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Increased feeding in response to decreased glucose utilization in the rat and monkey

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SMITH, GERARD P., AND ALAN N. EPSTEIN. *Increased feeding in response to decreased glucose utilization in the rat and monkey.* Am. J. Physiol. 217(4): 1083–1087. 1969.—The glucostatic hypothesis for the control of food intake predicts that decreased glucose utilization leads to increased food intake. Since the glucose analogue, 2-deoxy-D-glucose (2-DG), produces decreased intracellular glucose utilization of most tissues and particularly of brain, the injection of 2-DG into 5 monkeys and 28 rats provided an explicit test of the predicted relationship. Both monkeys and rats ate more after 2-DG. The most effective systemic doses of 2-DG were 300 mg/kg (iv) in monkeys and 750 mg/kg (ip) in rats. Larger doses ($\leq$ 1,000 mg/kg) produced drowsiness, stupor, ataxia, or retching, but not convulsions. When separate groups of rats were tested with either insulin (6 U Iletin) or 2-DG (750 mg/kg), they increased their mean food intake to approximately the same amount, but the rats treated with 2-DG ate sooner. Shorter latency of feeding with 2-DG is interpreted as resulting from the fact that 2-DG produces decreased glucose utilization of brain directly, whereas insulin reduces cerebral glucose utilization indirectly as a consequence of hypoglycemia. Since 2-DG produced marked hyperglycemia during the period of increased feeding, these results show that the abrupt onset of decreased glucose utilization, not hypoglycemia, is a sufficient condition for feeding in mammals.

feeding behavior; glucose metabolism; *Macaca mulatta*; brain metabolism; insulin-induced feeding; neural control of food intake; 2-deoxy-D-glucose; glucostatic control of food intake; hypothalamus

SINCE FOOD INTAKE AND GLUCOSE metabolism are enmeshed in the energy exchange of mammals, it is attractive to consider glucose metabolism as a major determinant of food intake. Seventeen years ago, Jean Mayer first proposed a glucostatic control of food intake (14, 15). In the ensuing debate, still current, three major characteristics of the glucostatic control of feeding have been specified: *1*) it operates over a relatively short epoch of hours or a day; *2*) the rate of cellular glucose utilization by brain tissue, especially the ventromedial area of the hypothalamus, is the critical parameter for food intake; and *3*) food intake and glucose utilization are related inversely.

Although a wide variety of data can be considered to support the glucostatic hypothesis (16), the demonstration that food intake varied inversely with glucose utilization as indicated by peripheral arteriovenous glucose differences is probably the most direct evidence (20). Since peripheral arteriovenous differences do not distinguish between glucose uptake and utilization and do not necessarily reflect cerebral arteriovenous differences, the evidence must be considered suggestive rather than compelling.

The analogue of glucose, 2-deoxy-D-glucose (2-DG), is a specific inhibitor of intracellular glucose utilization in brain, muscle, liver, and other tissues (3, 6). The decreased glucose utilization (glucoprivation) is a result of competitive inhibition of phosphohexoseisomerase by 2-DG (21) as well as a decrease of intracellular glucose uptake which may be secondary to the metabolic block (3). There are regional differences in the effect of 2-DG: it decreases glucose utilization of brain much more than that of striated muscle (22). (The term glucoprivation is introduced to emphasize the decrease in glucose utilization of cells as the critical intervening condition leading to increased food intake. Decreased intracellular glucose utilization has been called cytoglucopenia or metabolic hypoglycemia, but these terms refer to decreased glucose content of cells or blood and therefore miss the point. We believe glucoprivation is a more accurate term and we use it throughout this paper.)

Since 2-DG produces glucoprivation, we have used it to test one major prediction of the glucostatic hypothesis: decreased glucose utilization increases food intake. Our results in monkeys and rats confirm the prediction and they extend the similar observation in mice recently reported by Likuski et al. (11).

METHODS

Five male rhesus monkeys (*Macaca mulatta*), weighing 4.5–7.5 kg, and 38 male rats (Sprague-Dawley, 250–400 g) were the experimental subjects.

Monkey experiments. All animals were prepared with chronic inferior vena cava catheters and were adapted to chronic restraint in primate chairs. Details of preparation and care of macaques under these conditions were published previously (18). The macaques were fed in the morning (9–11 AM) and afternoon (1–3 PM). On experimental days, 2-DG (33–500 mg/kg) was injected intravenously (10 ml/min) just before the afternoon meal. Intake of pellets (Wayne Co.) was measured over the 2-hr morning meal and in each of the 2 hr of the afternoon meal. Tap water was provided during the meals and at night, but was withdrawn from 11 AM to 1 PM.

In some experiments, blood samples were obtained

through the venous catheter just before and during the afternoon meal.

Rat experiments. The rats lived in individual cages, with Purina pellets and tap water freely available, and were not deprived before the experiments. To test the effect of 2-DG on food intake, 28 animals were injected with 750 mg/kg (ip) in three separate experiments (N = 9 in two, N = 10 in the third) beginning at 10–11 AM. Hourly food and water intakes were measured for the next 6 hr. The food intake on the experimental day was compared to the spontaneous food intake during the same time on the day prior to the experiment.

To compare the feeding elicited by 2-DG with that elicited by insulin, food intake was measured as described above in a group of 9 rats receiving 6 U regular insulin (Iletin, Lilly Co.) subcutaneously and a second group of 9 rats receiving 750 mg/kg 2-DG intraperitoneally.

Insulin-induced feeding was also studied in 10 rats given 6 U regular insulin (Iletin) intraperitoneally. This experiment was performed to assure that the latency to the first bout of feeding after subcutaneous insulin was equal to or less than the latency after intraperitoneal insulin.

In experiments designed to test the effect of 2-DG on plasma glucose, each rat in an additional group of 10 received 2-DG (750 mg/kg ip) at the beginning of the experiment, and then tail vein blood samples were obtained at 1, 3, and 6 hr after the injection. Food and water were provided to only 4 of the rats during the experiments in which plasma glucose was measured.

2-Deoxy-D-glucose (Calbiochem or Sigma Corp.) was kept frozen and the required amount was mixed (with sterile water for monkeys or distilled water for rats) to provide a 5% solution (monkeys) or a 10% solution (rats) immediately before each experiment.

All blood samples from experiments with 2-DG were centrifuged immediately; plasmas were separated, frozen, and analyzed for glucose concentration by the glucose oxidase method and, in a few instances, for 2-DG by the method of Cramer and Neville (5).

RESULTS

Monkey experiments. When 2-DG was injected intravenously prior to the 2-hr afternoon meal, food intake increased two- to fourfold in four of the five monkeys in the 1st hr following the injection (Table 1). Food intake was not consistently increased during the 2nd hr of the meal. The most effective dose was 300 mg/kg. When a dose of 500 mg/kg was injected, transient drowsiness (<2-min duration), restlessness, and retching were frequently observed, but neither stupor nor convulsions were seen.

Fifteen minutes after 2-DG (300 mg/kg, iv) and the beginning of the meal, plasma glucose rose (Fig. 1). Plasma glucose continued to increase throughout the hour of increased food intake. Peak values of plasma glucose occurred 60–90 min after the injection of 2-DG. In all cases, plasma glucose was returning toward control values by the end of the 2nd hr. Since 2-DG reacts slowly with glucose oxidase, the plasma glucose measurements in Fig. 1 might be a mixture of glucose and 2-DG. This is not likely, however, because when 2-DG was measured directly in three experiments, less than 1 mg/100 ml remained in the plasma 15 min after the injection of 2-DG.

TABLE 1. *Food intake in monkeys after administration of 2-DG*

Monkey	Dose of 2-DG, mg/kg				
	0	33	100	300	500
	Food intake, g/hr				
39	19.7 (10) 8–30		44.3 (3) 30–53	58.3 (3) 46–72	110.0 (1)
42	28.0 (10) 18–47	44.2 (5) 33–56	52.0 (3) 42–68	53.3 (3) 38–76	57.0 (1)
43	39.3 (6) 23–57		38.0 (3) 34–42	77.3 (3) 65–91	
47	40.6 (12) 14–62	25.2 (6) 0–67	50.0 (3) 47–52	36.3 (4) 0–56	68.0 (1)
49	37.7 (13) 9–60	49.7 (6) 26–74	103.6 (3) 78–120	138.0 (3) 112–165	149.0 (3) 136–168

Mean values of food intake (g/hr) during the 1st hr after injection of 2-DG are presented at the specified doses of 2-DG for each monkey. Numbers in parentheses which follow mean value are the numbers of experiments from which the means were derived. Numbers below each mean value are the range of individual measurements.

No dose of 2-DG produced a consistent increase in food intake in *monkey 47*, although 2-DG (300 mg/kg) produced hyperglycemic curves similar to that displayed in Fig. 1. *Monkey 47* was also exceptional in that doses of 33 mg/kg or 300 mg/kg completely inhibited food intake in three experiments.

Rat experiments. Feeding in rats was also markedly stimulated by 2-DG. In three separate experiments 28 animals were studied (N = 9 in two, N = 10 in the third) using 750 mg/kg (ip), with which the best effects were obtained. The animals ate more and they ate sooner. Spontaneous intake of Purina pellets during the 6 hr of the test period on the day before 2-DG injection averaged 2.4 g (range: 0.0–6.8 g). During the 6 hr after 2-DG, food intake averaged 4.9 g (range: 0.0–11.4 g). Of the 28 animals, 23 ate more ($P < .01$, Sign test).

Latency to the first bout of eating was considerably shorter in rats given 2-DG. As is shown in Table 2 and Fig. 2, average latency measured in one group of nine rats decreased from 171 min (range: 23–360 min) on the day before injection to only 39 min (range: 9–198) after 2-DG. All of the nine rats ate sooner. Eight did so within 30 min after the injection (see Fig. 2). Only one of the animals had eaten spontaneously in less than 30 min (23 min) on the previous day. These latencies were also considerably shorter than those for the first bout of eating after a single subcutaneous dose of insulin (6 U Iletin) that produced comparable total food intakes for the 6-hr test (see Fig. 2). The average latency after insulin was 124 min (range: 24–360 min). Note also in Fig. 2 that there is considerable overlap between the sizes of the first meals eaten by the animals in both groups.

The insulin data in Fig. 2 are the results of subcutaneous insulin administration, because intraperitoneal insulin

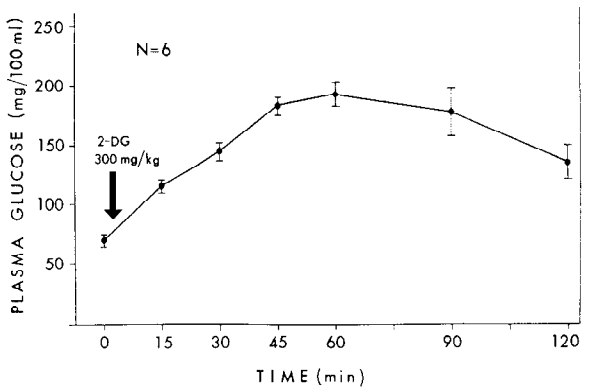

FIG. 1. Plasma glucose concentrations in six experiments on three monkeys. Following intravenous injection of 2-DG, monkeys were given food pellets and tap water. Note that marked hyperglycemia was present throughout 1st hr of increased food intake and persisted through the 2nd hr.

TABLE 2. *Latency of feeding in rats*

	Average	Range
Spontaneous	171	23–360
Insulin	124	24–360
2-DG	39	9–198

Latency in minutes to the first bout of eating in rats that were untreated (spontaneous), were given 6 units insulin, or were given 750 mg/kg 2-DG. No. of animals in each of the three groups = 9. Subcutaneous insulin is compared to intraperitoneal 2-DG because intraperitoneal insulin produced feeding with somewhat longer latency than did subcutaneous insulin (see text).

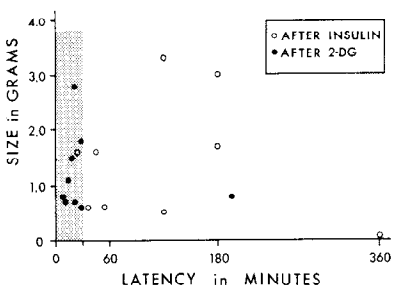

FIG. 2. Latency in minutes and size in grams of first meal in two groups of nine rats following 2-DG (750 mg/kg, ip) or insulin (6 U Iletin, sc).

elicited feeding after slightly longer latencies (N = 10, average latency = 165 min, range = 30–360 min). Despite the longer latencies to the first bout of eating, intraperitoneal insulin produced an increase of food intake comparable to that produced by subcutaneous insulin.

Water was available during all tests. The animals drank in association with feeding and water/food ratios were elevated after 2-DG. Average ratios for the group of nine rats in which they were calculated were 0.89 (range: 0.0–2.09) during spontaneous feeding and drinking and 2.22 (range: 1.73–5.40) after 2-DG.

As in the monkeys, the rats ate during a severe and sustained hyperglycemia. Changes in plasma glucose produced by 750 mg/kg 2-DG were studied in four rats permitted to eat (food intake shown at the bottom of Fig. 3) and in six deprived of food during the 6 hr after injection. The plasma results were not different between the animals that were given access to food and those that were not. They have been pooled and are shown at the top of Fig. 3. Mean resting plasma glucose was 99.9 mg/100 ml, rose to 319.3 mg/100 ml at the first 1-hr measurement, and remained elevated at 167.4 mg/100 ml at 6 hr after injection.

The rats ate and drank despite the stupor and ataxia that appeared within minutes of the injection and persisted for several hours. Lower doses of 2-DG (200–500 mg/kg) produced less depression but smaller and less consistent stimulation of feeding. Higher doses, up to 1,000 mg/kg, yielded severe and prolonged depression followed by eating and, in one rat, retching. All animals appeared normal the day after injection.

DISCUSSION

Rats and monkeys eat more shortly after an injection of 2-DG. Presumably, it is the metabolic state of decreased glucose utilization (glucoprivation) which drives the animals to eat more. Although glucoprivation was not demonstrated directly, the fact that 2-DG produced cerebral symptoms characteristic of hypoglycemia (drowsiness, stupor, and ataxia) despite marked hyperglycemia strongly suggests that both cerebral and peripheral glucoprivation occurred in the animals. We believe that the production of cerebral glucoprivation by 2-DG is the critical event in this phenomenon (see below), but the data do not exclude the possibility that peripheral glucoprivation is of equal or greater importance. It is also obvious that the experiments did not identify the neural or hormonal mechanisms which effect the increased feeding.

The threshold and dose-response relationship of the feeding effect of 2-DG varied in rats and monkeys as well as in individual animals of the same species. This variability is probably due to the fact that glucoprivation produced conflicting signals of varied intensity to the neural system which controls feeding. Glucoprivation not only produces a signal which drives animals to eat more, but glucoprivation also produces increased serum osmolarity (hyperglycemia)

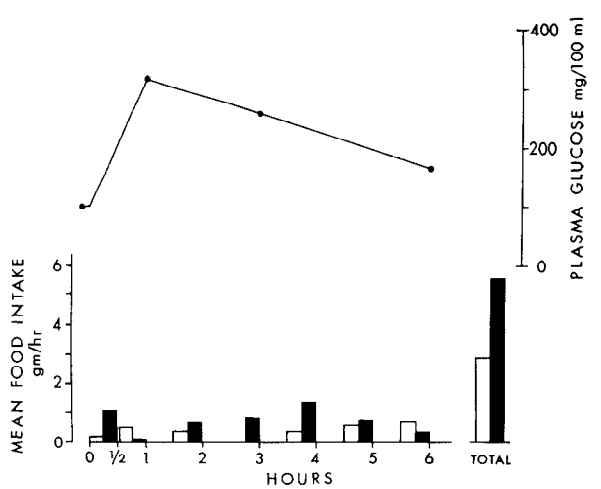

FIG. 3. Upper part of figure is mean plasma glucose concentrations of 10 rats just before and at 1, 3, and 6 hr after 2-DG (750 mg/kg, sc). Four rats had access to food and their mean food intake during 6 hr following 2-DG is depicted in lower part of figure.

and the complex neurohumoral excitement characteristic of an emergency state. Both hypertonicity and stress tend to inhibit feeding. The failure to observe increased feeding in five rats and one monkey is probably due to insufficient testing of small increments of 2-DG, so that the drive to eat never dominated the competing signals in these animals.

The other known short-term, metabolic stimulus for feeding in the rat is insulin hypoglycemia (2, 7, 12). In contrast to 2-DG, which produces decreased glucose utilization in both central and peripheral tissues, large doses of insulin produce increased glucose utilization in peripheral tissues but decreased glucose utilization of the brain (10). Since cerebral, but not peripheral, glucoprivation is common to both insulin and 2-DG, we believe that cerebral glucoprivation is the effective condition which elicits feeding after treatment with both materials. Insulin produces cerebral glucoprivation indirectly as a result of hypoglycemia; 2-DG produces cerebral glucoprivation directly. The possibility exists, however, that small doses of long-acting insulin alter food intake through metabolic effects other than cerebral glucoprivation because Mayer and Bates (15) demonstrated a small increase of food intake during treatment with NPH insulin in doses too small to produce hypoglycemia.

If cerebral glucoprivation is the effective condition which increases feeding, one would predict that animals would eat sooner after 2-DG than after large doses of insulin. This prediction was verified in the rat experiments (Fig. 2 and Table 2). This fact combined with the demonstrated severe hyperglycemia that accompanied the increased feeding after 2-DG makes it abundantly clear that the level of blood sugar is not a direct control or predictor of food intake. The association of hypoglycemia (7) with hunger and hyperglycemia with satiety, which were at best terminologic conveniences, are now clearly obsolete.

The fact that 2-DG produced increased feeding during marked hyperglycemia is reminiscent of the problem which the diabetic posed for the glucostatic hypothesis. It was this problem which originally focused attention on decreased glucose utilization as the critical variable rather than low blood sugar. Our results with 2-DG are much stronger evidence for the view that decreased glucose utilization is crucial for increased feeding because: *1*) The increased food intake seen after 2-DG occurs with an acute, reversible disturbance of glucose metabolism rather than a new, chronic metabolic state characteristic of diabetes. *2*) The type of diabetes which the metabolic and hormonal responses to 2-DG resemble is maturity-onset diabetes (19). Of 182 maturity-onset diabetics specifically questioned about increased appetite, only 17 patients acknowledged its presence (1). Furthermore, we know of no documented increase in food intake in maturity-onset diabetes. *3*) We know of no evidence for increased food intake in any type of diabetes which could not be explained as a compensation for the urinary losses of metabolic substrates.

In order to be an effective condition for feeding, cerebral glucoprivation must occur quickly. When it occurs gradually during a prolonged fast, brain metabolism shifts to the utilization of ketoacids as its main fuel (4, 17). This metabolic adaptation probably explains why patients with chronic hypoglycemia due to insulin secreting tumors do not have the cerebral symptoms or signs usually seen with acute hypoglycemia (13).

Our experiments with rats and monkeys, and those of Likuski and associates (11) in mice, are strong evidence for all but one of the predictions of the glucostatic hypothesis: feeding increased; it did so rapidly and for a short time during decreased glucose utilization produced by 2-DG. These results, however, are not relevant to the converse and most controversial prediction of the glucostatic hypothesis, i.e., that increased glucose utilization decreases food intake. This aspect of the hypothesis has been frequently criticized because infusions of glucose which produced hyperglycemia do not decrease food intake (9). These experiments, as well as those reporting positive results (15), are not, however, an adequate test because hyperglycemia produced by glucose injections does not produce increased brain glucose uptake or utilization (8). Until a specific manipulation that increases brain glucose utilization is tested for its effect on food intake, the prediction of decreased food intake by increased glucose utilization remains unproved.

On the basis of our experiments and those of Likuski and co-workers (11), an acute decrease of glucose utilization must now be considered one of the sufficient conditions for feeding in mammals. We contend, however, that acute glucoprivation is not a necessary condition for feeding. This view is based on the feeding behavior of the rat recovered from bilateral lesions of the lateral hypothalamus. This animal maintains constant food intake under normal laboratory conditions and it makes a precise adjustment of food intake to dietary dilution and to extremes of ambient temperature, but it does not increase its food intake during cerebral glucoprivation produced by insulin hypoglycemia (7). Acute glucoprivation is only one of several adequate conditions for feeding in mammals. When the feeding system is no longer activated by acute glucoprivation, other controls are sufficient to adjust food intake precisely.

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