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Threshold doses of 2-deoxy-D-glucose for hyperglycemia and feeding in rats and monkeys

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SMITH, GERARD P., JAMES GIBBS, ALAN J. STROHMAYER, AND PETER E. STOKES. *Threshold doses of 2-deoxy-D-glucose for hyperglycemia and feeding in rats and monkeys.* Am. J. Physiol. 222(1): 77–81. 1972.—Injection of the glucose analogue, 2-deoxy-D-glucose (2-DG), into intact mammals produces a metabolic state of glucoprivation characterized by extracellular hyperglycemia and decreased intracellular glucose utilization. Monkeys and rats eat more after 2-DG, despite marked hyperglycemia Smith, G. P., and A. N. Epstein. Increased feeding in response to decreased glucose utilization in the rat and monkey. *Am. J. Physiol.* 217: 1083–1087, 1969). Since the consequences of feeding and of hyperglycemia tend to diminish glucoprivation, both feeding and hyperglycemia may be considered homeostatic responses. We attempted to establish the physiological order of the responding systems which mediate feeding or hyperglycemia by investigating the sensitivity of each system to glucoprivation by seeking the threshold dose of 2-DG required to elicit feeding or hyperglycemia. In rats, the threshold dose of 2-DG for hyperglycemia was 100 mg/kg; threshold dose for feeding was >400 mg/kg. In monkeys, the threshold dose of 2-DG for hyperglycemia was 80 mg/kg; the threshold dose for feeding was 320 mg/kg. Thus, in both rats and monkeys the hyperglycemia system is at least 4 times as sensitive as the feeding system to the glucoprivation produced by 2-DG. If the glucoprivation produced by 2-DG is an adequate model for spontaneous glucoprivation, then these data are evidence that the glucostatic control of food intake operates only when glucoprivation is intense. Since intense glucoprivation has not been observed prior to a meal under routine feeding regimens, we conclude that the glucostatic control of food intake is not a dominant mechanism for the initiation of eating an ordinary meal.

Glucose metabolism; glucoprivation; glucostatic control

THE ANALOGUE of glucose, 2-deoxy-D-glucose (2-DG), inhibits glycolysis and the glucose utilization of a variety of tissues including brain by competitive antagonism of the phosphohexose isomerase reaction (1). When 2-DG is given to the intact mammal, a marked hyperglycemia occurs which is dependent on epinephrine released by the adrenal medulla (5). Despite the marked hyperglycemia, animals show signs of hypoglycemia, and human subjects have both the signs and symptoms of hypoglycemia (8). Thus, in the intact mammal, 2-DG uncouples extracellular glucose loading from intracellular glucose utilization. This kind of glucoprivation is useful in determining whether a response elicited by changes in glucose metabolism is linked to extracellular concentration or to intracellular utilization of glucose.

This property of 2-DG was exploited in a previous study of the effect of glucoprivation on food intake of monkeys and rats (12). Monkeys and rats ate more after 2-DG despite marked hyperglycemia. This result demonstrated that increased food intake was linked to the intracellular decrease in glucose utilization, not to the extracellular rise in glucose concentration. The result also confirmed one prediction of the "glucostatic" hypothesis for the control of food intake: the condition of decreased glucose utilization is associated with increased food intake (9).

Both hyperglycemia and feeding were elicited by an intracellular decrease in glucose utilization in the Smith and Epstein (12) experiment. The experiment reported here compares the sensitivity to decreased glucose utilization of the system mediating hyperglycemia to the sensitivity of the system for feeding by measuring plasma glucose or food intake after varying doses of 2-DG. The major finding is that the threshold dose of 2-DG for hyperglycemia is $\leq$ one-fourth the threshold dose for feeding in rats and monkeys.

METHODS

Rat experiments. Eighteen male albino rats (Sprague-Dawley, Hormone Assays, Chicago) weighing 250–400 g and housed in individual cages were subjects for the feeding experiments. Rat pellets (Purina) and tap water were available throughout the 24 hr, except for the 30–60 min prior to each experiment. On experimental days the pellets were removed about 10 AM. Intraperitoneal injections of test solutions were given approximately 30 min later using a 26-gauge needle. It usually took about 20 min to inject all animals. Following the last injection, a weighed portion of pellets was placed on the floor near the front of each cage. The quantity of food eaten at 30 and 60 min later was calculated by subtracting the weight of the remaining pellets plus the crumbs caught on paper beneath the cage floor from the initial weight of the pellets (10 g). All weighings were performed on a triple-beam balance (Ohaus Scale Corp.) which was accurate to 0.1 g.

Test solutions on control days and on treatment days were isovolumetric. This volume was set by calculating the volume of 2-deoxy-D-glucose solution (Calbiochem Co., N. Y. C.: 1% w/v in distilled water) which delivered 400 mg/kg of 2-deoxy-D-glucose (2-DG) to an individual animal. On days when smaller doses of 2-DG (100, 200 mg/kg) were given, the volume of 1% 2-DG solution sufficient to deliver the required dose was mixed with enough

1.8% saline (w/v) to make the final volume equal to the volume of 1% 2-DG required to deliver 400 mg/kg. On control days an isovolumetric injection of 1.8% saline was administered. All solutions were warmed to 37 C prior to injection. Solutions of 2-DG were mixed just before each experiment. The interval between 2-DG injections was never less than 48 hr.

Amount of food eaten at 30 and 60 min after an injection of 1.8% saline was compared to amount of food eaten during the same time periods on the next day after an isovolumetric injection of 2-DG (1%) or a mixture of 2-DG (1%) and saline (1.8%).

For the measurement of the plasma glucose response to 2-DG, food and water were removed from 95 intact and 22 adrenalectomized rats about 10 AM. Intraperitoneal injections of test solutions identical to those used in the experiments on food intake were administered and the rats were decapitated 30 or 60 min later. Samples of trunk blood were collected in tubes containing 10 mg fluoride and 10 mg potassium oxalate (Becton, Dickinson & Co.). Blood samples were centrifuged and plasma was frozen and later analyzed for glucose by a ferricyanide reduction method using the AutoAnalyzer (Technicon Corporation). 2-DG reacts in the reducing method used for measuring glucose, but 2-DG contributes only about 10% of the colorimetric reading of an equivalent concentration of glucose (unpublished observation). Plasma 2-DG was measured directly by the method of Cramer and Neville (2) in samples collected after doses of 2-DG which were threshold for hyperglycemia.

The adrenalectomized rats used in the hyperglycemia experiments were maintained on pellets and 0.9% saline. Adrenalectomies were performed by the vendor (Hormone Assays, Chicago). The perirenal and para-aortic areas of all adrenalectomized rats were carefully searched by one of us (*G.P.S.*) and no adrenal remnants were found.

Monkey experiments. Eight male rhesus monkeys (*Macaca mulatta*, 4–6 kg) were subjects for the feeding experiments. Monkeys were equipped with chronic inferior vena caval catheters, adapted to chronic restraint in primate chairs, and maintained in individual booths. Details of preparation and care of macaques under these conditions were published previously (13). The monkeys were fed in the morning (10–12 noon) and in the afternoon (2–4 PM). On experimental days 2-DG (5% w/v, 40–320 mg/kg) was injected intravenously by infusion pump (Harvard Apparatus Co.) at a rate of 10 ml/min just before the afternoon meal. On control days saline (0.9%) was given at the same rate and time. The injections were isovolumetric (36 ml). When the volume of 2-DG solution was less than 36 ml, enough saline (0.9%) was added to make up the remainder. All solutions were warmed to 37 C prior to injection. The quantity of food (Purina monkey chow, protein 25) eaten at 30, 60, and 120 min after saline or 2-DG injection was calculated by subtracting the weight of the remaining chow from the weight of the chow at the beginning of the meal (200 g). Chow was weighed to 1 g on a dietetic spring scale (no. 1140, Hanson Co.). Amount of food eaten after 2-DG was compared to the amount eaten after saline on the preceding day.

The plasma glucose response to 2-DG was studied in five of six monkeys which were subjects in the feeding experiments and in two additional monkeys. These experiments were carried out under the same feeding schedule. All injections of 2-DG (5% w/v, 40–100 mg/kg) were given just before the afternoon meal (2 PM). On experimental days the afternoon meal was delayed approximately 1 hr to permit blood sampling for glucose measurement when monkeys were not eating. Blood samples were collected from the inferior vena caval catheters, and the volume of blood was replaced immediately with an equal volume of saline. Blood samples were processed and measured for glucose and, in some instances, for 2-DG exactly as described above for rat blood samples. The interval between 2-DG injections was never less than 48 hr.

Statistical analyses of the effect of doses of 2-DG on plasma glucose in monkeys and rats were performed with Olivetti program no. 18 (*t* test). Feeding responses to 2-DG were analyzed by the sign test (4).

RESULTS

Rat experiments. Doses of 2-DG $\geq$ 100 mg/kg produced a significant increase in plasma glucose of intact rats at 30 and 60 min after intraperitoneal injection (Table 1). The increase of glucose was related to the dose of 2-DG over the dose range of 100–400 mg/kg. At the threshold dose of 100 mg/kg 2-DG, the error produced by plasma 2-DG in the measured concentration of plasma glucose did not exceed 1 mg/100 ml (range: 10% of 1 to 10 mg/100 ml, $n = 7$). The glucose response was apparently dependent upon a neuroendocrine mechanism involving the adrenal gland because adrenalectomy abolished the response to 100 mg/kg of 2-DG at both 30 and 60 min after injection. It should be noted in Table 1 that adrenalectomized rats had higher plasma glucose concentrations after saline injections than intact rats. We cannot explain this unexpected obser-

TABLE 1. *Plasma glucose response of intact and adrenalectomized rats to isovolumetric solutions of 2-DG or saline*

Dose of 2-DG, mg/kg	No. of Rats	Plasma Glucose, mg/100 ml
	30 min after injection	
Saline	18	103.3 ± 2.9
50	9	103.1 ± 6.3
100	9	129.8 ± 8.6*
Saline	5 (adx)	122.8 ± 4.9
100	5 (adx)	126.6 ± 0.5
	60 min after injection	
Saline	26	114.4 ± 2.1
100	8	125.9 ± 3.9*
200	8	150.9 ± 9.6*
400	17	310.1 ± 10.2*
Saline	7 (adx)	120.0 ± 4.5
100	5 (adx)	126.6 ± 4.7

Glucose values are means ± SE. Rats were decapitated 30 or 60 min after intraperitoneal treatment. Adx = adrenalectomized. *Glucose responses significantly different from the response to saline ($P < 0.05$, *t* test, one tailed). The responses at 60 min to 100, 200, and 400 mg/kg are significantly different from saline and from each other.

vation. Fortunately, this point does not affect the interpretation of the experimental results.

Identical injections of 2-DG failed to produce a reliable increase in food intake under the conditions of these experiments (Table 2). When 800 mg/kg were given under the same conditions, rats ate more over a 5-hr period (unpublished observations). This replicated the observation previously reported by Smith and Epstein (12). Thus, the failure to eat more at lower doses was not due to insensitive rats or odd conditions. Four of the 18 rats tested ate consistently more after doses of 2-DG $\geq$ 100 mg/kg (*rats 41, 42, 44,* and *45,* Table 2). Of this group, only *rat 42* showed a dose-related effect.

TABLE 2. *Comparison of food intake (g/hr) in hour after isovolumetric injections of saline or varying doses of 2-DG*

Rat	Dose of 2-DG, mg/kg					
	100		200		400	
	Control	2-DG	Control	2-DG	Control	2-DG
31	0.0	0.0		0.1	0.5	0.2
32			0.1	0.0	0.3	0.3
33	0.6	0.0	0.7	0.0	1.7	1.5
34	0.9	0.0	0.0	1.1*	2.0	1.3
35	1.6	1.7	1.4	1.6	3.5	2.0
36	1.7	0.0	0.7	1.0	0.7	1.0
37	0.0	0.5	1.2	0.1	1.2	0.1
38	0.0	0.1	0.0	0.7	0.0	0.7
39	1.7	0.1	1.1	0.0	1.1	0.0
40	0.1	0.0	0.0	1.5*	0.0	1.5*
41	0.0	1.9*	0.4	2.1*	0.0	1.8*
42	0.3	1.4*	0.1	2.3*	0.4	3.4*
43	0.2	0.6	0.5	0.6	0.3	1.1
44	0.1	2.4*	0.2	1.3*	0.2	1.8*
45	0.2	1.2*	0.2	0.3	0.2	1.3*
46	3.0	0.3	2.1	0.2	0.7	2.8*
47	0.1	1.0	0.3	0.1	0.2	0.9
48	0.5	0.1	0.2	2.8*	0.6	0.0

Saline injections (control) were always given on the day before 2-DG injections. Rats were not deprived before the tests. *Food intakes after 2-DG which were $\geq$1.0 g more than the control intakes. No dose of 2-DG produced an increase of food intake $\geq$1.0 g above control values in a sufficient number of animals to attain significance by the sign test. Analysis of the intakes during the first or second 30-min periods failed to uncover a reliable effect also.

TABLE 3. *Plasma glucose at intervals before and after isovolumetric injections of saline or 2-DG at doses specified*

Dose of 2-DG, mg/kg	Time, min.					
	−15	−5	0	+5	+15	+30
Saline	77.2 ±1.81 (31)	79.3 ±1.92 (27)	78.0 ±1.48 (31)	78.8 ±1.66 (31)	80.1 ±1.97 (30)	76.2 ± 1.90 (31)
40	76.9 ±3.91 (7)	76.6 ±2.95 (7)	74.6 ±3.80 (7)	79.1 ±1.68 (7)	76.6 ±3.85 (7)	78.4 ±4.69 (7)
80	82.7 ±6.38 (6)	81.3 ±6.83 (6)	80.0 ±5.37 (6)	92.8 ±6.57* (6)	98.8 ±8.81* (6)	94.2 ±11.70* (6)
100	79.6 ±4.55 (7)	81.1 ±4.23 (7)	83.0 ±4.80 (7)	91.3 ±5.23* (7)	113.0 ±4.93* (7)	128.6 ±7.91* (7)

Values are means ± SE. Injections were always given intravenously immediately after the sample at zero time was obtained. Numbers in parentheses are the number of experimental observations from which the mean ± SE was calculated. All tests were performed after 2 hr of food deprivation. Five of the seven monkeys studied here were subjects for the feeding experiments (see Table 4). *Glucose responses significantly different from the response to saline ($P < 0.05$, t test, one tailed).

TABLE 4. *Comparison of food intake (g/hr) in hour after isovolumetric injections of saline or varying doses of 2-DG*

Monkey	Dose of 2-DG, mg/kg							
	40		80		160		320	
	Control	2-DG	Control	2-DG	Control	2-DG	Control	2-DG
7	25	30	10 35	30 30	15	28	25	40
10	60	55	45	50	65	45	5	15
11	25	20	35	8	25	30	15	25
12	70	55	40	58	65	105	45	65
14			40	50	48	35	25	38
111			45	32	12	45	50	68

Saline injections (control) were always given on the day before 2-DG injections. Monkeys were deprived 2 hr before each experiment. The dose 320 mg/kg of 2-DG produced a significant increase in food intake (sign test), but smaller doses did not. Smaller doses were also not effective if food intakes were evaluated for the first or second 30-min periods of the hour.

Monkey experiments. The threshold dose for hyperglycemia after intravenous 2-DG in monkeys deprived of food for 2 hr was 80 mg/kg (Table 3). Plasma glucose increased significantly at 5 min after the injection. Hyperglycemia lasted longer than 30 min. The response was dose related; 100 mg/kg produced significantly higher plasma glucose at 30 min than 80 mg/kg produced. At the threshold dose of 2-DG, the error in the measurement of plasma glucose introduced by plasma 2-DG did not exceed 1 mg/100 ml.

The threshold dose for feeding under the same conditions was 320 mg/kg (Table 4). This was the most reliable dose in the five monkeys previously reported (12). *Monkey 7* ate more after 2-DG in four of five experiments. The response was not reliable at 80 mg/kg, however, and it was not dose related (Table 4).

DISCUSSION

The major result of this study is that the threshold dose of 2-DG that elicits hyperglycemia is $\leq$¼th the threshold dose that elicits increased food intake in rats and rhesus monkeys. The interpretation of this result rests on the assumption that the decrease in glucose utilization is directly related to the dose of 2-DG. This relationship has been demonstrated in vitro and in vivo (1). Thus, the major result of our experiments is evidence that the system producing hyperglycemia is at least 4 times as sensitive to activation by glucoprivation as the system for feeding. This estimate of the difference in sensitivity of the two systems is conservative because the conditions of testing were biased in favor of the feeding system. This bias resulted from the decision to determine the threshold for hyper-

glycemia only under those conditions in which the feeding response to 2-DG was reliable. Thus, we have omitted from this paper results of experiments in monkeys deprived of food for 16 hr because it has not been possible to demonstrate a reliable feeding response to 2-DG under this condition. Despite this fact, the system for hyperglycemia is more sensitive to glucoprivation by 2-DG (threshold dose is 20–40 mg/kg, unpublished data) than it was in the monkeys reported here.

The response of the hyperglycemia system to 2-DG is not only very sensitive, it is also graded. The response of the feeding system in the monkey does not seem to be dose related either in these experiments or in the ones reported previously (12). The lack of a dose-response relationship of the feeding system to 2-DG makes the interpretation of feeding responses to low doses of 2-DG in a minority of the animals difficult to evaluate.

The relatively high threshold of the feeding system appears to be fixed because the threshold dose of 2-DG for eliciting feeding was as high in one adrenalectomized monkey maintained on cortisone as the intact monkeys (unpublished observations). To be certain, this point requires further investigation.

The difference in sensitivity to activation by glucoprivation of the hyperglycemia system and the feeding system has important implications for the role of glucoprivation in initiating feeding behavior. Since the hyperglycemia system is more sensitive, it will respond first as glucoprivation develops. The hyperglycemic response will tend to diminish glucoprivation, particularly that of the brain. If the hyperglycemic response is insufficient, then glucoprivation will continue to increase until the threshold of the feeding system is exceeded. Thus, glucoprivation is a sufficient stimulus for feeding, but it is an unusual one. If the glucoprivation produced by 2-DG is an adequate model for spontaneous glucoprivation, then these data are evidence that the glucostatic control of food intake operates when glucoprivation is intense, i.e., when glucoprivation exceeds the response capacity of the hyperglycemia system. There is no evidence in any mammal at the present time that such intense glucoprivation occurs just prior to eating a meal when food is freely available (15).

Glucoprivation is an unusual stimulus for feeding and it is expendable. The expendability of glucoprivation as a stimulus for feeding under ordinary conditions has been demonstrated in the rat recovered from lateral hypothalamic lesions. Such animals regulate their body weight, despite permanent loss of the feeding response to intense glucoprivation induced by large doses of insulin (3). Furthermore, such rats regulate their body weight by eating more frequently; they nibble for prolonged periods (6). If glucoprivation were an important and sensitive cue for eating a meal, the predicted change in feeding behavior of an animal unable to respond to glucoprivation would be less frequent, not more frequent meals.

Although the experimental results do not support the hypothesis that the glucostatic control of food intake is a dominant mechanism for the initiation of eating an ordinary meal, they do not exclude a minor, facilitating role for the glucostatic control in the initiation of eating an ordinary meal. Experimental support for this logical possibility has not yet appeared.

Although we believe glucoprivation is an unusual and expendable stimulus for ordinary feeding behavior, the feeding response to glucoprivation induced in rats and monkeys by 2-DG occurs in a situation in which feeding is not essential for survival. This is simply demonstrated by withholding food from animals treated with doses of 2-DG sufficient to elicit feeding (12). Rats treated with 750–800 mg/kg of 2-DG and not given food show drowsiness, stupor, and ataxia for varying periods up to 4 or 5 hr, but they invariably survive and they have never convulsed. If food is withheld from monkeys treated with 300–500 mg/kg of 2-DG, there may be transient drowsiness or retching within the first 5 min. No other signs have been observed for up to 3 hr following injection. These observations suggest that feeding occurs when glucoprivation is intense, but not necessarily life threatening. Thus, feeding in response to glucoprivation is one of the array of mechanisms which is activated to restore metabolic homeostasis. This is an example of Richter's famous insight that some animal behaviors assist in the regulation of the internal environment (11). We can appreciate that insight more now that we understand the metabolic consequences of feeding during glucoprivation. Until feeding occurs in glucoprivation, glucose distribution is abnormal because the epinephrine released by activation of the hyperglycemia system not only promotes glycogenolysis, but it also inhibits insulin secretion (7, 10) and releases fatty acids from fat stores. Low insulin and high concentrations of fatty acids both tend to exclude glucose from fat and muscle cells. Thus, the hyperglycemic response alters glucose distribution in a pattern which conserves glucose for the brain. Feeding during glucoprivation not only provides exogenous glucose through ingestion and digestion of food, but it also tends to restore the normal distribution of blood glucose by stimulating insulin secretion (14).

These considerations lead to the view that feeding always provides important physiological signals for glucose homeostasis, but glucoprivation provides an unusual and expendable signal for feeding behavior.

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