Feeding: Satiety Signal from Intestine Triggers Brain's Noradrenergic Mechanism

Abstract. *Noradrenergic neurons in the hypothalamus involved in feeding and satiety are activated by gastrointestinal receptors. In the unrestrained rat, sites were first identified at which norepinephrine injected in the medial hypothalamus caused spontaneous feeding, or in the lateral hypothalamus caused no response. The activity of in vivo norepinephrine at these two sites was characterized by localized push-pull perfusion. When a nutrient was infused directly into the rat's duodenum, the synaptic release of hypothalamic norepinephrine was enhanced at lateral sites insensitive to norepinephrine, but suppressed at medial sites reactive to norepinephrine. Thus, signals from duodenal receptors are conceivably sent to the rat's brain to end feeding by way of noradrenergic inhibitory neurons in the hypothalamus.*

During the last 20 years accumulated evidence has implicated catecholamine-containing neurons in the hypothalamus in the central control of feeding (*1*). Norepinephrine (NE) applied directly to medial hypothalamic sites of several species evokes intense feeding in the fully satiated animal (*2, 3*). Recent physiological experiments show also that NE is released in vivo within the hypothalamus of a food-deprived rat or monkey not only as it consumes food (*4-7*) but also after 2-deoxy-D-glucose (2-DG) is given peripherally or centrally (*6, 8*). Pharmacological studies reveal further that NE infused into the lateral hypothalamic area can suppress feeding in the deprived rat (*9*). Thus, NE could not only inhibit cellular mechanisms in the lateral hypothalamus responsible for feeding, but also inhibit satiety mechanisms in the medial hypothalamus, which would result in spontaneous eating.

The relationship of alimentary mechanisms to NE-induced feeding as well as to the release of the amine is not known. We now report that a direct physiological stimulus to the gastrointestinal tract, which reflects a condition of "satiety" (*10*), differentially alters the pattern of NE release within the hypothalamus. In response to a duodenal signal, this catecholamine functions within the hypothalamic feeding system as a putative inhibitory transmitter, which apparently mediates satiety.

We infused either glucose or a commercial nutrient directly into the duodenum of the unrestrained animal in order to circumvent a host of variables associated with an eating response, including olfactory and gustatory cues, swallowing, and oropharyngeal stimuli (*11*). Six adult male rats of the Long-Evans strain were first acclimatized to a 12-hour light-dark cycle and maintained in individual cages with food and water freely available. Then, under pentobarbital anesthesia, a polyethylene catheter (*12*) was surgically inserted through the wall of the animal's stomach to rest 1.5 cm into the duodenum. The other end was externalized by cementing it onto the calvarium (*13*). In a second operation 1 week later, a 20-gauge stainless steel guide cannula was implanted stereotaxically 1.0 to 3.0 mm dorsal to the intended sites of perfusion in the animal's hypothalamus (*14*). Three to five days later, 2.5 μg of NE bitartrate were microinjected at successive depths below the tip of the guide tube (*15*) to identify hypothalamic sites at which the catecholamine evoked spontaneous feeding or failed to do so (*16*).

At the completion of NE screening injections and the subsequent designation of sites as NE reactive or nonreactive (*17*), the rat was deprived of food for 12 hours. Then 0.5 μl of L-[7-^{3}H]norepinephrine hydrochloride was injected into the site to label synaptic stores of the catecholamine in the hypothalamus (*18*). A concentric cannula assembly for push-pull perfusion (*4*) was lowered to the identified site 30 minutes later. Each site was perfused with an artificial cerebrospinal fluid (*12*) at a rate of 25 μl/min for 5 minutes with perfusates collected at successive 10-minute intervals. Within the 5-minute interval between the third and fourth perfusion, either 5.5 percent D-glucose or 27 percent liquid diet (Sustagen, Mead-Johnson; caloric density, 1.053 kcal/ml) was injected directly into the duodenum in a volume of 1.0 ml through the indwelling catheter. Then the push-pull perfusion sequence was continued for another 60 minutes. In the control experiments, an identical volume of 1.0 ml of 0.9 percent saline was infused into the duodenum under the same conditions of hypothalamic perfusion.

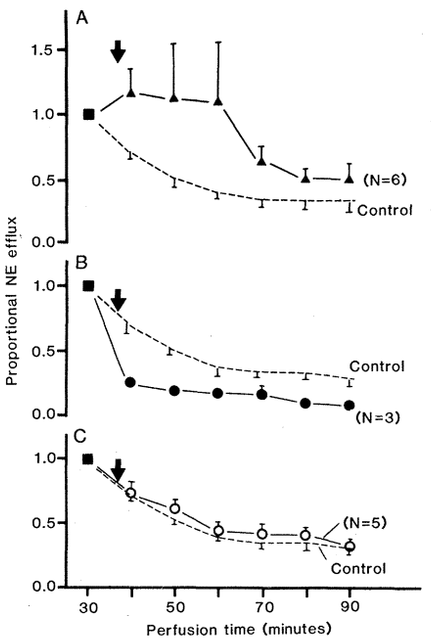

Fig. 1. Mean (± standard error) proportional efflux of ^{3}H-labeled NE from perfusion sites in the rat's diencephalon where earlier NE microinjection in satiated rats failed to alter food intake (A) or evoked feeding (B and C). After duodenal infusion of nutrient (arrow), NE efflux was (A) enhanced ($P < .05$), (B) suppressed ($P < .05$), or (C) unaffected. In control experiments (dashed line) 1.0 ml of saline was given ($N = 10$) instead of nutrient. The 30 minute sample value of labeled NE served as the baseline.

Replicate samples of each hypothalamic perfusate were assayed as follows: (i) an extracted portion of the sample (*19*) was separated on a Waters C18 reverse-phase high-pressure liquid chromatography column and the actual catecholamine content quantitated by electrochemical detection (*20*); or (ii) a 50- or 100-μl portion was added to PCS fluor solution, and the radioactivity was determined by liquid scintillation spectrometry. The Hall-Turner method (*21*) was used to determine the proportional changes in amine activity from a baseline, that is, the level of radioactivity contained in the third sample of the sequence taken at the 30-minute interval. A value of labeled NE in a given sample after the duodenal infusion of nutrient was considered significantly different from its corresponding sample in the control sequence of perfusions if it fell above or below 1.65 standard deviations ($P < .05$) of the mean proportion of the combined control perfusates.

Table 1 presents the results of the changes in NE release within the two anatomical regions of differential sensitivity to the catecholamine. At sites in the dorsolateral portions of the hypothalamus, including the zona incerta, NE release was enhanced by intraduodenal nutrient infusion. In this region NE failed to induce eating and has been found to inhibit feeding in other studies (*9*). This result contrasts with that observed within the medial region of the hypothalamus. Here, NE acted to stimulate feeding, but the nutrients given in the duode-

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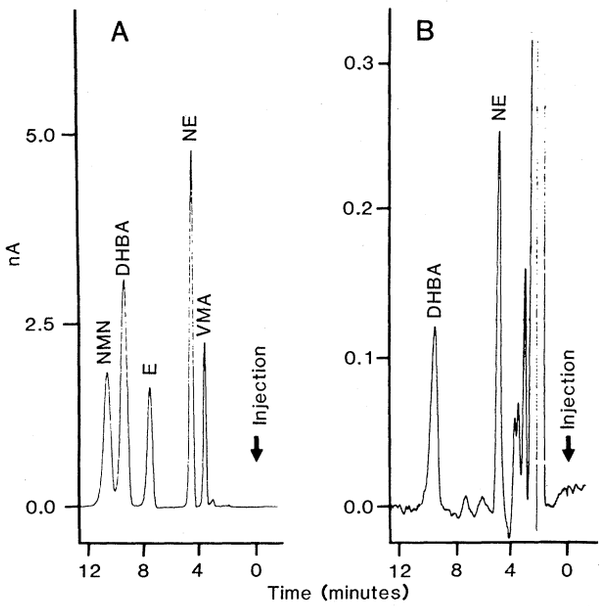

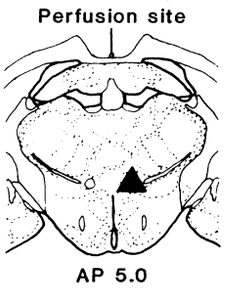

Fig. 2. High-pressure liquid chromatography records, after the injection (arrow), of (A) standards and (B) actual hypothalamic perfusate collected at diencephalic site depicted in histological inset. Standards were DL-4-hydroxy-3-methoxymandelic acid (*VMA*), *l*-norepinephrine HCl (*NE*), L-epinephrine bitartrate (*E*), 3,4-dihydroxy-benzylamine HBr (*DHBA*), and DL-normetanephrine HCl (*NMN*). Hypothalamic perfusate was extracted as in (*19*). Stationary phase: C_{18}-bonded silica; mobile phase: 0.05*M* citrate, 0.05*M* Na_2HPO_4, and 0.001*M* L-heptane-sulfonic acid sodium salt at a flow of 1.5 ml/min.

num caused either no change in NE release or a significant suppression of NE output. This effect occurred in the region of the paraventricular and ventromedial nuclei, where NE exerts its most intense effect (*22*) in inducing feeding. Within posteromedial sites, where NE is less effective, both glucose and Sustagen failed to alter the release of NE.

Either glucose or Sustagen infusion evoked an immediate efflux of NE from NE-sensitive sites in all six experiments (Fig. 1A). This enhanced efflux persisted during the collection of the next two samples of push-pull perfusate 25 minutes after the nutrients were infused. Thus, the influence of a duodenal signal on the hypothalamic activity of NE was not in the form of a single short-lived stimulus. Similarly, within medial hypothalamic sites at which NE injection induced feeding, the reduction in release of the amine caused by the nutrients was not only immediate but sustained over the next hour (Fig. 1B). At the more caudal NE-reactive sites, the output of NE was unaffected by the intraduodenal injection (Fig. 1C).

The amount of NE released in the sample collected from the posterodorsal edge of the medial hypothalamus (site IG2a in Table 1) immediately after intraduodenal Sustagen infusion corresponded to the value in the standard of 290 pg (Fig. 2A). In seven samples collected just after the intraduodenal infusion, the mean recovery of NE was 558 ± 174 pg, which is comparable to the concentrations of NE previously estimated in push-pull perfusates by radioimmunoassay (*7*).

It has been proposed that the presynaptic release of NE from noradrenergic terminals within the hypothalamus underlies, in part, the central neuronal control mechanism for feeding (*4–7*). The present results demonstrate for the first time that a functional signal from the gastrointestinal tract does alter catecholaminergic activity in the hypothalamus. The inverse relationship between anatomical sites that release NE after duodenal nutrient and those within which NE release is suppressed or unchanged is of special significance (*3*).

Electrophysiological evidence suggests that NE is predominantly an inhibitory transmitter in the central nervous system (*23*). Thus, when NE is injected into the medial hypothalamus, this exogenously applied NE would inhibit neurons that control satiety (*10*), thereby resulting in feeding. A nutrient introduced into the duodenum is a moderate physiological signal for satiety and thus reduces food intake (*10*). Therefore, the nutrient would have to reduce the synaptic release of NE from medial "satiety" neu-

Table 1. Relationship between anatomical site of NE test, sensitivity of locus to microinjected NE, and the evoked release (proportional) of the catecholamine into push-pull perfusate after direct infusion of nutrient into the rat's duodenum.

Animal site	Anatomical site of microinjection and perfusion (AP level)	Food intake after NE microinjection (g)	Labeled NE release from site after duodenal nutrient infusion
	NE-nonfeeders		
IG5a	Zona incerta; dorsolateral hypothalamus (5.0)	0.0	.89 ↑ * (glucose)
IG2a	Posterodorsal hypothalamus; zona incerta (5.0)	0.0	1.00 ↑ * (glucose)
IG2a	Posterodorsal hypothalamus; zona incerta (5.0)	0.0	.76 ↑ * (Sustagen)†
IG4a	Medial lemniscus; zona incerta (4.5)	0.0	.67 ↑ * (Sustagen)
IG4b	Far lateral hypothalamic area (4.5)	0.2	.91 ↑ * (Sustagen)
IG5b	Lateral hypothalamic area (5.0)	0.0	.70 ↑ * (Sustagen)
	NE-feeders		
IG2b	Posteroventromedial nucleus (5.0)	2.6	.41 (glucose)
IG2b	Posteroventromedial nucleus (5.0)	2.6	.55 (Sustagen)†
IG1b	Medial premamillary region (4.5)	1.4	.50 (Sustagen)
IG6b	Perifornical area (5.5)	1.0	.48 (glucose)
IG6b	Perifornical area (5.5)	1.0	.42 (Sustagen)†
IG7a	Paraventricular nucleus: medial perifornical (6.0)	2.9	.16 ↓ * (Sustagen)
IG7b	Anteroventromedial nucleus (6.0)	1.8	.18 ↓ * (glucose)
IG7b	Anteroventromedial nucleus (6.0)	1.8	.15 ↓ * (Sustagen)†
	Control (N = 10)		
All sites		0.2	.42 ± .05 (saline)

*$P < .05$. †Replication experiment with alternative nutrient at same site.

rons so that satiety would not be inhibited and the animal would not feed.

On the other hand, NE infused into the lateral hypothalamic area attenuates eating in the deprived animal (*3, 9*), presumably by inhibiting neurons that could be responsible for activating feeding. Consequently, the presence of a nutrient in the duodenum should evoke the synaptic release of NE from noradrenergic neurons in the lateral hypothalamus and thus prevent feeding. This is what occurs. Thus, the anatomically distinct output of NE from the medial noradrenergic neurons that activate satiety as well as lateral hypothalamic neurons yields the same functional result.

The neuronal pathway that affects both sets of hypothalamic cells in harmony may be the afferent vagal pathway, which arises from the gastrointestinal tract and projects to the diencephalon (*24*). This hypothesis would account for the finding (*10*) that a lesion placed in this lateral hypothalamic area eliminates the satiating effect of an intraduodenal infusion of a nutrient. Finally, cholecystokinin, a peptide-containing gut hormone implicated in satiety also differentially affects the release of NE from sites in the rat's hypothalamus (*25*). This would suggest that the suppression of feeding by cholecystokinin may be due to the dual action of the peptide on noradrenergic neurons in both the satiety and feeding systems in the animal's hypothalamus.

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12. The design of the catheter was provided by J. Gibbs and modified after D. S. Liebling, J. D. Eisner, J. Gibbs, and G. P. Smith [*J. Comp. Physiol. Psychol.* **89**, 955 (1975)].
13. A 25-cm length of polyethylene-50 tubing was fitted at the duodenal end with heat flanged collars between which silk suture was passed and knotted to hold the catheter in place. A 1-cm^2 piece of Silastic sheeting was cemented to the catheter with epoxy (Devcon) at the level of the gastric incision. A midline incision was made through the abdominal musculature, the catheter was inserted through a 1-mm incision in the stomach wall, and then subsequently attached to the ventral wall of the stomach by two silk sutures placed around the ends of the collars. Externally, the Silastic sheet was attached to the surface of the stomach by purse-string suture. The polyethylene catheter was brought out of the peritoneal cavity and drawn subcutaneously to the caudal portion of the head, where it was attached by Cranioplastic cement to the skull. A 1-ml bolus of 0.9 percent saline was infused through the catheter every day to maintain its patency.
14. Standard procedures were used [R. D. Myers, in *Methods in Psychobiology*, R. D. Myers, Ed. (Academic Press, London, 1971), vol. 1, pp. 247–280] in which a 23-gauge needle tubing, cut to a length of 1.5 cm, was cemented in place.
15. The NE salt was dissolved in a concentration of 3.5 μg/μl in an artificial cerebrospinal fluid containing the chloride salts of Na^+, K^+, Ca^{2+} and Mg^{2+}. The microinjector needle was 27-gauge stainless steel tubing connected with polyethylene-20 tubing to a 50-μl syringe (Hamilton) mounted on an infusion pump (Harvard). The volume of 0.75 μl was delivered to each hypothalamic site over an interval of 35 seconds.
16. We did not test to see whether NE injected into these "negative" sites would inhibit feeding, because the integrity of its tissue for subsequent perfusion studies was essential. Such an experiment is equally important.
17. A reactive site was so categorized if the previously fed animal consumed 1.0 g or more of freshly ground Wayne Lab Blox within 15 minutes after the microinjection of the NE solution.
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19. After the residual perfusate was dried under a stream of N_2, 1.0 to 10.0 ng of 3,4-dihydrobenzylamine-HBr (DBHA) and 30 mg of acidified alumina oxide were added to 0.4 ml of phosphate buffer. The solution was titrated to *p*H 8.6 and centrifuged to 10^4g, the alumina washed in distilled H_2O, and the catecholamines eluted with 100 μl of 0.1*M* perchloric acid.
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Mutagenic Activity in Photocopies

Abstract. *Extracts from several different photocopies were mutagenic in the Ames* Salmonella *assay. The mutagenic behavior was similar for extracts from copies and corresponding toners indicating that toners are directly responsible for the mutagenicity. The mutagenicity is caused by at least two classes of compounds which may be present either alone or in combination in any toner.*

We report that copies produced and toners used in several photocopying machines contained compounds that are mutagenic in the Ames *Salmonella* assay (*1*).

Extracts of copies with print and extracts of toners used in the machines were found to be mutagenic in the *Salmonella* tester strain TA98 in the absence of rat liver metabolic activation (Table 1). The mutagenic response decreased by addition of S9 from rat liver, but the presence of NADP$^+$ (nicotinamide adenine dinucleotide phosphate) in the S9 mix was not essential for this decrease. Heat-inactivated S9 mix did not decrease the mutagenic response. The mutagenicity was also detectable with the tester strains TA1538 and TA1537. No mutagenicity could be detected with TA1535, whereas a weak response was seen with TA100. Extracts of the plain photocopy papers did not show any detectable mutagenicity. Extracts of copies without intentional print (that is, copies that come out of the machine blank) were sometimes weakly mutagenic, probably as a result of the presence of unintentional print. The mutagenic response with different tester strains suggests that the compound (or compounds) are causing frameshift mutations. Mutagenicity in the absence of the metabolic activation shows that the compound (or compounds) are either directly acting mutagens or are converted to mutagens by the bacterial enzymes present.

There were wide differences in the mutagenic responses of extracts from copies and toners from different copiers. Copies from a 5a machine (we have coded copier manufacturers by number and copier models by letter) gave extracts with a mutagenic response corresponding to about 10^3 revertants per page with TA98, whereas copies from a 3b machine gave a response of about 10^5 revertants per page. The other copies

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