibowitz has suggested an σ excitatory and β inhibitor system for controlling food intake (8).

Despite repeated demonstration of noradrenergic effects on feeding behavior, two major problems have not been dealt with previously and have limited the appeal of the suggestion of a noradrenergic mechanism for control of feeding behavior. First, the doses of noradrenaline used to elicit eating have typically been two and 200 times the endogenous noradrenaline content of the rat brain (6 nmol) (10). The consistent use of nonphysiological doses of noradrenaline to elicit eating has led to the suggestion that noradrenergic effects on food intake are dose artifacts (11). Second, it has not been convincingly demonstrated that noradrenaline can potentiate normal, spontaneously initiated eating, or, stating the issue more precisely, that noradrenaline has an effect on meal size or meal frequency, which are the basic parameters of spontaneous ingestion. Examination of meal parameters is important because all adjustments in total food intake must be made either by changes in the number of meals eaten (meal frequency) or in the size of the individual meals (meal size). Recent work suggests that physiological challenges that influence food intake do so by altering one parameter while leaving the other unchanged (12). For example, the increase in food intake brought about by fasting results entirely from an increase in meal size (13).

We have addressed ourselves to both criticisms of a noradrenergic system for the control of food intake by exposing the rat brain to bilateral, intrahypothalamic infusions of noradrenaline during spontaneously initiated meals. With this maneuver we have succeeded in increasing meal size by 200–300% using noradrenaline at doses one to two orders of magnitude below the endogenous noradrenaline content of the rat brain.

METHODS

All experiments were performed using adult, male Sprague–Dawley rats weighing 350–400 g at the start of the experiments. Each rat was stereotaxically implanted with a double-barrelled cannula system in each side of the anterior forebrain. The design of the cannulae was similar to that described by Epstein (14).

A very liberal criterion was set for determining whether cannulae were positioned appropriately for noradrenergic effects on feeding. All rats were tested in their home cages with an infusion of 29.6 nmol (typical of doses used in previous work) of *l*-noradrenaline infused into each side of the brain. As in previous work, the injections were made when the animals were not eating. Nearly all animals responded by eating at least one gram more food (Purina pellets available on the cage floor) during the 1-hr period immediately following infusion than they had eaten when infused with the sodium bitartrate control solution on a preceding day. Rats that did not meet this criterion were not further tested.

After making their debut into the experiment, animals were tested in two ways. First, a group of eight rats was studied for the elicitation of feeding by noradrenaline, that is, they were injected in their home cages with the amine during an interval between meals (when they were *not* eating). Using this conventional strategy we attempted to find a minimal dose of noradrenaline that would initiate feeding behavior. The following doses were used in descending

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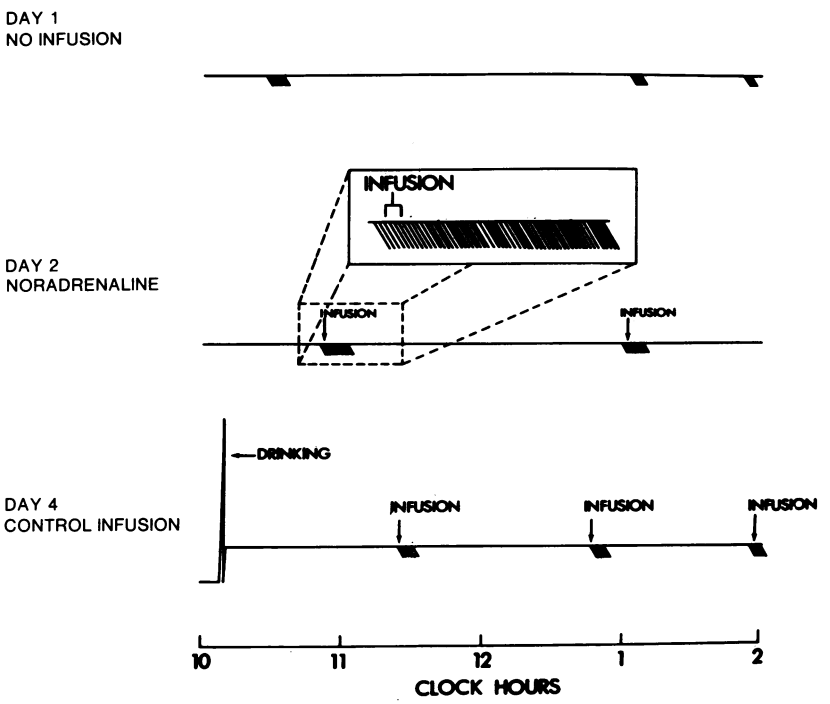

FIG. 1. Facsimile of cumulative record illustrating method of testing. Meals are comprised of groups of hatch-marks. Each hatch-mark indicates delivery of one 45-mg Noyes pellet. Note that the animal eats all food as discrete meals. Day 1 illustrates the meal pattern of a noninfused baseline day. The expanded panel of the Day 2 trace shows that delivery of the third food pellet triggers a 1-μl infusion of noradrenaline which lasts 30 sec. After at least 1 rest day, rats received within-meal infusions of a control solution (Day 4). The method of control infusion was identical to that used for noradrenaline infusion. The vertical pen excursion on the Day 4 trace typifies the appearance of a bout of drinking, sensed by a lick-detecting drinkometer. Drinking rarely occurred during the daytime test periods examined in these experiments.

order: 29.60 nmol, 1.48 nmol, 0.74 nmol, and 0.37 nmol of noradrenaline, and 29.60 nmol of sodium bitartrate control. The rats were then retested with 29.60 nmol of *l*-noradrenaline to determine whether any loss of responsiveness had occurred. All infusions were separated by at least 48 hr of rest during which no experimental manipulations were performed.

For the principal experiment, a second group of rats was used. They bore effective, bilateral cannulae and lived in Skinner boxes where they obtained all their food by pressing a lever for 45-mg Noyes pellets. One press delivered one pellet. Food intake was monitored electronically, yielding digital and graphic records. Water intake was measured to the nearest tenth milliliter and was continuously sensed by a lick-detecting drinkometer. The apparatus was arranged so that infusions could be made into the hypothalamus without disturbing the animal. Fine polyethylene tubing carried the solutions from remote microsyringes held in a Harvard infusion pump and set to deliver 1 μl in 30 sec when triggered automatically by the animal in the midst of its spontaneous ingestive behavior. Infusions were always bilateral.

This approach is, therefore, completely different from that taken by other investigators. Fig. 1 is a facsimile of a cumulative-recorder tracing that illustrates the method of testing. As can be seen, meals appear on the cumulative record as groups of hatch-marks. Each individual mark records delivery of one 45-mg morsel of food. The upper trace represents the animal's noninfused meal pattern. Meal onset was signalled when an animal earned three pellets during a 10-min interval bracketed by 10 min of no eating on either side. The expanded panel in the second trace shows that delivery of the third pellet triggered the infusion of *l*-noradrenaline or a control solution. All *l*-noradrenaline infusions were compared to at least 2 days of noninfused baseline (line one) and 1 day on which the control solution of either sodium bitartrate or *d*-noradrenaline bitartrate was infused at meal onset (line three). Experiments were always conducted during a 4-hr test period during daylight hours. The clock hours of this period varied among rats but were always the same for each rat.

Using the automated system for intrahypothalamic infusions we examined the effect of very low doses of noradrenaline on spontaneously initiated meals. A total of 20 rats were used. Three of these animals were tested with several noradrenaline doses ranging from 0.015 nmol to 29.6 nmol infused at the onset of a spontaneous meal. Eight of the animals were tested only at a dose of 0.37 nmol infused at the onset of a spontaneous meal. Five other rats received noradrenaline infusions (0.37 nmol) in combination with the noradrenergic receptor blocking agents, phentolamine (1.57 nmol) or propranolol (2.53 nmol), at the onset of spontaneously initiated meals.

The remaining four rats were tested both for elicitation of feeding and for effect of noradrenaline on spontaneous meals. First, they were tested in their home cages for elicitation of food intake during an intermeal interval. A dose of 0.15 nmol of *l*-noradrenaline was used. None of these rats ate. Then they were transferred to the Skinner boxes, where the effect of within-meal infusion of noradrenaline was examined. The rats were then tested twice for elicitation of eating. In these retests the same 0.15-nmol dose of *l*-noradrenaline was infused 60 min after termination of a spontaneously initiated meal. In other words, noradrenaline was administered during an intermeal interval to determine

Table 1. Exaggeration of spontaneous meal size by noradrenaline infused at meal onset

NA dose (nmol)	n	Meal size (g ± SEM) No infusion	Control infusion	NA infusion
0.0015	3	1.4 ± 0.1	1.3 ± 0.2	1.4 ± 0.5
0.0150	3	1.4 ± 0.1	1.3 ± 0.2	2.5 ± 0.3
0.1500	4	0.7 ± 0.1	1.0 ± 0.3†	2.8 ± 0.2*
0.3700	8	0.9 ± 0.2	1.1 ± 0.4	2.8 ± 0.4*
29.60	3	1.4 ± 0.1	1.3 ± 0.2	7.8 ± 0.4*

NA, noradrenaline.
* Significant difference from no infusion and from control infusion; $P < 0.01$.
† 2.6 nmol of *d*-noradrenaline bitartrate used as control infusion; all others utilized sodium bitartrate equimolal to the *l*-noradrenaline dose.

whether noradrenaline at this dose could initiate a *de novo* meal by shortening the intermeal interval after an uninfused meal. While still in the Skinner boxes, these four rats were finally retested with 0.15-nmol of noradrenaline infusions made at the onset of a spontaneous meal.

RESULTS

All of the rats used in the main experiment ate in response to the high, conventional dose of noradrenaline. The average amount of food intake elicited in eight rats tested with 29.6 nmol was 3.3 ± 0.2 g. Whereas all rats ate in response to an infusion of 29.6 nmol of *l*-noradrenaline, only three ate in response to 1.48 nmol and two of these ate less than 1 g. Below 1.48 nmol of noradrenaline, feeding could not be elicited in these rats although a retest with 29.6 nmol of noradrenaline showed that there was no significant loss of responsiveness.

In contrast to the low reliability of subnanomole doses of noradrenaline for elicitation of eating, examination of Table 1 reveals that infusion of very low doses of *l*-noradrenaline (0.15 or 0.37 nmol) at the onset of a spontaneously initiated meal produced large and reliable increases in meal size of over 200%. Control infusions of sodium bitartrate or 2.60 nmol of *d*-noradrenaline, the physiologically inactive optical isomer of noradrenaline, produced no change in meal size. As can be seen, a dose of only 0.015 nmol infused into each side of the brain caused an increase of 87% above noninfusion and control-infused meals. Although doses of 29.6 nmol produced very large increases in meal size, i.e., 400%, there were pronounced side effects such as ataxia and vocalization

Table 2. Failure of meal exaggerating doses of noradrenaline to alter feeding when infused between meals

Treatment	First meal size (g ± SEM)	Second meal size (g ± SEM)	Intermeal interval (min ± SEM)
No infusion	0.7 ± 0.1	1.5 ± 0.3	212 ± 14
l-NA 60 min after first meal	1.3 ± 0.4	0.8 ± 0.1	197 ± 50
d-NA during first meal	1.0 ± 0.3	0.6 ± 0.2	167 ± 140
l-NA during first meal	2.8 ± 0.2*	1.1 ± 0.3	257 ± 52

NA, noradrenaline.
* Significantly different from no infusion; $P < 0.01$.

Table 3. Blockade of meal size exaggeration by phentolamine*

No infusion	NA infusion	Phentolamine and NA infusion	Propranolol and NA infusion
1.03 ± 0.20	3.05 ± 0.56†	0.78 ± 0.21	2.41 ± 0.46†

NA, noradrenaline.
* Meal size given in grams ± standard error of mean for five rats.
† Significantly different from no infusion; $P < 0.01$.

at this dose. Such evidence of discomfort was never seen at doses below 1.48 nmol. Table 2 shows that doses of *l*-noradrenaline that produced a doubling or tripling of meal size elicited no eating when infused during an intermeal interval. *l*-Noradrenaline infusion produced no significant change in the length of intermeal intervals. Furthermore, intermeal infusion of *l*-noradrenaline did not alter the size of ensuing meals. However, since all intermeal intervals were longer than 90 min, the infusion of *l*-noradrenaline 60 min after termination of a meal never placed the infusion closer than 30 min to the onset of the next meal. Therefore, it cannot be said that these infusions would not have resulted in changes in meal parameters if they had occurred later in the intermeal interval.

Meal exaggeration by noradrenaline appears to be an α-receptor effect. It is blocked by phentolamine, but not by propranolol (Table 3).

DISCUSSION

Intracranial infusion of *l*-noradrenaline into the rat's diencephalon greatly exaggerated the size of spontaneously initiated meals. Reliable exaggeration of meal size occurred at doses that did not elicit eating when infused during an intermeal interval in the satiated animal. Exaggeration of meal size occurred at noradrenaline doses that are less than one-hundredth the endogenous noradrenaline content of the brain, and that are within the range of estimated NA concentrations in rat hypothalamic nuclei (10).

These findings strengthen the argument for a noradrenergic control of food intake in two ways. First, they show that it is not necessary to use doses of noradrenaline that are equal to, or orders of magnitude greater than, amounts that normally occur in the brain. Second, since exaggeration of meal size is an augmentation of a spontaneous, natural behavior, it seems less likely that the observed increase in food intake is due to some nonspecific artifactual effect of noradrenaline.

The fact that noradrenaline can exaggerate spontaneously occurring meals at lower doses than those necessary for elicitation of eating suggests that the infused noradrenaline may be imitating the effects of noradrenaline released from endogenous stores during eating. On the other hand, it is possible that brain noradrenergic neurons can be activated for the control of food intake both during and between meals, but that meal size is more susceptible than is meal frequency to change by small alterations in noradrenergic neuron activity.

All alteration in food intake must be made through changes in either the frequency with which meals are initiated or through alterations in the size of individual meals. Recent work (12) suggests that meal size and meal frequency changes occur, at least in part, independently to alter food intake in response to specific environmental or metabolic needs. For example, meal frequency appears to be in-

creased during short-term cold stress, while size remains unchanged (15). Conversely, meal frequency seems to be inert when food intake must be increased in order to meet a metabolic demand such as lactation (16), prior food deprivation (13), or caloric dilution of a liquid diet (17, 18). In these situations the increased total intake is entirely the result of increased meal size. The option of control of total food intake by increasing meal size should be of considerable adaptive value. Increasing the amount of intake in a single bout of ingestion would allow animals to take advantage of food that became available irregularly.

Alterations of meal frequency suggest the operation of a control that initiates meals, a control of hunger. Alteration of meal size, on the other hand, necessitates a control that terminates feeding, a control of satiety. Our demonstration that noradrenaline can double or triple meal size at doses that fail to initiate feeding suggests that the release of endogenous noradrenaline during feeding increases food intake by a suppression of the latter, that is, by an inhibition of satiety.

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