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Action of Excess Calcium Ions in the Brain on Motivated Feeding in the Rat: Attenuation by Pharmacological Antagonists[1]

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MYERS, R. D. AND BENDER, S. A. *Action of excess calcium ions in the brain on motivated feeding in the rat: Attenuation by pharmacological antagonists.* PHARMAC. BIOCHEM. BEHAV. 1(5) 569–580, 1973. – The intraventricular infusion of 5 μl of a physiological solution in which the concentration of calcium ions was adjusted from 1.26 mM to 151.2 mM caused spontaneous feeding and drinking in the satiated rat. The ingestive response was observed both when pellets and water were freely available, in which case food consumption exhibited a dose-response relationship increasing with the millimolarity of calcium in the infusate, and when operant responses at FR 2 or FR 10 were required for food reward. The rate of bar pressing by an animal at these two schedules of reinforcement did not differ from that observed when the same animal was deprived of food and water for 23 hr. The alpha-adrenergic antagonist, phentolamine (16 μg, 32 μg), did not appreciably attenuate Ca^{2+}-induced feeding during either ad lib or motivated conditions, though these doses drastically reduced food intake induced by the intraventricular infusion of 10 μg norepinephrine HCl. The pre-infusion of a number of additional blocking agents had variable yet consistent effects upon Ca^{2+}-induced feeding; hexamethonium (8 μg, 25 μg), propranolol (6 μg, 28 μg) and methysergide (8 μg) each caused a statistically significant enhancement in the magnitude of the Ca^{2+} response while atropine (6 μg, 15 μg) significantly attenuated feeding after Ca^{2+}. This suggests an interaction between a number of pharmacologically distinct fiber pathways that may mediate the Ca^{2+} feeding response. The modulation of feeding by Ca^{2+} ions supports an ionic theory for the regulation of body weight in the rat, in which neurons that are involved in ingestive responses and which pass through the hypothalamus and other structures along the ventricular lumen are selectively sensitive to the concentration of calcium in the extracellular fluid.

Ca^{2+}-induced feeding Pharmacological antagonism Motivation Hypothalamus Transmitter release Norepinephrine

INHERENT set-point mechanisms within the hypothalamus have been proposed which integrate the physiological signals pertaining to an animal's state of energy balance as well as its thermal enviroment [12, 17, 30]. In studies on the control of feeding and body temperature, the neuronal activity which mediates each of these intrinsic regulatory processes has been manipulated experimentally. In 1970, Myers and Veale demonstrated that the core temperature of a cat could be elevated or lowered by a slight alteration in the ratio of Na^+ to Ca^{2+} ions at discrete sites within the animal's hypothalamus. Later it was shown in similar experiments that a primate would thermoregulate about this newly established body temperature when exposed to peripheral heat or cold stress [33]. This indicated that the animal's internal thermostat may indeed be set at a new level following a central perturbation in the ratio of these two essential cations.

A resetting of the central mechanisms which control the body weight of the rat was first demonstrated by Kennedy [17]. Hyperphagia and a marked increase in the quantity of body fat was observed after a lesion of the ventromedial hypothalamus. When the caloric content of the diet was altered, the animal nevertheless consumed a specific quantity of food in order to maintain its new level of body weight. In recent studies, preoperative obesity or food deprivation attenuates substantially the classical hyperphagic or aphagic syndromes[34]. Thus, an abnormal feeding response may actually be an active process by which an animal alters and then defends its weight at a new level [13,16].

The intrinsic ratio of two endogenous cations in the hypothalamus has also been implicated in ingestive behavior [31]. In a satiated cat, stimulus-bound feeding was observed when a physiological solution containing an excess of Ca^{2+} ions was perfused within isolated sites of the hypothalamus. Similarly, excess Ca^{2+} ions infused into the cerebral ventricles cause spontaneous feeding in the satiated rat [26] and sheep [39].

The purpose of the current investigation was to examine further the phenomenon of Ca^{2+}-induced feeding in the rat. The response was observed in a free-feeding (ad lib) situation and where the animal had to emit an operant response to obtain a pellet of food. In an attempt to characterize the nature of the receptor sites involved in Ca^{2+}-induced feeding, pharmacological antagonists were also administered via the ventricular route. To examine the possible inter-

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action between Ca^{2+} feeding and the level of motivation of the animal, different schedules of reinforcement were employed.

METHOD

Adult male rats of Long-Evans strain, each weighing from 300–400 g, were housed individually in wire mesh cages. The animals were maintained on a 12 hr light–dark cycle, 7 a.m.–7 p.m., at a temperature of about 23°C, during which time they had free access to water and powdered Wayne Lab Blox.

After the rat was anesthetized with 35 mg/kg sodium pentobarbital, a 21 gauge stainless steel guide cannula was implanted either unilaterally or bilaterally just above the lateral cerebral ventricle [27] at stereotaxic coordinates AP, +5.4; H, +2.5; L, +1.7. To verify placement of the guide during surgery, a 26 gauge injector was fitted into a piece of PE 20 tubing and then lowered 1.0 mm below the guide tip to a depth at which artificial CSF flowed out freely into the ventricle. After surgery, a 26 gauge stylet that was bevelled at the same angle as the guide tube was inserted in the guide cannula to prevent its occlusion. A seven to ten day recovery period elapsed prior to any experimental infusions.

An infusion was made after a 26 gauge injector was lowered into the lateral ventricle of the rat. A 5.0 μl volume of the infusion fluid was delivered through the attached calibrated PE 20 tubing either by gravity flow or via a 100 μl Hamilton syringe set in a Sage pump. In both cases, the infusion lasted from 15–30 sec, at which point the tubing was clamped, the injector gently removed and the stylet replaced.

The pellet and water intake of each rat was recorded for a 60 min period or longer after the infusion. However, since most feeding responses terminated within 30 min, intake for the first 40 min was ordinarily used in the data analysis. Whenever the inflow of the test solution into the ventricle became irregular as a result of necrotic tissue at the guide tip or other possible factors, the animal was discarded from the study. Upon completion of a sequence of experiments, each animal was sacrificed by an overdose of sodium pentobarbital, and 5–8 μl of 0.2% solution of bromophenol blue were infused into the ventricle. The position of the injector was verified by careful dissection of the brain and by examination of the structures that were stained by the dye.

The test solutions were: the chloride salts of Ca^{2+}, Na^{+}, K^{+}, and Mg^{2+}, norepinephrine hydrochloride (NE) and artificial CSF containing 5 ions [37]. Infusions of NE in both free-feeding (ad lib) and operant conditions served as intake controls for determining the relative effect of the adrenergic blocking agents upon Ca^{2+}-induced feeding. The pharmacological antagonists used were phentolamine hydrochloride (Ciba), hexamethonium chloride dihydrate (Mann), propranolol hydrochloride (Ayerst), atropine sulfate (Fisher) and methysergide maleate (Sandoz). Both the test solutions and the blocking agents were prepared daily in pyrogen-free artificial CSF, except for solutions of sodium and phentolamine which were prepared with sterile distilled water. Pyrogen-free glassware was used when preparing all solutions, during which time the pH of each was adjusted to 5.4–7.0. Immediately before each solution was infused, it was passed through a 0.22 μ Swinnex millipore filter. Each antagonist was injected into the cerebral ventricle in a 5.0 μl volume 30 min prior to the infusion of either NE or a solution of excess Ca^{2+} ions.

Free-Feeding

In this part of the investigation, a total of 33 rats with unilateral intraventricular guide cannulae was used. The test chamber consisted of a clear plastic box measuring 28 × 19 × 13 cm. An inverted graduated water tube with a metal drinking spout and a food well containing 45 mg Noyes pellets were fastened to one wall of the chamber. A 4 cm cube of wood was also present in the chamber to permit gnawing behavior. Before an experiment, the animal was placed in the chamber and allowed to eat and drink freely. A criterion period of at least 30 min was required immediately prior to the infusion during which the rat did not ingest food or water.

A counterbalanced experimental design was used in which the sequence and concentration of excess Ca^{2+} or other cation and the control vehicle were varied. For an animal's response to be included in the data analysis, a feeding response after infusion of excess Ca^{2+} was required. Each feeding response evoked by an infusion of excess cation was used in the dose-response calculations; however, only a rat that consumed at least 25 pellets in two successive experiments following 50.4 mM Ca^{2+} was used in the blockade study. The order and concentration in which each blocking agent was administered prior to the Ca^{2+} infusion were determined randomly. At least 24 hr were allowed between the infusion of a cation or antagonist. To rule out a nonspecific toxic or cumulative effect of an antagonist, each rat's feeding response had to be at or near the prior control level of intake on the day following the infusion of a blocking agent.

Motivated Feeding

Prior to the implantation of bilateral intraventricular cannulae, each of 9 rats was trained on an FR 10 schedule of reinforcement to obtain food pellets and water. The experimental chamber consisted of a 22 × 27 × 29 cm Plexiglas box with a grid floor. At one end a lever was mounted above a metal tray into which 45 mg pellets were delivered from the attached Noyes pellet dispenser. A second lever was mounted similarly on the other side of the same wall and was above a recessed pan into which droplets of water fell from a calibrated liquid dispenser (Lafayette Instrument). So that the animal did not develop a position habit, the reinforcement delivered by each lever was reversed on alternate days of training. Postoperatively, each rat was tested on the FR 10 schedule to reinstate its lever-pressing performance.

Prior to an infusion, the animal was brought to the test chamber and allowed to press the levers freely for food and water. As in the free-feeding condition, a period of at least 30 min was required in which neither lever was pressed. After an infusion, simultaneous cumulative records of the animal's responses for both food and water were obtained from a Gerbrands Cumulative Recorder and Sodeco printout counter.

A solution of 50.4 mM Ca^{2+} ions was used since this concentration was found to evoke the most consistent feeding response in the free-feeding condition. Phentolamine was administered in the motivated paradigm because of its potent antagonism of feeding induced by NE injected into the cerebral ventricle of the rat [1]. As in the ad lib condition, the rat had to consume at least 25 pellets to be included in the blockade study. At least 24 hours

elapsed between an infusion of Ca^{2+} or the injection of an antagonist. A verification of the Ca^{2+} response was also performed on the day following the administration of the antagonist.

The animals were assigned to two groups of four and five rats each and tested thereafter either on a FR 2 or FR 10 schedule of reinforcement, respectively. Upon completion of the sequence of experiments with the antagonists, 5 μl of 50.4 mM Ca^{2+} were infused on subsequent days in three rats in the FR 2 group, during which time the schedules of reinforcement were FR 2, FR 5 and FR 10.

RESULTS

The infusion of a solution of excess Ca^{2+} ions in the cerebral ventricle evoked marked feeding and drinking in the satiated rat. Initially, the animal became aroused and often explored the chamber before approaching either the food well or the lever which it pressed to obtain food pellets. The alpha-adrenergic antagonist, phentolamine, failed to consistently block feeding induced by 50.4 mM Ca^{2+} during either free-feeding (ad lib) or operant conditions of feeding. In both cases, phentolamine significantly reduced food intake induced by 10 μg NE HCl in the same animals.

Free-Feeding

As illustrated in Fig. 1, the magnitude of the feeding response induced by Ca^{2+} was directly related to the concentration of the cation in the infusate. The slopes of the curves for the intake of pellets and water rose rapidly after Ca^{2+} was elevated above its endogenous level (1.26 mM) and declined only at the highest Ca^{2+} concentration (151.2 mM). Though at this millimolarity it appeared that the animal attempted to eat, ataxia, loss of coordination and even hyperexcitability were often observed.

The latency for pellet intake, presented in Table 1, was inversely related to the amount consumed. At the lowest concentration the latency was 13 minutes, whereas for Ca^{2+} concentrations ranging from 50.4 mM to 151.2 mM, the latency to eat was about 4 min. The drinking responses appeared to be prandial, since the latencies for water intake were almost always greater than those for pellets. The variability in the response to intraventricular Ca^{2+} ions was rather substantial since excess Ca^{2+} failed to evoke a consistent feeding response in one of every five animals tested. Probably the differences in the spread of the injected solution as well as in the capacity of the cation to penetrate the ependymal wall of the ventricle are two of the factors which would account for the variations in the feeding response.

As shown in Table 2, the infusion of the 5-ion vehicle or a solution in which there was an excess of Na^{+}, K^{+} or Mg^{2+} ions had little or no effect on ingestive behavior. There were isolated instances during which an animal became aroused after an infusion containing an excess of these

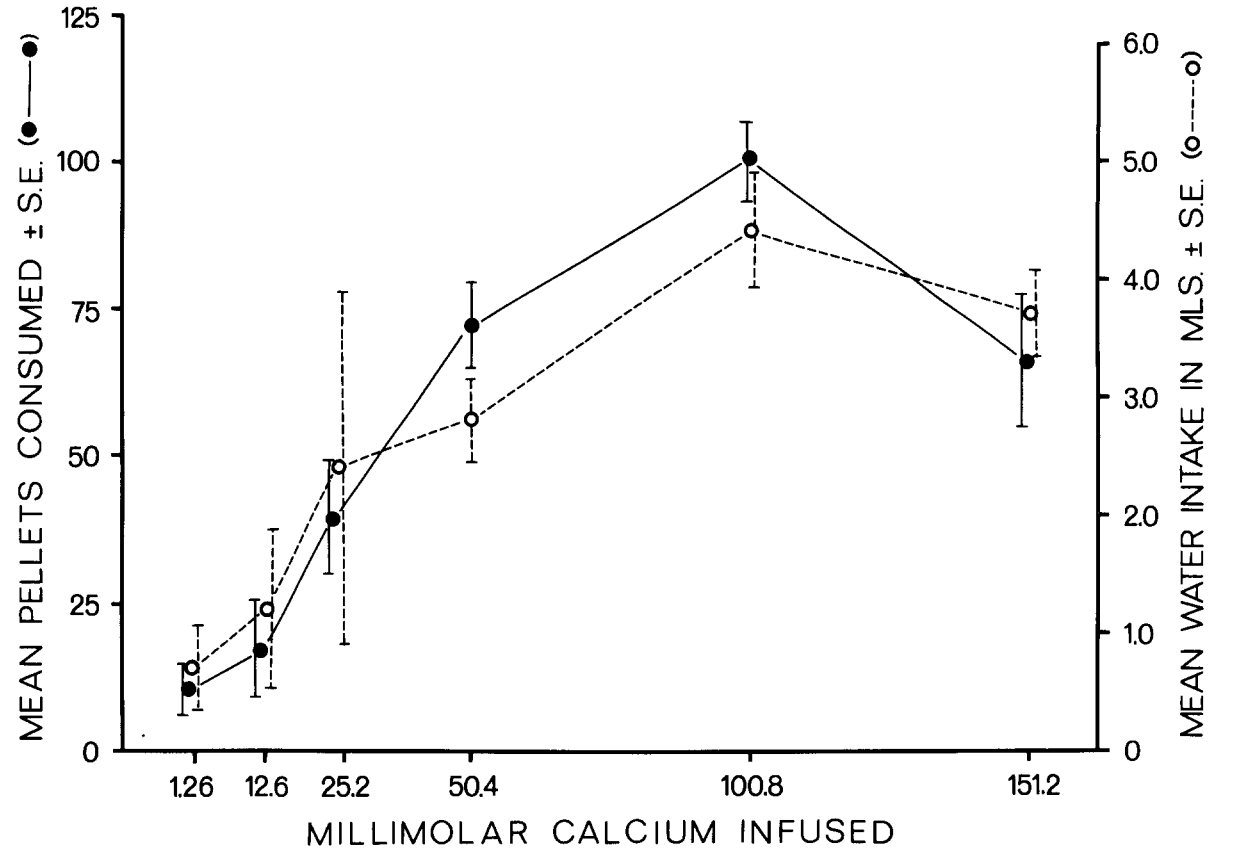

FIG. 1. Mean pellet (•) and water intakes (o) with increasing concentration of Ca^{2+} in the infusate. Standard errors are indicated by the vertical bars.

TABLE 1

MEAN PELLET (45 mg) AND WATER INTAKES (ml) FOR INCREASING CONCENTRATIONS OF Ca^{2+} IN THE INFUSATE. THE CORRESPONDING LATENCY (IN MIN) IS INDICATED IN THE PARENTHESES.

Ca^{2+} Concentration	Number of Experiments	Pellets	Water
1.26 mM	13	12 (13.3)	0.7 (20.8)
12.6 mM	9	18 (8.8)	1.2 (13.0)
25.2 mM	18	40 (6.6)	2.4 (8.2)
50.4 mM	23	71 (4.7)	2.8 (13.0)
100.8 mM	28	100 (4.0)	4.4 (7.2)
151.2 mM	6	66 (3.6)	3.7 (10.6)

TABLE 2

MEAN PELLET (45 mg) AND WATER INTAKES (ml) FOR CONTROL SOLUTIONS. THE CORRESPONDING LATENCY (IN MIN) IS INDICATED IN THE PARENTHESES.

Infusate		Number of Experiments	Pellets	Water
5-ion		13	12 (13.3)	0.7 (20.8)
K^+	12.5 mM	6	0	0
	25.0 mM	6	0	0
Na^+	153.0 mM	3	1 (11)	0.3 (16)
	187.0 mM	4	0	0.3 (2)
	221.0 mM	4	0	0
Mg^{2+}	5.0 mM	5	0	0
	20.0 mM	13	8 (17.6)	0.6 (2.7)
	40.0 mM	13	14 (3.2)	1.3 (2.9)

cations. As reflected in the intakes presented in Table 2, one animal did eat and drink in response to a solution of 40 mM Mg^{2+}. However, this response was not obtained in other rats.

Pharmacological Blockade of Free-Feeding

Each of the five antagonists used had a significant effect upon Ca^{2+}-induced feeding. The prior infusion of 15 μg of atropine SO_4 or 16 μg or 32 μg of phentolamine HCl resulted in an attenuation of the Ca^{2+} response. Conversely, 6 μg or 28 μg of propranolol HCl, 25 μg of hexamethonium chloride or 8 μg of methysergide enhanced the consumption of pellets observed after an infusion of Ca^{2+}. Table 3 shows the effects of four of these agents upon levels of Ca^{2+}-induced feeding and drinking. As indicated by the percent change in pellet intake at each dose, the degree of the blockade depended upon its concentration in the infusate. However, it cannot be concluded that the observed changes in water consumption were due to a direct action of the antagonist upon a central pathway influencing fluid intake since drinking appeared to be prandial.

In examining the effect of a compound that inhibits feeding produced by intrahypothalamic or intraventricular NE, it was found that phentolamine attenuated food intake only partially after an infusion of 50.4 mM Ca^{2+}. This is in contrast to the almost complete reduction in the consumption of pellets if phentolamine was infused prior to NE. Figure 2 shows the mean control pellet intakes for both NE and Ca^{2+} feeding plotted with the intakes recorded after the prior infusion of either 8 μg, 16 μg or 32 μg of phentolamine. Each animal served as its own control for both NE and Ca^{2+}-induced feeding. At each dose of phentolamine there was no statistical difference between the control levels of intake after the infusion of either agonist. However, there were significant differences ($p<0.01$) between the level of Ca^{2+} and NE feeding after each of the three doses of the antagonist (8 μg, $t = 3.42$, $df = 17$; 16 μg, $t = 3.00$, $df = 20$; 32 μg, $t = 3.30$, $df = 11$).

The blockade by phentolamine of these two feeding responses is expressed in Table 4 (top). The mean percent attenuation of the pellet intake observed in each experimental sequence was calculated. After 8 μg of phentolamine, NE-induced feeding was reduced by over 75%, while only 28% of the Ca^{2+} response was blocked. When 32 μg of the antagonist were injected, the NE feeding response was reduced by 89%, whereas Ca^{2+}-induced feeding remained at 50% of its control level. At the two higher doses of the blocking agent, there was usually a marked reduction in locomotor activity prior to and after the infusion of Ca^{2+} or NE. Also presented in Table 4 (bottom) are the latencies for feeding induced by NE and Ca^{2+} during the control experiments and when one of the three doses of phentolamine was infused prior to either of the other two solutions. The control latency after Ca^{2+} was significantly greater than that for NE-induced feeding ($p<0.01$, $t = 2.50$, $df = 34$). However, after 16 μg of phentolamine were infused, the mean feeding latency following the injection of intraventricular NE increased significantly ($p<0.05$, $t = 1.80$, $df = 28$); on the other hand, the latency for Ca^{2+}-induced feeding did not show a significant increase until the prior infusion of 32 μg of the antagonist ($p<0.05$, $t = 2.00$, $df = 22$).

Motivated Feeding

When placed in an operant situation, a satiated rat infused with a solution of excess Ca^{2+} ions pressed the two levers spontaneously for both pellets and water in a sustained fashion. Before the rat approached either lever, there was some initial activity about the chamber, as in the free-feeding condition. Generally, the animal consumed the droplet of water or pellet without any interruption in the rate of lever pressing. After a rapid succession of responses at one lever, the rat often switched to the adjacent bar and began pressing. There were numerous instances during which the animal momentarily sniffed about the chamber between groups of responses.

Figure 3 illustrates the average number of lever responses

TABLE 3

MEAN PERCENT CHANGES FROM CONTROL LEVELS OF PELLET AND WATER CONSUMPTION FOR EACH ANTAGONIST INFUSED PRIOR TO Ca^{2+}. THE DENOMINATOR OF THE FRACTION INDICATES THE TOTAL NUMBER OF EXPERIMENTS FOR EACH ANTAGONIST FROM WHICH THE MEAN PERCENT CHANGE WAS CALCULATED. THE NUMERATOR INDICATES THE NUMBER OF EXPERIMENTS WHERE EITHER AN ENHANCEMENT OR ATTENUATION IN PELLET INTAKE WAS OBSERVED, AS INDICATED BY THE DIRECTION OF THE ARROW.

Blocking Agent	Number of Experiments and Direction of Change	Percent Change in Intake	
		Pellets	Water
Hexamethonium			
8 μg	2/5 ↑	+16.4	+20.0
25 μg	13/17 ↑	+29.0*	+40.0
Propranolol			
6 μg	6/7 ↑	+18.4†	−6.0
28 μg	6/9 ↑	+29.3*	−8.0
Atropine			
6 μg	2/4 ↓	−1.0	+26.0
15 μg	11/11 ↓	−45.5†	−4.1
Methysergide			
8 μg	5/9 ↑	+33.1*	+120.0

t-test: $*p<0.05$, $\dagger p<0.01$ from mean control intakes in the same animals

for pellets (top) and water (bottom) with the corresponding intakes (right axis) following an infusion of 5 μl of 50.4 mM Ca^{2+}. The mean number of pellets consumed at FR 2 and FR 10 was significantly different ($p<0.05$, $t = 2.21$, $df = 40$). On the FR 10 schedule of reinforcement, the rat responded over 300 times compared to less than 100 on the FR 2 schedule. The mean intake of pellets during either of the operant schedules was significantly less than induced by the same concentration of Ca^{2+} in the free-feeding condition. No significant difference was observed in the mean quantities of water consumed between the two ratio groups ($p>0.05$, $t = 0.60$, $df = 37$). The mean water consumed during the free-feeding and operant paradigms was also not significantly different. It is interesting, however, that the mean latencies in fluid consumption of 6.3 min and 2.7 min for FR 2 and FR 10, respectively, were both significantly less than those observed during the free-feeding condition.

Figure 4 presents representative cumulative records obtained from two rats of the lever responses emitted for both pellets and water. After an infusion of 50.4 mM Ca^{2+} at time zero, the rat's initial rate of responding on the FR 2 and FR 10 schedules was very similar to that rate generated when the same animal was deprived of food and fluid for 23 hr.

A representative cumulative record for an individual rat tested on three schedules of reinforcement, FR 2, FR 5 and FR 10, and superimposed on one axis, is presented in Fig. 5. A direct relationship exists between the ratio of reinforcement and the animal's rate of pressing for pellets. Following the intraventricular infusion of 50.4 mM Ca^{2+}, the rat's response was relatively stable on each schedule of reinforcement for approximately 15 min or even longer.

Pharmacological Blockade of Motivated Feeding

As indicated in Table 5, the infusion of 16 μg of phentolamine into the cerebral ventricle of the rat 30 min prior to an infusion of NE caused a mean attenuation of 69.7% of the responses on FR 2. This is in marked contrast to a blockade of only 40.5% of Ca^{2+} feeding by the alpha blocker under the same schedule of reinforcement. Similarly, on the FR 10 schedule, the rat's mean consumption of pellets after NE was attenuated 77.5% by phentolamine, while feeding after Ca^{2+} remained over 40% of its control level.

A close agreement existed between the percent blockade of the Ca^{2+}-induced feeding response during the FR 2 and free-feeding conditions. However, when the rat was on the FR 10 schedule, the antagonism of its feeding was even greater. An increase in the efficacy of the blockade at FR 10 in comparison to the FR 2 schedule was also seen during NE-induced feeding. The antagonism of NE feeding in the ad lib condition was somewhat different than that of the operant conditions. That is, at the FR 10 schedule of reinforcement the percent blockade just approached the percent attenuation during free-feeding.

A comparison of the average number of pellets consumed under both schedules of reinforcement following alpha-adrenergic blockade is presented in Fig. 6. The levels

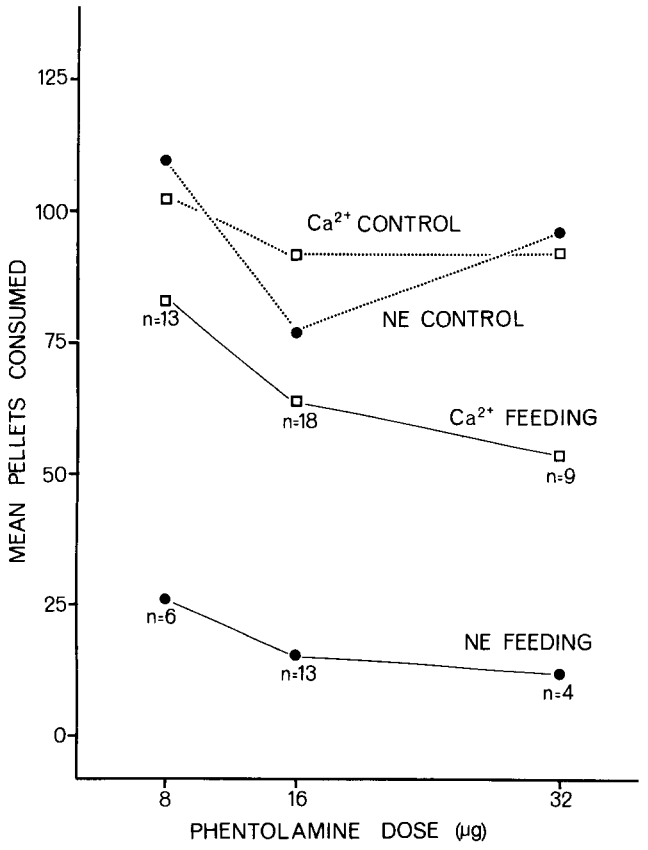

FIG. 2. Mean pellet intake induced by Ca^{2+} (□) or NE (●) after the prior infusion of three doses of phentolamine. The upper two curves indicate the control levels of feeding in the same animals induced by either Ca^{2+} or NE alone. The number of experiments is indicated by n.

of intake within each ratio group were not significantly different during Ca^{2+}- or NE-induced feeding. However, following the infusion of 16 µg of phentolamine, the magnitude of blockade of cation and amine feeding was significantly different under both of the schedules (FR 2, $p<0.05$, $t = 1.72$, $df = 22$; FR 10, $p<0.01$, $t = 2.80$, $df = 20$). Again, the Ca^{2+} blockade was nearly half as great, and it should be noted that although the magnitude of blockade is different, the pattern of the blockade is highly similar. Furthermore, the relationship of alpha-adrenergic blockade

TABLE 4

MEAN PERCENT BLOCKADE (TOP) OF THE FREE-FEEDING INDUCED BY EITHER NE OR Ca^{2+} AFTER THE PRIOR INFUSION OF THREE DOSES OF PHENTOLAMINE. THE MEAN CONTROL AND FEEDING LATENCIES AFTER PHENTOLAMINE (BOTTOM) ARE ALSO SHOWN FOR THE SAME ANIMALS. THE NUMBER OF EXPERIMENTS IS INDICATED IN THE PARENTHESES.

Condition	Control	Percent Blockade by Phentolamine		
Free-Feeding	CSF	8 µg	16 µg	32 µg
10 µg NE HCl	–	77.1 (6)	84.2 (13)	89.1 (4)
50.4 mM Ca^{2+}	–	27.8 (13)	36.3 (18)	50.2 (9)
		Latency to Feed (min) After Phentolamine		
10 µg NE HCl	2.6 (20)	3.0 (5)	4.8 (10)*	4.6 (4)*
50.4 mM Ca^{2+}	4.7 (16)	5.6 (13)	4.5 (16)	7.5 (8)*

*$p<0.05$ from control latency

TABLE 5

MEAN PERCENT PHENTOLAMINE (16 µg) BLOCKADE OF FEEDING INDUCED BY NE AND Ca^{2+} DURING AN FR 2 OR FR 10 SCHEDULE OF REINFORCEMENT (LEFT). THE MEAN CONTROL AND POST PHENTOLAMINE FEEDING LATENCIES ARE SHOWN ON THE RIGHT. THE NUMBER OF EXPERIMENTS IS INDICATED IN THE PARENTHESES.

Condition	Percent Blockade by Phentolamine 16 µg	Latency Control	Latency Post Phentolamine 16 µg
FR 2			
10 µg NE HCl	69.7 (12)	1.9 (7)	5.4 (12)*
50.4 mM Ca^{2+}	40.5 (15)	4.5 (6)	3.8 (15)
FR 10			
10 µg NE HCl	77.5 (11)	2.9 (8)	3.2 (11)
50.4 mM Ca^{2+}	58.9 (11)	3.6 (8)	4.3 (6)

*$p<0.05$ from control latency

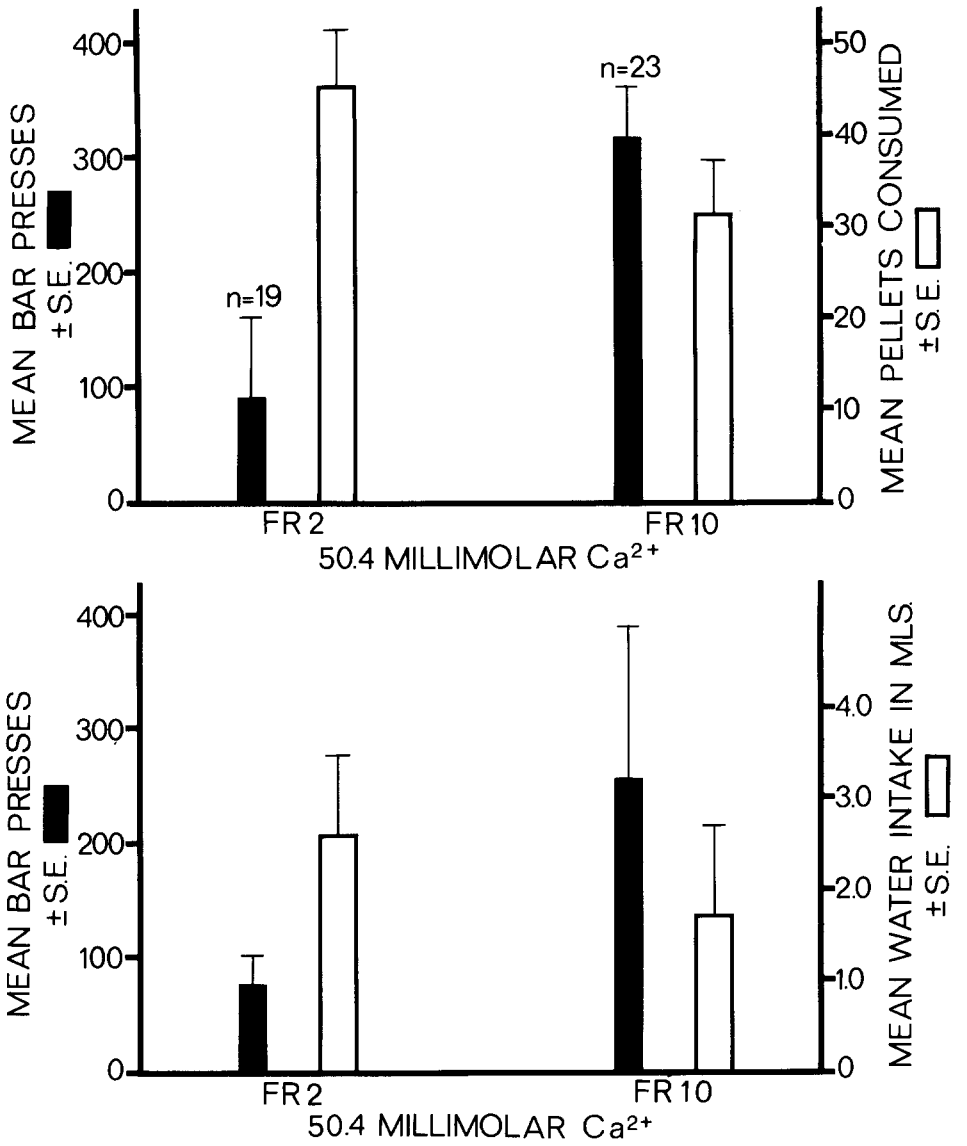

FIG. 3. Mean lever-press responses (■) for pellets (top) and water (bottom) at an FR 2 and FR 10 schedule of reinforcement. The corresponding number of pellets or ml of water consumed is indicated on the right axis (□).

during the operant conditions is very similar to the antagonism of free-feeding illustrated in Fig. 2.

Records of lever responding for pellets consumed following the intraventricular infusion of 50.4 mM Ca^{2+} and 10 μg NE are presented in Fig. 7. The representative cumulative records, under both FR 2 and FR 10 schedules of reinforcement, are also contrasted with those following the infusion of 16 μg of phentolamine. Though the slopes generated by the rats are different under the two schedules, the rates of response emitted by the rats at each ratio are very similar following an infusion of NE or Ca^{2+}. However, 30 min after the infusion of phentolamine, the slope of NE-induced feeding under FR 2 is much less steep than that observed following Ca^{2+}. On FR 10 the slopes are nearly identical but NE-induced feeding did not continue nearly as long.

The latencies for Ca^{2+} and NE feeding are compared to those after phentolamine blockade in Table 5. As in the free-feeding condition, the control latency for NE feeding at FR 2 was substantially less than that observed after an infusion of Ca^{2+}. When the rats were given 16 μg of the antagonist at this schedule, only the latency of NE eating increased significantly ($p<0.01$, $t = 1.80$, $df = 28$). On the FR 10 schedule, the latencies for both responses increased after phentolamine, but neither was significantly different.

DISCUSSION

The ingestive behavior following the intraventricular infusion of a solution of excess calcium ions seemed to resemble a response to natural hunger. That is, the animal

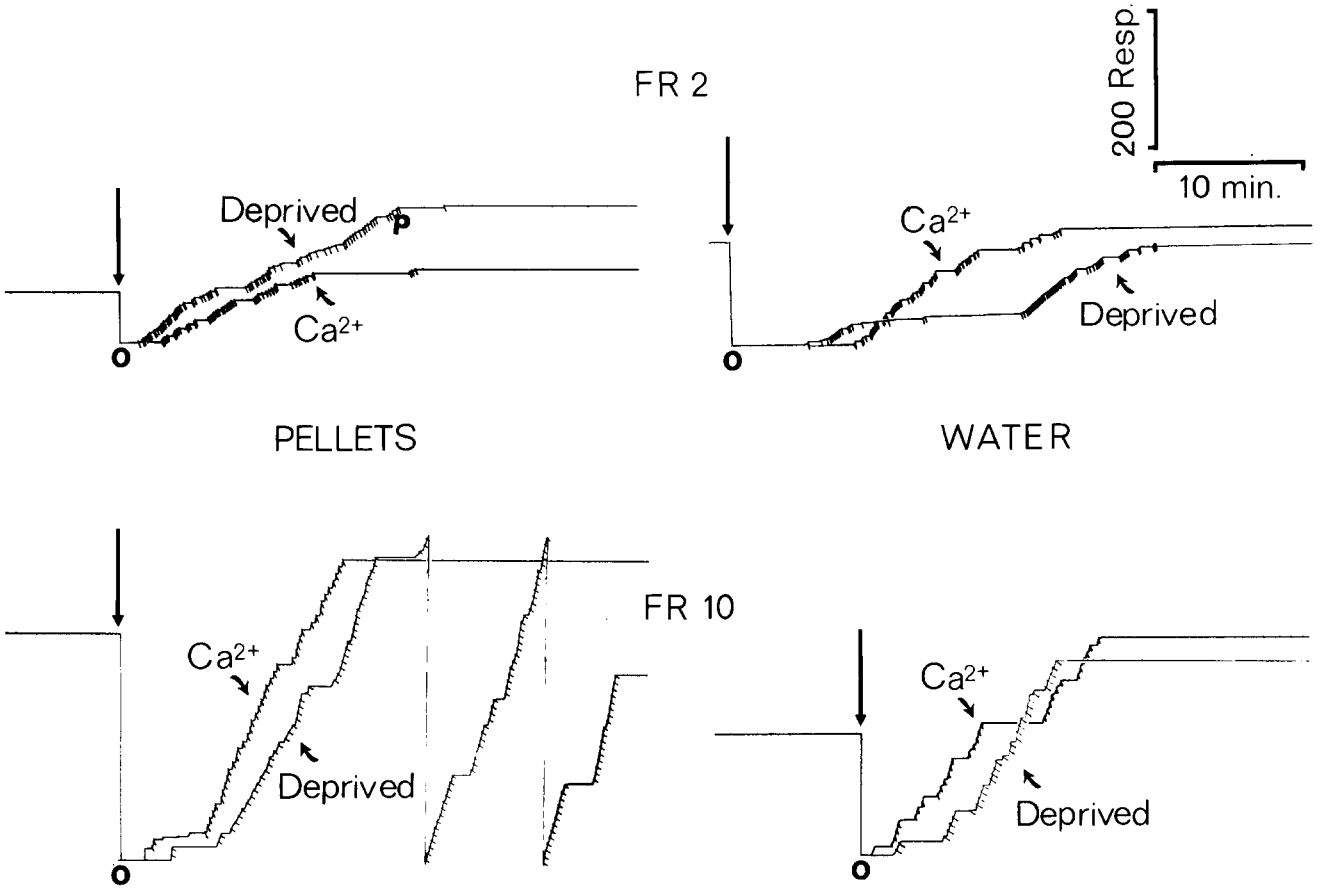

FIG. 4. Representative cumulative records of lever-press responses by two animals at FR 2 or FR 10 for 45 mg. Noyes pellets and 0.10 ml of water. The rat's responses after 50.4 mM Ca^{2+} is compared in each case to when the same animal was deprived of food and water for 23 hr. The two tracings were placed on the same horizontal axis and the vertical arrow at time zero indicates both the point of infusion and when the hungry animal was placed into the chamber.

related the cues associated with its internal environment to a previously learned task in order to reduce this drive state [22]. As a result, the animal approached a lever and depressed it to obtain pellets at a rate equal to that observed after food deprivation. Thus, the infusion of Ca^{2+} appears to involve a central physiological imbalance that is alleviated by the motivated response to acquire food.

Although the mechanism by which excess Ca^{2+} in the brain produces feeding is unknown, Ca^{2+} may evoke a state of behavioral arousal similar to the effect of electrical stimulation of the lateral hypothalamus [10]. Allowing for the variation in the structures reached by the Ca^{2+} solution and for differences in the rat's internal drive state at the time of the infusion, this could account for the absence of feeding in some animals that nevertheless did exhibit other behavior. However, the consistency and magnitude of feeding in many animals indicate that the reaction is far more specific.

An alternative explanation is that the cation causes an alteration in permeability of the neuronal membrane resulting in the depolarization of neurons that are involved in the initiation of ingestive activity [42]. However, this would not account for the fact that a solution of Na^{+}, Mg^{2+} or particularly K^{+}, which in the concentrations used are also able to alter the excitability of the cell, did not stimulate feeding. Furthermore, Ca^{2+} has been found to have a stabilizing effect upon the neuronal membrane when infused into the ventricle of a cat [45]. Several investigators have shown that the effect of Ca^{2+} upon transmitter release is due to a direct action upon the synaptic vesicles rather than on fiber conduction [4,15].

Although the structure upon which Ca^{2+} acts is unknown, its possible effect on the ventromedial nucleus of the hypothalamus should be considered since this structure lies in close proximity to the third ventricle. For example, if Ca^{2+} released norepinephrine from the ventromedial nucleus or produced a temporary functional lesion of that structure, the net effect of the cation would be directly upon cells thought to play an inhibitory role in feeding behavior, rather than upon first order neurons responsible for the initiation of an ingestive response. Our behavioral observations do not support this notion, however, since neither a decrease in random activity [44], nor the lack of motivated activity for food that is characteristic of an animal with a ventromedial lesion [23,44] occurred after the infusion of Ca^{2+}. In fact, locomotor activity clearly increased following the Ca^{2+} infusion although this admittedly was a transient change. Further, the rat's lever pressing for pellets during a thirty minute period after an infusion of Ca^{2+} equalled 25% of the total responses for 12

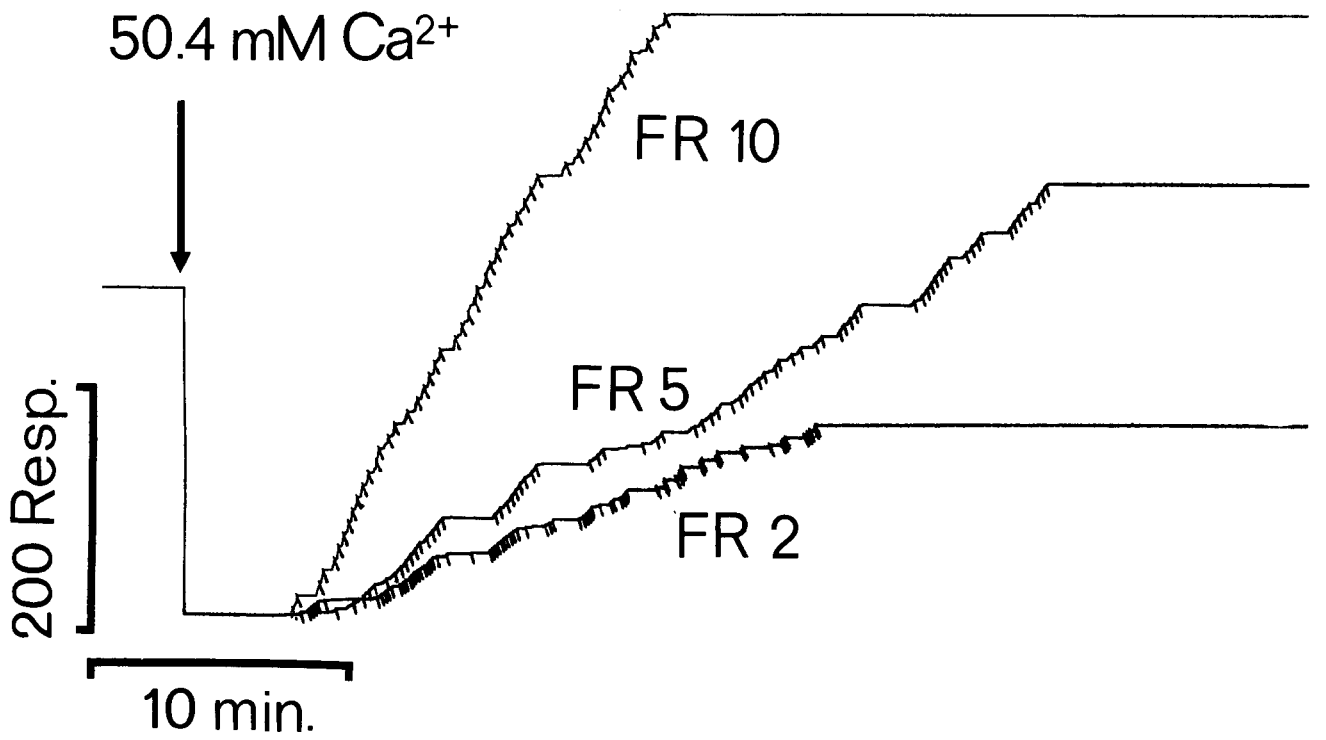

FIG. 5. Lever-press responses for pellets by one rat at increasing schedules of reinforcement. The vertical arrow indicates the time of Ca^{2+} infusion in all three experiments.

hr observed by Teitelbaum [44] in dynamic and obese hyperphagic animals that were on the same schedule of reinforcement. The apparently high level of motivation for food after an elevation in brain Ca^{2+} is not consistent with behavior following the elimination of ventromedial function.

An appealing explanation of the Ca^{2+}-induced feeding is that the cation affects the presynaptic membrane of a noradrenergic fiber system subserving feeding behavior. Peripherally, Ca^{2+} acts at the terminal bouton to mobilize stored norepinephrine [2], as well as in the hypothalamus to release this catecholamine from the vesicles [35]. From our observations, this alone does not seem to be the sole mechanism responsible for the rat's intense feeding after an intraventricular infusion of Ca^{2+}.

Whereas the alpha-adrenergic antagonist drastically reduced the ingestive response observed after the intraventricular injection of norepinephrine during both the free- and operant-feeding conditions, the amount consumed after the Ca^{2+} infusion remained at 65%, 60%, and 40% of the ad lib, FR 2 and FR 10 control levels, respectively (Tables 4 & 5). These striking differences in the magnitude of alpha receptor blockade suggest that the effect of Ca^{2+} is mediated through an additional pathway in the diencephalon that is distinct but as yet unknown neuropharmacologically.

The near parallelism of the NE and Ca^{2+} blockade curves in Fig. 2 and the longer latency of the Ca^{2+} response could reflect a common mechanism of action. If this were so, corresponding increases in both the Ca^{2+} and NE feeding latencies should arise after phentolamine blockade. However, after 16 μg of the antagonist were infused during the free-feeding and FR 2 conditions, orly the latency for norepinephrine-induced feeding showed a significant increase (Table 5). Perhaps phentolamine did not occupy a sufficient number of the same type of receptor sites onto which NE is released; however, Kleinrok and Zebrowska-Lupina [18] showed that 10 μg of phentolamine infused intraventricularly blocked significantly the stimulating effect of systemic amphetamine upon locomotor activity. Since the excitatory effect of amphetamine is presumably mediated by the release of NE in the brain [9] the alpha-antagonist in this comparatively low dose does ostensibly occupy a sufficient number of receptors onto which NE is liberated.

The possibility that a noradrenergic mechanism is not entirely responsible for feeding has already been raised [19]. Neurons located in the lateral hypothalamus contain a chemoceptive profile through which a number of agents may stimulate feeding. Thus, the role of NE as either an excitatory or inhibitory transmitter in ingestive behavior has been questioned. For example, a rat ate spontaneously after the intrahypothalamic injection of cyclic AMP [3], the nucleotide through which the action of catecholamines are thought to be mediated both peripherally [43] and centrally [14]. However, these ingestive responses depended upon taste preferences that had been paired previously with the injections of cyclic AMP. Thus, the effect of NE upon the feeding system in the rat's diencephalon may be secondary to its alteration of local cellular metabolism.

Nevertheless, the partial reduction of feeding by phentolamine along with the enhancement of the Ca^{2+} response by propranolol certainly indicate some alpha-adrenergic receptor component in the response. This agrees with the finding that norepinephrine induces feeding after the prior

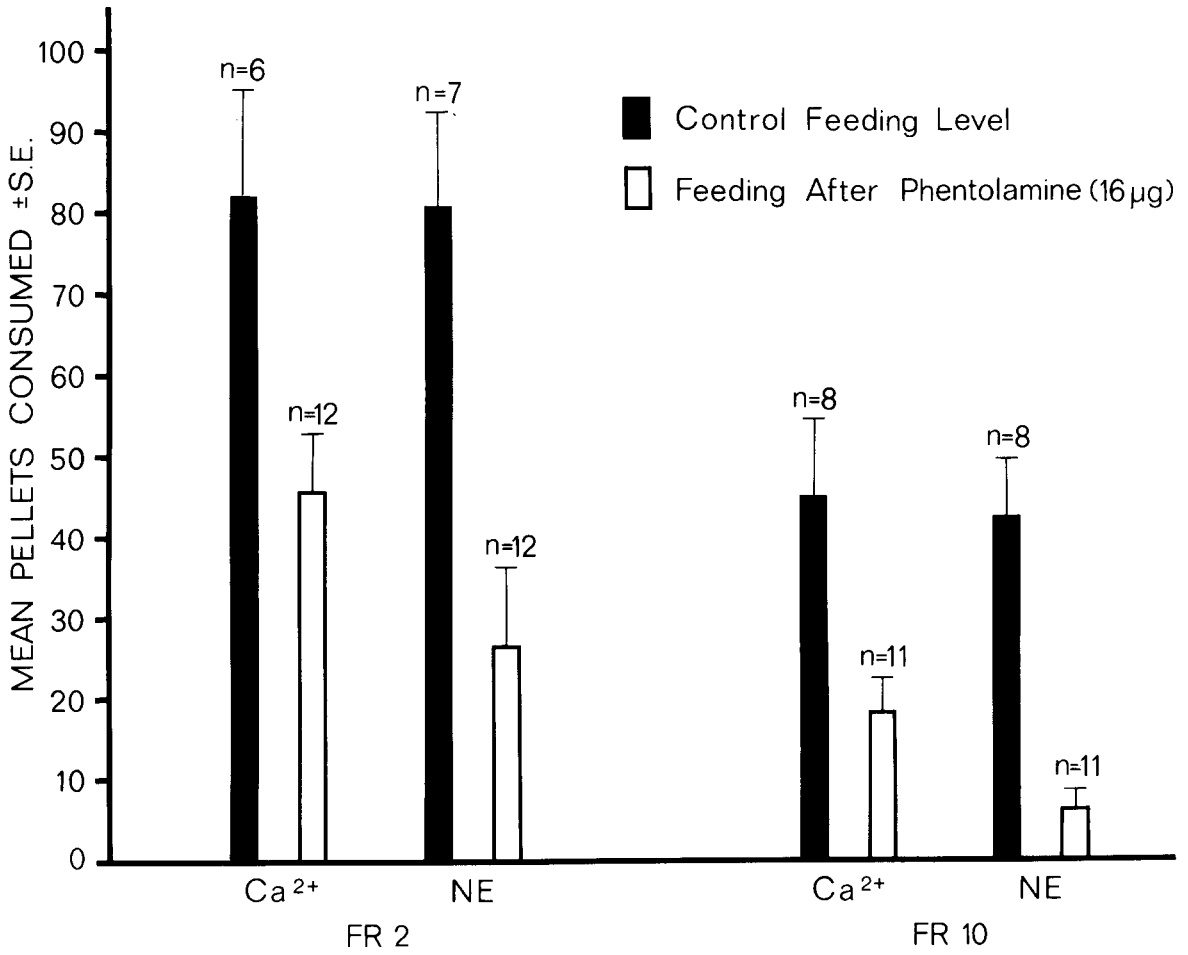

FIG. 6. Mean pellet intake induced by Ca^{2+} and NE during control experiment (■) and after the preinfusion of 16 μg of phentolamine (□). The number of experiments is indicated by n. $p<0.05$ ($t = 1.72$, $df = 22$) at FR 2 and $p<0.01$ ($t = 2.8$, $df = 20$) at FR 10 between the post phentolamine levels of feeding induced by NE and Ca^{2+}.

infusion of propranolol [1] as well as with the idea that alpha- and beta-adrenergic receptors could be involved in pathways subserving hunger and satiety [20].

The reliable attenuation of Ca^{2+} feeding by intraventricular atropine points to a possible cholinergic link in the feeding circuit. On the one hand, carbachol applied to the hypothalamus can evoke feeding in the rat [6, 8, 46] and rabbit [40], whereas the systemic or intracranial administration of atropine may partially inhibit both induced feeding and natural hunger [11,21]. Inasmuch as Ca^{2+} does release acetylcholine in the brain [36], its potent effect upon feeding may in part be dependent upon a cholinergic pathway [25,28].

Even though the cation may affect cholinergic synapses in the brain, there is some question pertaining to the specificity of atropine in causing a reduction in feeding. An intraventricular injection of atropine in the cat resulted in an EEG of high voltage slow waves and a general depression of motor activity [5]. Rinaldi and Himwich [38] proposed that the thalamic system for behavioral arousal depended upon the integrity of the cholinergic pathways. Consequently, the significant inhibition of food intake after atropine is administered systemically may be due to its attenuation of a required state of vigilance [41]. For these reasons, the enhancement of Ca^{2+}-induced feeding observed after a prior infusion of hexamethonium was unexpected considering that atropine also blocks the effect of acetylcholine on autonomic ganglia. However, if the proposed inhibitory synapses from the ventromedial to lateral hypothalamic area are nicotinic rather than muscarinic in character, hexamethonium would be able to block this influence and release the facilitating effects of Ca^{2+}.

Although the intraventricular injection of serotonin has only a minor effect upon feeding [32], methysergide may alter the rat's level of arousal. A systemic injection of 5-HT reduces daily food intake of a rat by as much as 20% [41], and methysergide, by its known action in small amounts [7], may have blocked the inhibitory influences of some tryptaminergic system. Thus, when the serotonin antagonist was administered prior to Ca^{2+}, the amount of feeding that followed was enhanced.

Whether or not the neurons that are involved in the Ca^{2+} eating phenomenon have a functional role during natural hunger is unclear at present. Further, it remains to be determined whether neurons within the limbic forebrain – limbic midbrain feeding circuit described by Morgane [24]

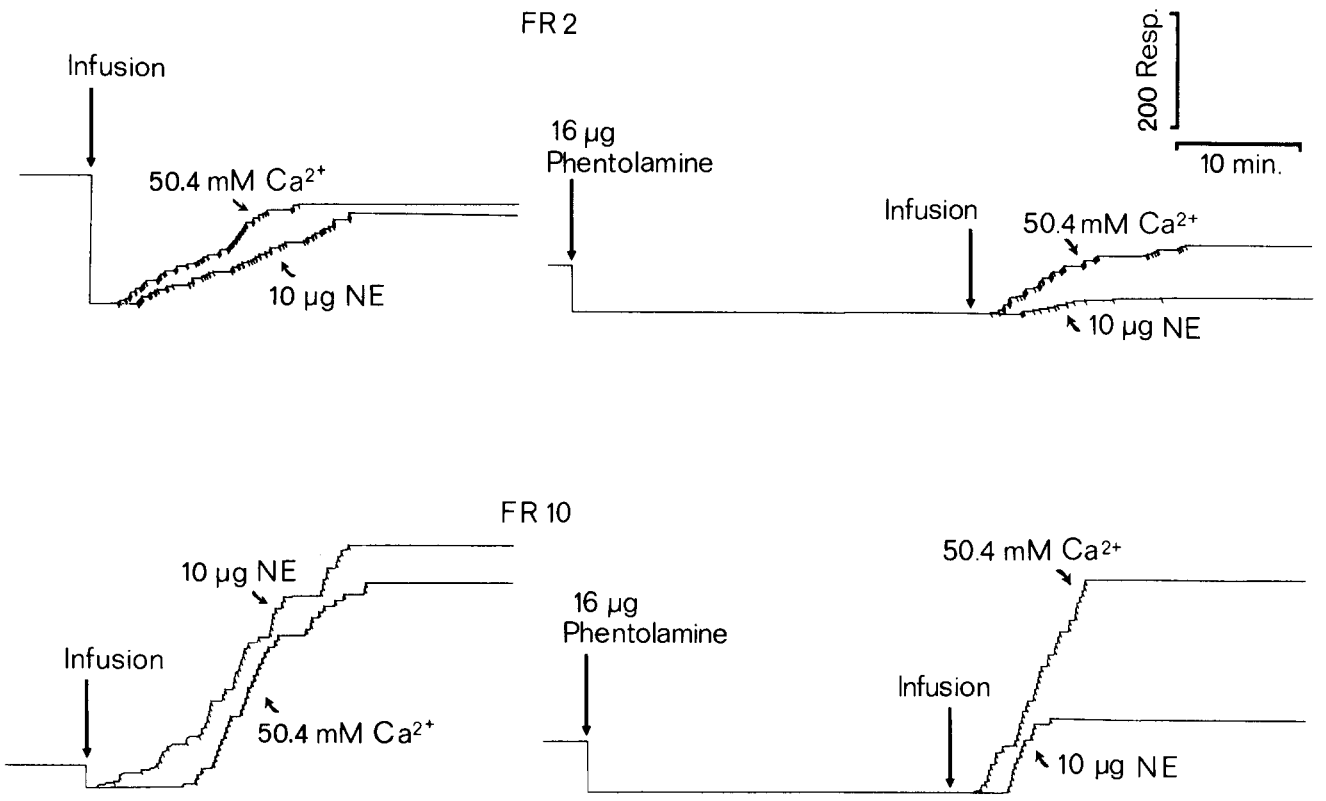

FIG. 7. On the left are superimposed lever-press responses for pellets by two animals at FR 2 and FR 10 induced by the infusion of either Ca^{2+} or NE. Responses by the same animals when phentolamine was infused 30 minutes prior to Ca^{2+} or NE are shown on the right. A vertical arrow indicates the time of phentolamine infusion or of the infusion of either Ca^{2+} or NE.

are selectively sensitive to the concentrations of Ca^{2+} in the extracellular fluid. If so, this may be similar to the intrinsic activity of Na^{+} and Ca^{2+} ions in the posterior hypothalamus which is thought to establish the set-point for body temperature in the cat and monkey [30,33]. Recently, Myers and Tytell [29] observed that the endogenous levels of these two cations shifted in a reciprocal fashion within the cerebrospinal fluid during the development of a fever. If the same sort of alteration in the affinity of hypothalamic cells for endogenous Ca^{2+} could be demonstrated in cases of chronic obesity, more substantive evidence for the presence of an ionic mechanism underlying the set-point for body weight would be provided.

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