Feeding Produced in the Satiated Rat by Elevating the Concentration of Calcium in the Brain

Abstract. *When the concentration of calcium ions in the cerebral ventricles is elevated, a fully satiated rat eats voraciously. This feeding response is not prevented by prior intraventricular administration of alpha- or beta-adrenergic blocking agents, or other pharmacological antagonists. This supports the concept of an independent ionic mechanism, rather than a neurotransmitter one, for modulating a "set-point" for weight or hunger.*

In a fully satiated cat, an imbalance in the ratio of essential cations in the extracellular fluid in the hypothalamus evokes voracious feeding (*1*). A hyperphagic response occurs when calcium in excess of its normal physiological concentration is perfused by means of push-pull cannulas in the ventromedial region, or when excess sodium ions are perfused in the lateral hypothalamus. It has been postulated therefore that an ionic mechanism may govern a "set point" for hunger (*2*) within the central neural circuit that is delegated to feeding behavior; this might be similar to the proposed sodium-calcium mechanism, in the posterior hypothalamus, which establishes the "set point" for body temperature (*3*).

We now report that spontaneous feeding occurs in the satiated rat when the intracerebral ratio of Na^+ to Ca^{2+} is artificially reduced by elevating the concentration of calcium. This ingestive response does not depend on adrenergic, cholinergic, or other humoral systems, since intracerebral Ca^{2+} induces feeding even after the administration of adrenergic and other blocking agents.

In each of 15 male rats of the Long-Evans strain (*4*), a 21-gauge guide cannula was implanted stereotaxically just above the lateral cerebral ventricle according to procedures described earlier (*5*). After this operation, a 26-gauge injector cannula was lowered just to the level at which artificial cerebrospinal fluid (CSF) flowed into the cannula. A physiological solution (*6*) was infused by gravity flow over an interval of 15 to 45 seconds. We added the chloride salt of Na^+, Ca^{2+}, Mg^{2+}, or K^+ in graded amounts to the solution to be infused intraventricularly in order to alter the normal concentration of an essential cation in the rat CSF (*7*).

Before an experiment began, each rat was brought to its test chamber, where water and 45-mg Noyes pellets were freely available. When the animal discontinued feeding, the content of the food well was adjusted so that ten Noyes pellets (0.45 g of food) were always present. A block of wood was placed on the floor of the cage to ensure that the eating response was not due to nonspecific gnawing behavior. No ionic solution was infused until the rat failed to eat or drink for at least 30 to 60 minutes.

If either the physiological control solution (*6*) or one containing an excess in Na^+, Mg^{2+}, or K^+ (*8*) was infused intraventricularly, there was virtually no activation of ingestive behavior. However, when an excess of Ca^{2+} (2.5 to 151.2 m*M* above normal) was infused, the rat consumed food pellets and drank water for an interval lasting usually for 20 to 40 minutes but never longer than 40 minutes. The magnitude of the feeding response depended on the concentration of excess Ca^{2+} in the infusate; the latency period between infusion and feeding was inversely related to the concentration of calcium ions (Fig. 1, top). When the calcium concentration was increased to 50.4 m*M*, the latency period fell sharply and was relatively constant at 3 minutes even when the concentration was increased further. When the calcium concentration was increased to 151.2 m*M*, the intake of food declined, not because the rat did not feed (*Latency*, Fig. 1, top), but rather because of the relatively adverse side effects such as ataxia, hyperactivity, twitching, and convulsive-like motor symptoms (*9*).

Water intake was directly related to the concentration of calcium in the infusate (Fig. 1, bottom). Drinking appeared in most instances to be prandial as indicated by latency periods that were much longer than those for feeding.

We then attempted to determine if a neurohumoral factor could be implicated in the feeding induced by Ca^{2+} because: (i) The Ca^{2+} ion may release transmitters, including norepinephrine, serotonin, and acetylcholine, in the central nervous system (*10*); (ii) an adrenergic system in the brainstem has been postulated to mediate the feeding response in at least two species, the rat (*11*) and the monkey (*12*). Prior to the infusion of excess calcium ions into 18 rats that showed the feeding response to excess Ca^{2+}, we administered, intraventricularly, pharmacological antagonists which block alpha-adrenergic, beta-adrenergic, serotonergic, and cholinergic receptors.

Only two of these antagonists (*13*) had any notable effect on feeding induced by Ca^{2+}. Propranolol, a beta-adrenergic blocking agent, injected intraventricularly 30 to 60 minutes before the infusion of 50.4 or 100.8 m*M* calcium, not only failed to inhibit the feeding response but rather enhanced eating by an average of 38.1 percent in

Table 1. Differential blocking of spontaneous feeding in a satiated rat. Results are expressed as the mean percent by which an injection of phentolamine blocks the feeding induced by subsequent infusion of excess Ca^{2+} (50.4 or 100.8 m*M*), or of norepinephrine (NE) (10 μg), into the cerebral ventricles. The numbers in parentheses indicate the number of animals tested.

Feeding induced by	Blockage at phentolamine dose (μg) of		
	8	16	32
Ca^{2+}	28% (13)	36% (18)	50% (9)
NE	77% (6)	84% (13)	89% (4)

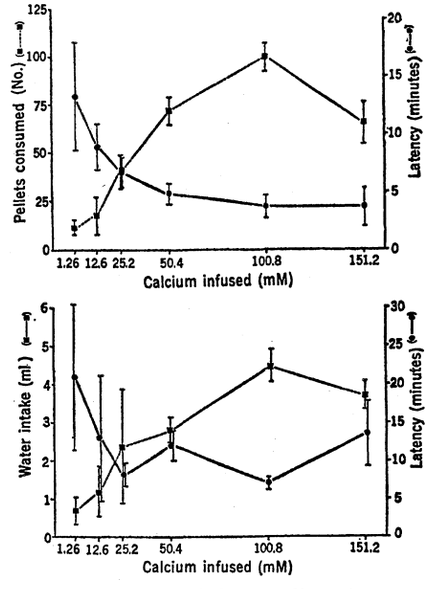

Fig. 1. Amount of food pellets (average number) (top) and of water (average milliliters) (bottom) consumed by 15 rats. The latency periods for each, in response to an infusion of Ca^{2+} into the cerebral ventricle, are indicated on the right-hand axes. The normal CSF concentration of 1.26 m*M* or five concentrations in excess of this value was given in a volume of 5 μl. The standard errors are indicated by the vertical lines.

8 of 11 rats. Phentolamine, an alpha-adrenergic antagonist, partially attenuated the feeding induced by Ca^{2+}, but only in some animals. (Table 1 presents the average percent of blocking, by 8, 16, or 32 μg of phentolamine, of eating induced by Ca^{2+}.) Even though 16 μg of phentolamine given intraventricularly approaches a toxic level (*14*), the Ca^{2+} infusion still evoked feeding. In spite of uncoordinated motor movements after administration of 32 μg of phentolamine, the animal nevertheless struggled over to the food well and consumed food pellets in an amount that was 50 percent of that ordinarily eaten after calcium infusion.

These results add to the doubt already expressed by Krebs and Bindra (*15*) pertaining to the role of norepinephrine or a related adrenergic substance as the sole transmitter involved in the diencephalic coding of feeding, either in the rodent or primate (*11, 12*). When a noradrenergic antagonist virtually inhibited the feeding in response to norepinephrine in the rat, excess Ca^{2+} infused after alpha-adrenergic blockade still produced a feeding response that was 65 to 75 percent of the normal feeding induced by Ca^{2+}. The beta-adrenergic antagonist failed entirely to reduce the induced eating (*16*).

It could be argued that Ca^{2+} acts in the central nervous system to release norepinephrine presynaptically onto postsynaptic receptor sites not occupied by the alpha- or beta-adrenergic blocking compounds; feeding would thus still occur. A possible alternative explanation is that a dissociation exists between a humoral circuit for motivated feeding behavior and the mechanism proposed here in which a "set point" for hunger (*2*), weight regulation (*17, 18*), or lipid metabolism (*19*) is established. If this is the case, norepinephrine could mediate the former, whereas an ionic ratio could modulate the latter. Evidence in favor of this latter view includes:

1) One of the principal homeostatic "set points," that for body temperature, seems to depend on the constant ratio of Na^+ to Ca^{2+} in the caudal hypothalamus (*3*). When this ratio is altered in the posterior hypothalamic area, the temperature of an animal may be stabilized at a new level that is as high as 40°C, or as low as 32°C, for many hours. At these temperatures, the animal will nevertheless thermoregulate around the new "set point" (*20*). Although the anatomical locus for a "set point" for hunger or weight in the rat is unknown at the present time, it is likely that some distinct region of the hypothalamus is involved. Because the cat feeds after a slight elevation of Ca^{2+} in the ventromedial hypothalamus (*1*), the relatively short latency period of the feeding induced by Ca^{2+} in the rat would suggest that this cation is acting on a structure such as the ventromedial nucleus lying close to the ependymal wall of the third ventricle.

2) Powley and Keesey have postulated that a "set point" mechanism for weight regulation exists in the hypothalamus (*17*) and can be altered selectively by a discrete hypothalamic lesion. When a rat is starved prior to the ablation of the lateral hypothalamus, the classical aphagic syndrome (*21*) is reduced substantially if not entirely abolished. Such a basic "set point" function would have to be controlled by a very fundamental biological mechanism based on an intrinsically stable property of the nervous system.

One could envision that as the dimension of the lesion is increased, more and more cells whose steady-state firing rates tend to maintain the inherent weight regulation "set point" are destroyed. At the same time, the ionic influence on these cells would be reduced. Thus, the constancy in the balance in the ratio of sodium to calcium could provide the milieu whereby such a steady-state activity of the neurons sets the level of weight or hunger independent of those hourly changes in the degree of appetite or satiety (*22*).

R. D. MYERS
S. A. BENDER
M. K. KRSTIĆ*
P. D. BROPHY†
Laboratory of Neuropsychology,
Purdue University,
Lafayette, Indiana 47907

References and Notes

1. R. D. Myers and W. L. Veale, *Physiol. Behav.* **6**, 507 (1971).
2. W. L. Veale and R. D. Myers, *ibid.* **7**, 601 (1971).
3. R. D. Myers and W. L. Veale, *Science* **170**, 95 (1970); *J. Physiol.* **212**, 411 (1971).
4. Royal Victoria and Sprague-Dawley rats were used as control strains, and several females were also tested as a control for sex differences.
5. R. D. Myers, G. Casaday, R. B. Holman, *Physiol. Behav.* **2**, 87 (1967); R. D. Myers, in *Methods in Psychobiology*, R. D Myers, Ed. (Academic Press, London, 1971), vol. 1, pp. 247–280. The stereotoxic coordinates (in millimeters) were: anterior-posterior, 5.4; lateral, 1.7; horizontal, +2.5. At the conclusion of the experiments, the position of each injector cannula was verified by the standard procedure of injecting 5.0 μl of bromophenol blue dye into the ventricle.
6. This substitute CSF was prepared in pyrogen-free glassware with sterile distilled water and contained: Na^+, 127.6 m*M*; K^+, 2.5 m*M*; Ca^{2+}, 1.3 m*M*; Mg^{2+}, 1.0 m*M*; and Cl^-, 134.5 m*M*. When infused intraventricularly, the solution has no observable physiological or behavioral effects (R. D. Myers and P. D. Brophy, *Neuropharmacology*, in press).
7. D. J. Reed, C. D. Withrow, D. M. Woodbury, *Exp. Brain Res.* **3**, 212 (1967); J. G. Chutkow, *Proc. Soc. Exp. Biol. Med.* **128**, 555 (1968).
8. The excess of each cation in the infusate was in the following ranges: Na^+, 11.0 to 88.0 m*M*; K^+, 4.8 to 23.6 m*M*; Mg^{2+}, 2.4 to 23.9 m*M*.
9. The reliability of the feeding induced by Ca^{2+} varies from day to day, and unlike the feeding induced by norepinephrine injected in the same way, certain rats (about one out of four) fail to eat after an infusion of excess Ca^{2+}, even though the dye injection verifies that the cannula is unobstructed and the solution is dispersed throughout the ventricle. The reasons for these results are unknown and could be due to: (i) the volume of the microinfusion (smaller volumes of Ca^{2+} solution evoke more reliable feeding); (ii) an apparent tachyphylaxis to repeated infusions with Ca^{2+}; (iii) the placement of the cannula in the ventricle (we have seen a "streaming" effect in which infusate appears to have flowed dorsal to the massa intermedia); (iv) the kinetics of the infusion in terms of the spread of infusion, anatomical sites reached, rapidity of spreading, and unknown penetration of Ca^{2+} through the ependymal wall of the rat's ventricle.
10. M. Bjegović, M. Randić, A. Padjen, *Jugoslav. Physiol. Pharmacol. Acta* **4**, 81 (1968); B. A. Hemsworth and J. F. Mitchell, *Brit. J. Pharmacol.* **36**, 161 (1969); R. D. Myers, W. L. Veale, D. B. Beleslin, *Experientia* **26**, 1324 (1970); A. Philippu, G. Heyd, A. Burger, *Europ. J. Pharmacol.* **9**, 52 (1970).
11. S. P. Grossman, in *Thirst*, M. J. Wayner, Ed. (Pergamon, New York, 1964), pp. 487–514; N. E. Miller, *Science* **148**, 328 (196 → J. N. Coury, *ibid.* **156**, 1763 (1967); D. A. Booth, *J. Pharmacol. Exp. Ther.* **160**, 336 (1968); R. D. Myers and T. L. Yaksh, *Physiol. Behav.* **3**, 917 (1968); J. L. Slangen and N. E. Miller, *ibid.* **4**, 543 (1969).
12. R. D. Myers and L. G. Sharpe, *Physiol. Behav.* **3**, 987 (1968); R. D. Myers, *Ann. N.Y. Acad. Sci.* **157**, 918 (1969); L. G. Sharpe and R. D. Myers, *Exp. Brain Res.* **8**, 295 (1969); T. L. Yaksh and R. D. Myers, *Amer. J. Physiol.* **222**, 503 (1972).
13. Each drug was again given in a random order in a volume of 5 to 10 μl and was dissolved usually in the five-ion vehicle solution. The doses of each compound used were: atropine 4, 6, 8, 10, and 20 μg; methysergide 5 and 10 μg; hexamethonium 8, 20, and 30 μg; phenoxybenzamine 16 μg; phentolamine 8, 16, and 32 μg; and propranolol 4, 8, 24, and 32 μg.
14. Z. Kleinrok and I. Zebrowska-Lupina, *Psychopharmacologia* **20**, 348 (1971).
15. H. Krebs and D. Bindra, *Nature* **229**, 178 (1971).
16. Berger *et al.* also report that propranolol injected intraventricularly enhanced the feeding in the rat produced by norepinephrine also injected intraventricularly [B. D. Berger, C. D. Wise, L. Stein, *Science* **172**, 281 (1971)].
17. T. L. Powley and R. E. Keesey, *J. Comp. Physiol. Psychol.* **70**, 25 (1970).
18. M. Cabanac, R. Duclaux, N. H. Spector, *Nature* **229**, 124 (1971).
19. G. R. Hervey, *ibid.* **222**, 629 (1969).
20. R. D. Myers and T. L. Yaksh, *J. Physiol.* **218**, 609 (1971).
21. D. R. Williams and P. Teitelbaum *J. Comp. Physiol. Psychol.* **52**, 458 (195 → P. Teitelbaum and A. N. Epstein, *Psychol. Rev.* **69**, 74 (1962).
22. The site of action of Ca^{2+} has not yet been identified in the hypothalamus or in other structures in the brainstem.
23. Supported in part by NSF grant GB 24592, ONR contract N00014-67-A-0226-0003, and NIMH training grant T1 MH 10267. We thank P. Curzon for his technical assistance.

* Present address: Institute of Pharmacology, Medical Faculty, University of Belgrade, Yugoslavia.
† Present address: Department of Psychology, Southern Illinois University, Carbondale.

27 January 1972; revised 10 March 1972