Feeding induced by intracerebroventricular 2-deoxy-d-glucose in the rat

RICHARD R. MISELIS AND ALAN N. EPSTEIN

Department of Biology and School of Veterinary Medicine, and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, Pennsylvania 19174

MISELIS, RICHARD R., AND ALAN N. EPSTEIN. Feeding induced by intracerebroventricular 2-deoxy-d-glucose in the rat. Am. J. Physiol. 229(5) : 1438–1447. 1975.—Feeding was induced with lateral intracerebroventricular (ICV) injections of 2-deoxy-d-glucose (2-DG), a glucose analogue causing glucoprivation. Feeding increased rapidly by an average of 2.6 g following ICV 2-DG (2.9 or 5.8 mg per rat), but did not increase after ICV d-glucose or sucrose. At the same doses, 2-DG did not increase feeding when given peripherally. Core temperature dropped in a dose-dependent manner with doses of 2-DG sufficient to induce feeding after peripheral administration, but did not occur with ICV injections. The 2-DG (0.006–1.219 M) did not stimulate feeding when infused bilaterally into the lateral hypothalamus, the preoptic area, or the anterior lateral hypothalamus. Nor did it produce feeding when injected into the ventromedial hypothalamus at the same sites and in the same rats in which procaine HCl caused increased feeding. The brain, therefore, is directly sensitive to glucoprivation in the control of feeding, and glucoprivation alone is sufficient to mobilize feeding behavior. The specific site of sensitivity to glucoprivation and the mode of action of the glucoprivic system in the brain are unknown.

Feeding induced by intracerebroventricular 2-deoxy-D-glucose in the rat. Am. J. Physiol. 229(5) : 1438–1447. 1975.—Feeding was induced with lateral intracerebroventricular (ICV) injections of 2-deoxy-D-glucose (2-DG), a glucose analogue causing glucoprivation. Feeding increased rapidly by an average of 2.6 g following ICV 2-DG (2.9 or 5.8 mg per rat), but did not increase after ICV D-glucose or sucrose. At the same doses, 2-DG did not increase feeding when given peripherally. Core temperature dropped in a dose-dependent manner with doses of 2-DG sufficient to induce feeding after peripheral administration, but did not occur with ICV injections. The 2-DG (0.006–1.219 M) did not stimulate feeding when infused bilaterally into the lateral hypothalamus, the preoptic area, or the anterior lateral hypothalamus. Nor did it produce feeding when injected into the ventromedial hypothalamus at the same sites and in the same rats in which procaine HCl caused increased feeding. The brain, therefore, is directly sensitive to glucoprivation in the control of feeding, and glucoprivation alone is sufficient to mobilize feeding behavior. The specific site of sensitivity to glucoprivation and the mode of action of the glucoprivic system in the brain are unknown.

Feeding induced by intracerebroventricular 2-deoxy-D-glucose in the rat. Am. J. Physiol. 229(5) : 1438–1447. 1975.—Feeding was induced with lateral intracerebroventricular (ICV) injections of 2-deoxy-D-glucose (2-DG), a glucose analogue causing glucoprivation. Feeding increased rapidly by an average of 2.6 g following ICV 2-DG (2.9 or 5.8 mg per rat), but did not increase after ICV D-glucose or sucrose. At the same doses, 2-DG did not increase feeding when given peripherally. Core temperature dropped in a dose-dependent manner with doses of 2-DG sufficient to induce feeding after peripheral administration, but did not occur with ICV injections. The 2-DG (0.006–1.219 M) did not stimulate feeding when infused bilaterally into the lateral hypothalamus, the preoptic area, or the anterior lateral hypothalamus. Nor did it produce feeding when injected into the ventromedial hypothalamus at the same sites and in the same rats in which procaine HCl caused increased feeding. The brain, therefore, is directly sensitive to glucoprivation in the control of feeding, and glucoprivation alone is sufficient to mobilize feeding behavior. The specific site of sensitivity to glucoprivation and the mode of action of the glucoprivic system in the brain are unknown.
they were allowed to recover 3-7 days in their home cages before testing. Food and water intakes and body weights were measured daily. The rats were maintained under an artificial 12:12 light:dark cycle at an ambient temperature of 22°C.

Surgery. After adaptation to their home cages, the rats were given the following preoperative medication: 0.5 ml per rat of atropine sulfate (ip), 0.2 ml pcrr rat of procaine penicillin G suspension (300,000 U/ml), and 0.2 ml per rat oxytetracycline (50 mg/ml im). With the use of a stereotaxic instrument, the rats were fitted with a permanently implanted, unilateral intracranial cannula described by Epstein (15) that had these modifications. The outer 23-gauge cannula was shortened to 8 mm. The principal modification was in the injector, which was made from tubing of a 1.5-inch, 30-gauge stainless steel needle. The sharp, beveled tip of the needle was retained as the tip of the injector, and when in place extended 1 mm beyond the outer cannula to ensure penetration of the injector into the cerebrospinal fluid space. It had been observed that chronic intracerebroventricular (ICV) cannulas became unreliable either because the tip of the outer cannula displaced the corpus callosum downward during surgical placement rather than perforating it, or because they became blocked by choroid plexus and/or proliferating ependyma. The sharp, extended tip of the injector was designed to perforate these obstructions just before each injection.

The outer cannulas were implanted at the following coordinates: anterior to the ear bars (A) 8.3-8.6 mm; right lateral to the sagittal sinus (L) 1.5 mm; down from the dura (D) 3.0-3.1 mm. Placement of the cannula within the lateral ventricle was assessed at the end of the surgery by occluding the jugular veins at the thoracic inlets with digital pressure, thereby raising intracranial pressure and driving cerebrospinal fluid (CSF) up the cannula and into view.

Intracerebroventricular injection of saccharides. Three injection saccharides were used in equimolar concentrations: 2-DG (500 mg/ml distilled H2O), D-glucose (549 mg/ml distilled H2O), and sucrose (1,043 mg/ml distilled H2O). The approximate molarity of the solutions was 2.36 M. Glucose and sucrose were used as osmotic control substances and for possible effects of their own.

The first experiment was designed to compare the effectiveness of the test solutions in increasing a rat's feeding over its spontaneous feeding observed during the same time period (11 AM to 3 PM) on the previous base-line day. Five Sprague-Dawley males weighing 355-365 g and five Sherman females weighing 270-290 g were the subjects in this study. A base-line day preceded each injection day. The observation period was 4 h. Each rat was given four injections (2-DG twice, and glucose and sucrose each once), each at intervals of 6 or 7 days. The sequences of injection days were counterbalanced among the 10 rats and arranged so that either a glucose or sucrose injection day intervened between the two 2-DG injection days. Two rats, however, never received sucrose injections. One rat died before receiving glucose.

On an injection day solutions were freshly prepared and the injection apparatus (injector, tubing and microsyringe) was filled with the test solution. The rats were removed from their cages, obturators were removed, and each injector was inserted into its outer cannula. The rat was then replaced in its cage with fresh pellets and tap water and given 10-15 min to accommodate to the attached tubing, which permitted free movement by the rat in its cage. The rats were given two 7-μl injections (for a total of 5.8 mg 2-DG pcrr rat) of a test solution 5 min apart, or one 7-μl injection (2.9 mg 2-DG/rat) of a solution. Each 7-μl volume was infused by hand from a remote 50-μl syringe over a 60-s period. Food and water intakes were measured to an accuracy of 0.1 g and 0.5 ml, respectively, from the beginning of the injection, at 1.5 h after the injection, and at the end of the 4-h test. Food spillage collected in trays beneath the cages was subtracted from the intake. Notes were made of each rat's behavior from the beginning of the injection to the end of the 1.5-h period in order to obtain latencies and to record general behavioral effects. At the end of the 4-h measurement, the injectors were removed, the obturators were replaced, and the injection apparatus was flushed with distilled water.

Histology. At the end of the experiments, the rats were sacrificed and examined histologically to determine the injection site of the test solutions. The rats were killed with an overdose of sodium pentobarbital given intraperitoneally and were perfused intracardially, first with isotonic saline and then with 10% Formalin in isotonic saline. The brains were removed, fixed in 10% Formalin, and then embedded in celloidin. Sections were made at 40-μm thickness and stained with Loyez fiber stain.

Peripherally administered 2-DG. To determine that the ICV-injected 2DG was not escaping the CNS via the venous system and possibly stimulating peripheral receptors, 2-DG was administered intravenously and subcutaneously in two separate groups of rats at the same dose used for the ICV injections.

Intravenous injection. Three naive male Sprague-Dawley rats were prepared with PE-10 jugular catheters under ether anesthesia. The catheter was placed with the tip terminating in the superior caval vein. The opposite end was threaded subcutaneously to exit from the dorsal surface of the neck. Between injection days the catheter was kept filled with a 10% heparin solution in isotonic saline.

Solutions of 2-DG at the same concentration used in the previous experiment (500 mg/ml distilled H2O) and isotonic saline were injected. Behavioral observations and food and water measurements were made over a 4-h period, at the same time of day, on each injection day and on the baseline day. A single base-line day was followed by 3 injection days separated by 2 rest days between each injection day. On an injection day rats were hooked up to the same remote injection apparatus used in experiment 1 and given 10-15 min to accommodate to the apparatus. The heparin solution in the jugular catheter was flushed into the rat in order to fill the catheter with test solution. On the 1st injection day the rats were tested with 4 small-volume injections at 1-h intervals. Volumes of 7, 7, 14, and 25 μl of 2 DG solution were infused intravenously, allowing 1 h for observation after each injection. The respective doses were 2.9, 2.9, 5.8, and 10.4 mg infused at the rate of 7 μl/min. This served to mimic the ICV injections given in the first experiment. On the 2nd injection day the rats were infused over a 3-min period with 500 μl of isotonic saline. On the 3rd injection day the rats...
At the conclusion of the study the rats were sacrificed for ICV injections. In addition to glucose, urea (183 mg/ml distilled water) was present. Intraventricular injections were given a high dose of 2-DG (equivalent to 500 mg/kg in 475 μl of solution) to verify the adequacy of the preparation. The low dose was equivalent to the ICV dose of the first experiment. There were 3 injection days, 3-4 days apart. On the 1st injection day half the rats were given isotonic saline solution subcutaneously in a volume equal to the volume of 2-DG solution (dose = 25 mg/kg). On the 2nd injection day the solutions were reversed. On the last injection day the six rats were given the high 500 mg/kg dose of 2-DG to verify their responsiveness to glucoprivation.

Core temperature effects of 2-DG. When given systemically 2-DG is an antimetabolite that transiently lowers metabolic rate (36, 53). The decreased metabolism should result in decreased heat production and can be expected to lower core temperature. The hypothermia may underlie the increased feeding. Groups of rats were therefore tested for changes in colonic temperature after peripheral or ICV injection of 2-DG.

Eight naive, Sprague-Dawley rats (four males and four females) weighing between 330-375 g were divided into male-female pairs and tested with intraperitoneal injections of 2-DG (750 mg/kg, 325 mg/kg, 75 mg/kg) and glucose (750 mg/kg). Core temperature was recorded using a Yellow Springs Instrument telethermometer and thermistor probe inserted through the anus approximately 5 cm into the colon. The rat was removed from its cage and placed in a restraining tunnel to facilitate use of the thermistor probe. Each rat was probed 5 times during the 1-h period preceding the intraperitoneal injection to establish a base-line temperature, and 10 times in the 3-h period after the injection to measure its effect. Food and water were not available while core temperatures were being monitored.

Five naive, Sprague-Dawley males and two naive, Sherman females were prepared with lateral ventricular cannulas as described in experiment 1. They were tested with 2-DG and glucose, using the same injection procedure as described for the ICV injections. In addition to glucose, urea (183 mg/ml distilled H₂O) was also used as a control since it leaves the CSF more rapidly than glucose and therefore mimics a more transient osmotic effect. A base-line core temperature was assessed as described for the intraperitoneal injections. Each rat was tested once with 2-DG and once with a control substance. There was a 6-day rest period between injection days. Three of the rats (LV-c 36, 37, and 39) were then tested for feeding on separate injection days with ICV 2-DG in the same manner as the rats in experiment 1. At the conclusion of the study the rats were sacrificed by an overdose of sodium pentobarbital immediately after a 10-μl ICV injection of methyl green dye (25 %) was made through the cannula. The presence of the dye in the ventricular system confirmed the location of the cannula tip in the lateral ventricle.

Results: Experiment 1

Intracerebroventricular injections. Table 1 compares for each rat its food intake during the base-line day with its intake during the same 4 clock hours on subsequent injection days. Glucose and sucrose injections did not increase food intake. After 2-DG injections, on the other hand, feeding increased 2.6 g above base-line feeding and the increase occurred within the first 1.5 h of the test period. The average latency to the initiation of feeding was 13.0 min on the 1st 2-DG injection day and 17.9 min on the 2nd injection day. The most frequent latencies were between 6 and 10 min, and the range was from 6–33 min. In the majority of cases the increased feeding occurred as a single uninterrupted meal.

After an intracerebroventricular injection, rats usually aroused from their resting or sleeping position and moved about the cage. Locomotion and exploratory behavior appeared normal. In many cases the rats first took a drink of water. This occurred in 11 of the 20 2-DG injections, in five of eight sucrose injections, and one of nine glucose injections. The average latency-to-drink was between 1 and 2 min and an average of 1.5 ml of water was drunk. With the sucrose and glucose injections, motor activity continued for 5–25

### Table 1. Food intake in response to intracerebroventricular injections of 2-DG, D-glucose, and sucrose

<table>
<thead>
<tr>
<th>Rat</th>
<th>Sucrose Control</th>
<th>D-Glucose</th>
<th>2-DG Day 1</th>
<th>2-DG Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>I</td>
<td>Δ</td>
<td>B</td>
</tr>
<tr>
<td>LV-c 12</td>
<td>0.7</td>
<td>0.0</td>
<td>-0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>LV-c 13</td>
<td>0.0</td>
<td>1.0</td>
<td>+1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>LV-c 14</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>LV-c 15</td>
<td>0.0</td>
<td>1.0</td>
<td>+0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>LV-c 16</td>
<td>0.2</td>
<td>1.3</td>
<td>+1.1</td>
<td>1.5</td>
</tr>
<tr>
<td>LV-c 17</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>LV-c 18</td>
<td>1.7</td>
<td>2.7</td>
<td>+1.0</td>
<td>2.7</td>
</tr>
<tr>
<td>LV-c 19</td>
<td>0.5</td>
<td>0.0</td>
<td>-0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>LV-c 10</td>
<td>1.3</td>
<td>2.1</td>
<td>+0.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Values given are in grams. B, base-line day; I, injection day; Δ, Difference (I - B). * Combined sucrose and glucose, + 0.2 g; combined 2-DG, + 2.6 g, P < 0.01.
The motor activity induced by 2-DG was followed by varying severities and durations of ataxia and stupor. These symptoms did not correlate with the onset of feeding. In 11 tests with 2-DG eating began before signs of stupor or ataxia were observed. The animals had either moved about the cage or had just moved from a normal stationary stance to a pellet before beginning to eat. In six cases the eating began and/or continued while the rat showed signs of stupor and ataxia. In two cases the rats began eating after having gone through a phase of slight stupor and ataxia. In the last case, the rat ate without exhibiting stupor or ataxia.

Histological examination confirmed the location of the cannula tips within the lateral cerebral ventricle in all animals (Fig. 1). In the vicinity of the cannula tip, there usually was a slight hydrocephalus and localized parenchymal destruction.

Peripheral administration of 2-DG. When given intravenously the low-dose equivalent to the effective ICV dose did not increase feeding in rats. However, all three rats ate at higher intravenous doses of approximately 200 mg 2-DG (equivalent to 500 mg/kg). The average increase in feeding above base line was 3.8 g. There was no significant change in feeding following the isotonic saline injection (Table 2). Postmortem examination verified the proper placement and patency of the catheters.

The six rats given the low dose of 2-DG subcutaneously (25 mg/kg) showed no significant change in feeding. After the 500-mg/kg dose of 2-DG, however, each rat increased its feeding above the base line. The average increase in feeding was 2.5 g (P < 0.01, t test for correlated means) (Table 3).

Core temperature effects of 2-DG. As shown in Fig. 2, 2-DG produced a dose-dependent drop in core temperature after peripheral injection. With the maximum dose (750 mg/kg) core temperature fell 3°C. At one intermediate dose of 2-DG there was a 1.5°C drop, and with the lowest 2-DG dose and the glucose dose of 750 mg/kg there was very little change. Core temperature began falling between 10 and 20 min after the intraperitoneal injection. It fell at a maximal rate at about 30 min. Core temperature remained depressed for more than 3 h at the maximal 2-DG dose. This may be an indicator of the duration of glucoprivation.

In the rats prepared with ICV cannulas, base-line core temperatures were stable but elevated compared to normal rats. Figure 3 shows that ICV 2-DG, glucose and urea produced no consistent effects on core temperature. There were no precipitous temperature drops as seen with the intraperitoneal injections. Two rats had an immediate 1°C fall in core temperature; however, one temperature drop followed a 2-DG injection and the other followed a glucose injection. Regardless of the substance tested, ICV injections produced no consistent changes in core temperature. Rats LV-c 36, 37, and 38 were tested with ICV 2-DG injections for feeding to confirm their identity with the animals studied in Experiment I. Their feeding increased by an average of 2.2 g (range, 1.6–2.6 g).

Discussion

Intracerebroventricular injections. This experiment shows clearly that 2-DG injected into the lateral cerebral ventricles will increase feeding. The amount eaten is equivalent to a large, single, spontaneous meal for the rat (26) and it occurs with a short latency. The same absolute increase in feeding observed by Smith and Epstein (45) with peripherally administered 2-DG was observed here with direct central application, but at a dose (2.9 or 5.8 mg per rat) that was between 1/50th and 1/100th of their peripheral dose (750 mg/kg body wt) and with one-half the latency. The increased feeding appears, then, as an extra, spontaneous meal shortly after the 2-DG injection. This contrasts with the increased feeding caused by exogenous insulin administration. Insulin-induced feeding occurs with a longer latency (8, 45) and seems to be due to an increase in the size of a meal that would have occurred had the insulin injection not been made (6).

| Table 2. Average food intake in response to intravenous 2-DG and isotonic saline |
|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Base Line | 2-DG, 1-h Intervals | 4-h Total | Isotonic Saline | 2-DG, 475 μl |
| 7 μl | 7 μl | 14 μl | 25 μl | Total |
| μl | μl | μl | μl | μl | μl |
| 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| μl | μl | μl | μl | μl | μl |
| 1.9 | 0.3 | 0.6 | 0.3 | 0.4 | 1.6 |
| μl | μl | μl | μl | μl | μl |
| 5.7 | 5.7 | 5.7 | 5.7 | 5.7 | 5.7 |

Values given are in grams. * P < 0.01 for difference against all other means.

| Table 3. Average food intake in response to subcutaneous 2-DG and isotonic saline |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| Base Line | Isotonic Saline | 2-DG | 2-DG | 2-DG |
| 25 mg/kg | 500 mg/kg | (500 mg/kg) − Base Line |
| g | g | g | g | g |
| 1.3 | 1.8 | 1.5 | 3.8 | 2.5 |

Values given are in grams. n = 6.
Equivalent injections of glucose and sucrose did not increase feeding, eliminating colligative effects of the injected solutions as the cause of feeding. Such effects have been observed to increase feeding in sheep (44). However, an immediate drinking response was observed after both 2-DG and sucrose injections, probably due to osmotic dehydration of thirst osmosensors (5).

Peripheral administration of 2-DG. Since both the intravenous and subcutaneous doses of 2-DG that were equivalent to the dose administered centrally did not increase feeding, 2-DG...
could not have been stimulating feeding by escaping from the cerebral ventricles via the venous system and stimulating peripheral receptors. Both groups of rats increased their feeding when given high doses of 2-DG, demonstrating that the preparations were adequate to test the effects of 2-DG.

Core temperature effects of 2-DG. The dose-dependent drop in core temperature seen with peripherally administered 2-DG suggests an alternative explanation for the 2-DG feeding response observed. This is strengthened by the fact that core temperature was found to be dropping most rapidly at approximately the time that the rats began eating in the Smith and Epstein study (45). However, there were no consistent changes in core temperature following the ICV injections of 2-DG, and rats clearly eat after such injections. Therefore, a decrease in core temperature is not a necessary precondition for eating in response to glucoprivation. It is also unlikely that local variations in brain temperature that may have occurred with ICV injections of 2-DG play a role in the feeding response. No correlation has been observed between falling brain temperature and the initiation of feeding or between peak temperature rise in the brain and satiety (1, 20, 41). In addition, it has been shown that local cooling in the rat brain initiates heat conservation mechanisms that result in a rise in core temperature (43). Since there were no consistent rises in core temperature with the ICV injections of 2-DG, the occurrence of local brain cooling can be excluded.

EXPERIMENT 2: EXPLORATION FOR SPECIFIC GLUCOPRIVIC SITES WITHIN BRAIN PARENCHYMA

Is there a specific site within the brain of high sensitivity to 2-DG that is involved in initiating feeding during glucoprivation? Several regions within the hypothalamus have been suggested for this role. The gold thioglucose work (29, 30) suggests that it may be the ventromedial hypothalamic (VMH) area. Small increases in feeding have been reported after 2-DG injections into the lateral hypothalamus (LH) (4, 19). In the latter case the increases were confounded by feeding that followed glucose injections. Lesion studies that demonstrate the abolition of glucoprivic feeding of peripherally administered 2-DG suggest the perifornical region (37), the lateral hypothalamus (35, 52), and the lateral preoptic area (6).

Rats were therefore prepared withymmetrically-aimed, bilateral cannulas to explore the VMH area, the length and width of the LH area and the preoptic (PO) area for high sensitivity to 2-DG in producing feeding. In addition the rats prepared with cannulas in the VMH area afforded the opportunity to test for nonspecific depressive effects of 2-DG. Since 2-DG depresses metabolism, it could be expected to depress neural activity and might, therefore, release feeding behavior by depression of an inhibitory circuit. The VMH contains a feeding-inhibitory circuit which, when depressed by a local anesthetic, produces feeding (15). The same feeding effect would be expected using 2-DG if it has neural depressive action.

Methods

2-DG tests at LH, anterior hypothalamus (ALH), and PO hypothalamic sites. Three areas of the lateral zone of the hypothalamus were tested with direct injections of 2-DG and glucose in both crystalline form and in solutions of various concentrations. Rats were prepared with bilaterally symmetrical cannulas (11 mm) by a technique previously described (15). The injectors resembled those described in experiment 1 except that they did not have beveled tips and the injector terminated flush with the outer cannula tip.

LH RATS. Six Sprague-Dawley male rats and five Sherman female rats weighing between 253-400 g were implanted with cannulas in the lateral hypothalamus at the following coordinates: A 6.4-6.8 mm, L 1.8-2.0 mm, D 7.0-8.0 mm. They were tested with crystalline 2-DG and crystalline glucose that were pressed into the tips of the 30-gauge injectors and with 2-DG solutions of the following concentrations (wt/vol): 100, 200, 500, and 2,500 mg/100 ml (0.006-0.132 M). The volumes injected ranged from 0.5-3 ml bilaterally, so that the doses of 2-DG per rat were from 0.001 to 0.150 mg. Injections were made over a period of 5-10 s by hand with a remote microsyringe in a manner similar to that described for experiment 1. Generally, feeding was observed for a 1-h period after an injection. In many cases multiple injections of 2-DG (multiplying the above milligram doses) were tried if feeding did not occur with a single injection.

Five of the Sprague-Dawley males were also tested with procaine HCl (50 mg/ml) after overnight food deprivation to inhibit feeding and thereby verify the location of the cannulas within the feeding area of the LH. The overnight food deprivation began in the late afternoon. On the following day, the rats were given wet mash (1 part by weight Purina rat chow powder to 1.5 parts water) after being prepared for remote injection. The procaine injections began when a rat had begun to eat. They were given 0.5-10 mg, normally 2-ml bilateral injections. The duration of the inhibition of feeding was measured and a second injection given once the rat resumed eating. In some cases three injections were given.

ALH AND PO RATS. Two groups of rats were prepared with bilateral cannulas aimed for either the LH at the level of the anterior hypothalamus or for the preoptic area. There were seven Sprague-Dawley rats (five males and two females) weighing between 318 and 403 g prepared with PO cannulas and three Sprague-Dawley males weighing between 363 and 390 g prepared with ALH cannulas. The respective coordinates for the PO cannulas and the ALH cannulas were: A 8.5-9.2 mm, L 1.1-1.5 mm, D 7.1-7.5 mm, and A 7.8-8.1 mm, L 1.4 mm, D 7.4 mm. Both groups of rats were tested with 2-DG solutions of the following concentrations (wt/vol): 500, 5,000, 7,500, and 15,000 mg/100 ml (0.030-0.915 M). Volumes of 1-3 ml were infused bilaterally over a period of 5-10 s with a Harvard Apparatus infusion pump. The 2-DG doses were 0.010-0.90 mg per rat. Several rats were tested with more than one infusion. Feeding and drinking behavior was observed for 1 h after an infusion.

At the end of the study the rats were sacrificed and the brains were removed for gross anatomical diagnosis of the cannula-tip locations.

2-DG and procaine tests in VMH area. Three naive, Sprague-Dawley female rats weighing between 242-293 g were prepared with bilateral cannulas aimed at the VMH. The coordinates used were: A 6.0 mm, L 0.6 mm, and D 8.3 mm. After a recovery period of several days the rats were placed
on a feeding schedule in which wet mash (described above) was offered during the afternoon along with the pellets. Each rat was studied with bilateral injections of procaine-HCl (50 mg/ml isotonic saline), two doses of 2-DG (75 mg/ml isotonic saline and 200 mg/ml isotonic saline), and a base-line (no injection) test.

At the beginning of each session, the rat was removed from its cage and an injector was inserted into each cannula. Fresh wet mash was placed in the cage and the rat was replaced and given 10-15 min to accommodate to the presence of the wet mash and the injection apparatus. The bilateral injections were made remotely using 10-μl microsyringes. Volumes of 3 or 4 μl were infused bilaterally over a 30-s period with a Harvard infusion pump. The rats received from 0.2-0.8 mg 2-DG per rat. Their behavior was observed for 1.5 h after the injections. Food and water intakes were measured at the end of this period. The rats were tested first with the procaine-HCl, then with a solution of 2-DG at a concentration of 75 mg/ml, and again with a 2-DG solution but at a concentration of 200 mg/ml. There were 3 or 4 days of no testing between injection days. Finally, to verify their capacity to respond to 2-DG, the rats were tested with a 600 mg/kg dose of 2-DG intraperitoneally, and feeding was observed over a 4 h period. At the conclusion of the study, the rats were sacrificed and a routine histological examination was done to determine the location of the cannula tips.

In addition, two Sprague-Dawley male rats were prepared with bilateral VMH cannulas and tested for a feeding response to crystalline 2-DG and glucose packed into the tips of the injectors. They were observed for feeding and drinking for 1.5 h after insertion of the injectors into the cannulas.

Results: Experiment 2

LH rats. The LH-cannula rats showed no significant feeding effects after 2-DG injections in either crystalline or solution form. The procaine-HCl tests following overnight deprivation did succeed in suppressing eating after 0.5- to 2-μl injections. The average duration of inhibition of feeding was 8.0 min (range, 0.5-20 min) for 13 injections in the five rats. See Fig. 4 for location of injection sites within the hypothalamus.

ALH and PO rats. There was one instance of feeding in the PO group and four examples of drinking. Feeding in this rat, however, could not be replicated. In the ALH preparation three rats ate small amounts of food (less than 1.0 g) with latencies of 4.5 to greater than 30 min. In two rats feeding could not be replicated. The third rat ate twice but less than 0.5 g each time. Each of these rats also drank at least once. Drinking, when it occurred, ranged from 1 min 20 s to 14 min in latency and never exceeded 1.5 ml of water intake. See Fig. 4 for locations of injection sites within the ALH and PO.

VMH rats. Figure 5 presents data from this experiment. On base-line days the rats ate an average of 2.7 g (range, 0.9-3.7 g) wet mash. Each rat was tested twice with procaine-HCl, once with 4 μl of 75 mg/ml 2-DG and twice with 4 μl of 200 mg/ml 2-DG. The 2-DG infusions at concentrations of either 75 mg/ml or 200 mg/ml produced no increase in feeding. The average 24-h intake subsequent to the 2-DG injections (17.6 g) was not different from the average intake 0 mg/kg.

<table>
<thead>
<tr>
<th>Proline</th>
<th>2-DG</th>
<th>No Inj.</th>
<th>2DG I.P.</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mg/ml</td>
<td>75 mg/ml</td>
<td>200 mg/ml</td>
<td>Baseline</td>
</tr>
<tr>
<td>WET MASH</td>
<td>WET MASH</td>
<td>WET MASH</td>
<td>WET MASH</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>
24-h intake preceding the 2-DG injection day (17.5 g). However, after infusion of procaine-HCl, the rats ate an average of 7.0 g (range, 5.1–8.2 g) wet mash with an average latency of 1.9 min. The response to procaine-HCl confirms the location of the cannula tips within the vicinity of the VMH feeding-inhibitory circuit. The rats ate an average of 6.9 g (range, 6.1–7.8 g) of pellets in response to the 600-mg/kg dose of 2-DG intraperitoneally. This confirms their capacity to eat in response to 2-DG. The male rats tested with crystalline 2-DG and glucose did not increase feeding following placement in the VMH.

Histological examination showed that the cannulas terminated within the VMH between the fornices. In one rat the cannulas were perfectly symmetrical and terminated in both VM nuclei. The other two cannulas were slightly asymmetric to the right and left, respectively, but both terminated in the vicinity of the ventral posterior portion of the VM nuclei (Fig. 4).

**GENERAL DISCUSSION**

Glucoprivation produced by the glucose analogue, 2-DG, and confined to the brain alone stimulates the intake of an additional spontaneous meal of normal size and it does so with a short latency. Therefore, the central nervous system contains a mechanism for the detection of glucoprivation and the mobilization of a feeding response presumably to combat the deficit in glucose metabolism produced by 2-DG. In an effort to localize this mechanism more definitively within the brain, direct intraparenchymal injections of 2-DG were made into the ventromedial and lateral hypothalami and the preoptic areas. They did not produce increased eating, in contradiction to the report of Balagura and Kanner (4) and Gonzalez and Novin (19). This leaves the exact nature and anatomical location of the glucoprivic system within the brain unspecified. Several recent lesion studies, however, demonstrate the involvement of the medial forebrain bundle in glucoprivic feeding (6, 33, 37) and implicate the frontal cortex as part of its neural system (8).

The demonstration that detection of glucoprivation by the brain alone is sufficient to mobilize feeding confirms the Smith and Epstein (45) hypothesis that the peripheral administration of 2-DG increases feeding by inducing central glucoprivation. This work does not exclude a role for peripheral glucose detection in food intake (19, 38, 42), but it does show that central glucoprivation alone is sufficient to produce feeding. Confinement of 2-DG to the brain alone was achieved by intracerebroventricular injection of small volumes of test solutions, and the reliable central dose was completely inert by intravenous and subcutaneous administration. Control experiments excluded the possibility of induction of feeding by nonspecific colliagative properties of the injected solutions. In addition, it is unlikely that central hypothermia secondary to the decreased metabolic rate which is seen after peripheral injection of 2-DG was an important stimulus, first because no behavioral effects of brain cooling were seen, and second because core temperature did not change after 2-DG injection into the brain.

The hypothalamus was explored with injected 2-DG in order to identify the anatomical substrate for the detection of glucoprivation. In spite of intensive efforts to find a reliably sensitive site within the hypothalamic, intraparenchymal injection of 2-DG in both crystalline form and in solutions ranging in concentration from 100 mg/100 ml-20,000 mg/100 ml in VMH, LH, and PO sites failed to produce feeding. Separate independent evaluations confirmed the location of the cannula tips within both the VMH inhibitory circuit and the LH excitatory circuit for feeding, and verified the efficacy of the preparations for behavioral testing with 2-DG. The same rats that increased feeding after the injection of a nonspecific neural depressant (procaine) into the vicinity of the only well-established inhibitory circuit for feeding (15), the VMH area, did not increase their feeding after 2-DG injections through the same cannulas. This excludes the interpretation that feeding may be a consequence of release of the feeding system from inhibition by nonspecific depression of neural activity (25). Feeding in rats with cannulas in the LH could be suppressed by the injection of procaine but feeding could not be stimulated by injection of 2-DG through the same cannulas and over a wide range of doses. Since procaine had differential effects depending on its site of injection within the hypothalamus, it is unlikely that it induced feeding by diffusing to a distal site via the ventricles.

The exploration of the hypothalamus for a specific site sensitive to 2-DG provides further evidence against the suggested role for the VMH in glucoreception relevant to food intake (29–31). It confirms other work (33, 39) showing that 2-DG injected into the VMH does not increase feeding. It complements lesion studies which demonstrate that the rat that has become hyperphagic as the result of ventromedial hypothalamic lesions still has the capacity to mobilize feeding in response to glucoprivation (24, 33, 37). The work with gold thioglucose (29–31) formed an early foundation for the concept that the VMH is the site of glucoreceptors for feeding. These results have recently been reexamined and refuted by the demonstration that gold thioglucose is a vascular poison (3). The lesions of the VMH and of other cerebral sites that it produces are now understood to be the result of an inflammatory response of hypothalamic vasculature that results in nonspecific, ischemic damage to neural tissue (11, 12) distal to the damaged blood vessels.

The failure to find a specific site within the hypothalamus sensitive to glucoprivation does not support recent work that suggests that specific hypothalamic detectors can mobilize feeding (4, 19). In the Balagura and Kanner study (4) only small increases in feeding (+0.72 g) were found when 2-DG was injected into the perifornical area, and they obtained increased feeding (+0.42 g) with their control injections, making the interpretation of their effects difficult. Gonzalez and Novin (19) reported increased feeding after 2-DG injections into the LH of the rabbit. The increases, however, were only significant when compared to mock injections, but were not significant when compared to control injections of glucose or isotonic saline.

The glucoprivic feeding system does not appear in the ontogeny of the rat until well after weaning, in spite of the presence of the hyperglycemic response to glucoprivation from the earliest time tested in the sucking rat pup (23). The possible involvement of the frontal cortex (8) and the...
demonstrated need of the median forebrain bundle (MFB) (33) for the feeding response to glucoprivation correspond to its late appearance in ontogeny, since the cortex does not reach functional maturity (14) and the hypothalamus, including the MFB, has not attained complete myelinization (10) until after 20 days. In addition, glucoprivic feeding requires an alpha-adrenergic synaptic system (34, 54) whose full maturity may be necessary for a feeding response requiring the presence of specific motivation and appetitive behaviors. These facts, in addition to rationalizing the late appearance of the glucoprivic control in the ontogeny of feeding, suggest that its mechanism is qualitatively different than that for the autonomic-endocrine controls of blood sugar-homeostasis. It may be telencephalic and alpha adrenergic rather than dependent on specific hypothalamic receptors.

How does the glucoprivic control play a role in normal feeding of the adult rat? There is no definitive answer. The thresholds for feeding and hyperglycemia seem to be within the same range (16). This conflicts with Smith et al. (46) who found that the feeding threshold was higher than the hyperglycemic threshold. They reasoned that the glucoprivic control of feeding must be only an emergency system, since in normal metabolic circumstances the hyperglycemic response will be triggered sooner than feeding and will reverse the glucoprivic site automatically from glucose reserves. The difference in thresholds requires more complete study. Account should be taken of the view that comparison may be unwarranted between the threshold for an automatic reflex like the autonomic-endocrine hyperglycemic response and the threshold for a behavioral response which requires specific motivational arousal and the mobilization of appetitive behaviors for its expression.

Blass and Kraly (6) also conclude that glucoprivation must contribute very little to control of normal feeding. They find that rats that lack the glucoprivic feeding response after lateral preoptic damage are nevertheless still capable of body weight regulation and feeding responses to other challenges to food intake. This is not a compelling argument. Given the concept of multiple controls in physiological regulations (2, 18, 50), one would not expect to see disruption of food intake adjustments when only one control of a redundant system is eliminated. Other controls take over and are sufficient to maintain the regulation (17, 18, 21, 48). A good example is provided by the rats with brain lesions that eliminate drinking to the point of intracellular dehydration, one of the best-established physiological controls for thirst. These rats still drink normal amounts of water and maintain a normal hydration state (3, 40) on ad libitum access to water.

Various studies of the control of food intake suggest an important role for the central detection of some postabsorptive nutrient (27, 47). The glucoprivic system could play this role. Alternatively, the animal may detect changes in the metabolism of the other macronutrients or, as has been suggested by Nicolaidis et al. (16), it may monitor energy production which is the common result of the metabolism of all three nutrients rather than detecting the catabolism of a single nutrient. This is a reasonable hypothesis since glucose metabolism is intricately related within a system of fuel homeostasis to the metabolism of lipids and protein, with the hormone insulin playing the important signal role in the periphery (13). In summary, this work is a direct demonstration of the presence within the brain of the rat tissue sensitive to glucoprivation that is involved in the control of feeding, and it shows that glucoprivation alone is sufficient to mobilize feeding. The hypothalamic locus of specific sensitivity could not be found in spite of extensive testing in the VMH, the LH, and the PO area. We urge the retention of the possibility of a role for glucoprivation in normal feeding, and consider it premature to relegate glucoprivic feeding to an emergency category. But we also stress that it is unreasonable to assign it a predominantly hypothalamic locus and to burden it with the control of all feeding behavior as has been the tendency in the past.

We thank Jeffrey W. Peck for helpful discussion. The hospitality and support of S. Nicolaides and J. Le Maguen at the Collège de France, where the final draft of this paper was written, are gratefully acknowledged.

This research was supported by Public Health Service Grant NDS 03469 to Alan N. Epstein and by Public Health Service Grants to the Institute of Neurological Sciences, University of Pennsylvania. R. R. Miselis was a trainee of the Veterinary Medical Scientist Training Program, National Institutes of Health Grant GM-02051, at the School of Veterinary Medicine, University of Pennsylvania. This work was presented by R. R. Miselis as partial fulfillment for the PhD degree at the University of Pennsylvania, 1973. Preliminary results of this investigation were reported, in part, in abstract form in The Physiologist 13: 262, 1970; American Zoologist 11: (4), 1971; and at the Fourth International Conference on the Regulation of Food and Water Intake, Cambridge, England, 1971.


Received for publication 11 November 1974.

REFERENCES


