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Glucoprivic (2DG) Eating in Rats Despite Knife Cut Induced Hyperphagia[1]

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HOUPT, K. A. AND R. M. GOLD. *Glucoprivic (2DG) eating in rats despite knife cut induced hypothalamic hyperphagia.* PHARMAC. BIOCHEM. BEHAV. 3(4) 583–588, 1975. – Two weeks after parasagittal hypothalamic knife cuts, baseline eating was elevated and 300 mg/kg 2 deoxy-D-glucose (2DG) did not further stimulate food intake. Five weeks postoperatively the food intake baseline had fallen and an eating response to 300 mg/kg 2DG was now seen ($p<0.005$). In this delayed (static) phase intake was also stimulated by 150 mg/kg 2DG ($p<0.005$). 600 mg/kg did not stimulate intake in the lesioned rats at any time, although sham-operated rats always responded positively to this high dose. In conclusion, the neural substrate damaged in hypothalamic hyperphagic rats does not appear to mediate eating in response to glucoprivation. The eating response is masked by high baseline intake in the dynamic phase, but reappears in the static phase.

Glucoprivation Parasagittal knife cuts Hypothalamic hyperphagia Obesity 2-Deoxyglucose

IN intact rats glucoprivation produces an increase in food intake. Glucoprivation can be produced by the administration of 2-deoxy-D-glucose (2DG) which inhibits glucose utilization within the cell [5]. Systemic 2DG stimulates food intake [26]. Intraventricular [19] and intracranial 2DG [3] also stimulate food intake. A second way frequently used to produce glucoprivation is by the administration of insulin. Insulin has also been shown to stimulate food intake [18,28].

Rats which have recovered from the aphagia that follows lateral hypothalamic brain lesions eat neither in response to 2DG [29] nor in response to insulin [28]. This suggests that lateral hypothalamic cells or fibers of passage are involved in responding to a decrease in metabolizable glucose. Selective depletion of brain dopamine by intraventricular 6-hydroxydopamine [27] or medial forebrain bundle lesions [4] also prevent the response to 2DG. This supports the fiber of passage interpretation because the nigrostriatal dopamine bundle passes through the lateral hypothalamus [2,10]. It has been suggested that the medial hypothalamus may also play a role in the glucoprivic eating response, but the data supporting this suggestion are inconsistent. On the one hand, rats obese as a result of medial hypothalamic lesions increase their short term food intake following insulin [28], intraventricular 2DG [20], or intravenous 2DG [23]. Müller *et al.* [22], on the other hand, report that rats with medial hypothalamic lesions do not eat in response to systemic 2DG. In the present study we report that 2DG produces a robust eating response in hyperphagic obese rats. Furthermore, we demonstrate how the particular experimental conditions used by Müller *et al.* [22] could easily explain failure to obtain a reliable eating response. The apparent contradiction may be due to differences in the size or location of the medial hypothalamic lesions in the various studies. Also, rats with particularly effective lesions may have such a high base rate of food intake, especially in the rapid weight gain or dynamic phase, that eating in response to 2DG is masked. Finally, high doses of 2DG computed on a per kg of rat basis may be an overdose for obese rats.

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To deal with the lesion size problem, in the present study hypothalamic obesity was produced with bilateral parasagittal knife cuts. This procedure minimizes damage to nuclear areas, most critically the lateral hypothalamus, and also spares the rostrocaudal fibers of passage within the medial forebrain bundle [11,13].

To deal with the possible ceiling effects from a high baseline of food intake by hyperphagic rats, the food intake following 2DG glucoprivation was measured both in the early postoperative dynamic stage when baseline intakes are high, and again 5 weeks postoperatively when baseline food intakes were reduced, at which time a response to 2DG may more easily be detected.

To deal with the possible overdosing of obese rats, several dose levels were used.

METHOD

Animals and Diets

Female Carworth rats were housed individually and fed a high fat diet [6,12] from 8 cm dia. glass bowls secured to a corner of each cage. Spillage, which was minimal, was collected on papers placed beneath the cages. Water was available ad lib.

Surgery

Parasagittal knife cuts were made under ether anesthesia using a retracting wire knife made from a Hamilton microliter syringe [14]. The knife was stereotaxically lowered into the brain at anterior 8.5 mm, lateral ± 0.9 mm. When the knife tip was 6.5 mm below the dura, the cutting wire was extended 3 mm caudally. The extended wire knife was then lowered until it hit the bony floor of the skull.

Procedure

2DG (Sigma) was prepared and sterilized as a 5 percent (wt/vol of H_2O) solution. Doses were administered intraperitoneally and ranged from 150–600 mg/kg body, weight. Volumes injected ranged from 1 to 7 ml depending on the weight of the rats and the dose. On control days sterile 0.9 percent NaCl was administered in equivalent volumes. Food intake was measured until 4 hr after injections. On some test days 2DG and control injections were preceded and followed by 1 hr of food deprivation. The deprivation period was imposed for two reasons (1) to avoid the inhibitory effects of gastric fill on food intake especially in the dynamic phase hyperphagics and (2) to allow the 2DG-treated rats to recover from CNS depression before presenting food. On other test days there was no deprivation period. There were no trends or significant differences between the no deprivation ad lib 4 hr intakes and the 3 hr intakes following deprivation.

The first group of rats (13 lesioned and 4 sham operated) were tested (300 mg/kg) 7 weeks postoperatively at which time obesity was well advanced, the mean body weight of the knife cut rats being 575 ± 14 g vs 265 ± 7 g for the sham operated controls.

A second group of rats (7 lesioned and 3 sham operated) was tested preoperatively (300 and 600 mg/kg); two weeks following surgery during the phase of rapid (9 g/day) weight gain (300 mg/kg); and again 5 weeks following surgery (150, 300, and 600 mg/kg), by which time the lesioned rats were obese (527 ± 13 g vs 289 ± 23 g, for the controls) and their rate of weight gain had slowed to 3 g/day.

The blood glucose response to 2DG was measured in both lesioned and sham operated rats. After an overnight fast a preinjection sample was taken from the tail vein under light ether anesthesia. 2DG (300 mg/kg) or an equivalent volume of 0.9 percent NaCl were then given IP. A post injection blood sample was taken an hour later. Whole blood was analyzed by the glucose oxidase method (Worthington Biochemicals).

RESULTS

The first group of rats were in the slow gaining or static phase of hypothalamic hyperphagia. The baseline (NaCl) food intake was higher for the obese rats than for the sham operated controls, but not so high as to prevent a significant increase in food intake in response to a moderate dose of 2DG (300 mg/kg). (Fig. 1.) The knife cut group (4.3 ± 0.5 g/3 hr) did not differ significantly from the sham operated group (3.9 ± 0.4 g/3 hr $p > 0.05$) in the amount of food eaten following 2DG. Glucoprivic eating in response to 2DG thus appears to be possible in hypothalamic hyperphagic rats.

In addition to an increase in food intake, rats respond to 2DG with a profound hyperglycemia [26]. In the sample of hyperphagic obese rats tested the hyperglycemic response to 2DG was undiminished (Table 1).

In the second group of rats tested the preoperative administration of 300 and 600 mg/kg 2DG produced the predicted increase in food intake. The data for the 300 mg/kg dose are seen in Fig. 2 (left section) and the response to both 300 and 600 mg/kg doses are shown in Fig. 3. (preoperative data).

Seven to 10 days after surgery baseline (NaCl) intake was very high (5.2 ± 1.0 g) and 300 mg/kg 2DG did not further stimulate food intake (4.2 ± 0.3 g, $p > 0.05$) (Fig. 2 middle section). By 5 weeks after surgery the baseline response to saline had receded sufficiently (2.0 ± 0.4 g) to reveal a significantly enhanced food intake in response to 300 mg/kg 2DG (4.0 ± 0.5 g, $p < 0.005$), (Fig. 2 right section). A smaller dose, 150 mg/kg 2DG, also stimulated intake significantly (3.1 ± 0.3 g vs 1.7 ± 0.3 g $p < 0.005$). A larger dose, 600 mg/kg, failed to stimulate intake in the obese rats (2.0 ± 0.4 g vs 1.6 ± 0.5 g $p < 0.05$, (Fig. 3, static obese) whereas sham operated controls continued to show an increase in food intake in response to all of the doses of 2DG (Fig. 3, sham operated).

To summarize, glucoprivic eating can be demonstrated in the static phase obese rats when moderate or low doses of 2DG are used, but the response is masked by the high baseline intake of the dynamic stage of hypothalamic hyperphagia and is not seen in obese rats when high doses of 2DG are used.

Anatomical Findings

The knife cuts were typically 0.8–1.0 mm from the midline, which coincides with the fornix. Rostro-caudally the knife cuts extended for 2.5–3.0 mm and they invariably included the area alongside the paraventricular nucleus. Dorso-ventrally the cuts extended from the ventral edge of the thalamus to the base of the brain. In several cases much of the area lateral to the ventromedial nucleus of the hypothalamus was spared. (Fig. 4).

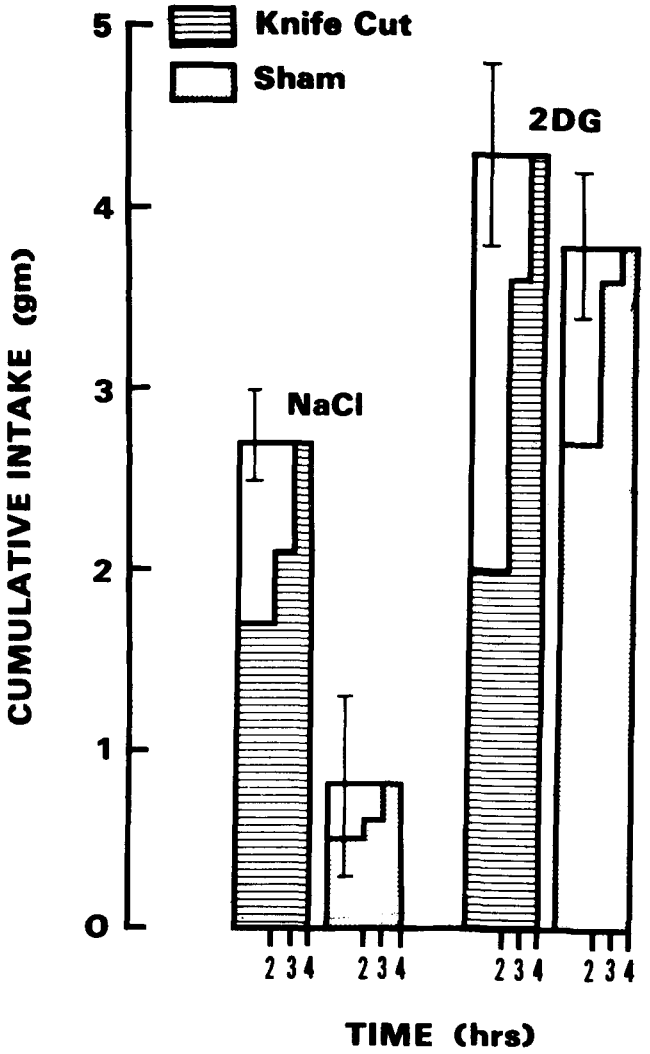

FIG. 1. Eating in response to 300 mg/kg 2DG in static phase obese rats. The pair of colums on the left represent baseline intake following 0.9% NaCl injection for knife cut (lined column) and sham operated (open column) rats. The pair of columns on the right represent intake following 300 mg/kg 2DG. The total height of the columns is total three hour food intake, (4 hr postinjection). The steps within the columns represent cumulative hourly intake. In this case the rats were deprived 1 hr before and one hour after injection.

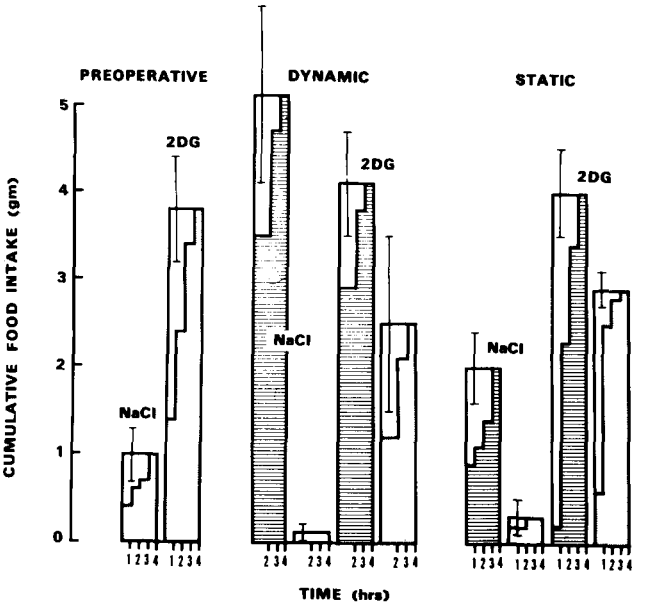

FIG. 2. The response to glucoprivation at various stages of hypothalamic hyperphagia. 2DG (300 mg/kg) was given preoperatively to both groups of rats. The data is combined in the left section. 2DG (300 mg/kg) was given during the dynamic phase of the obesity (middle section) and during the static phase of the obesity (right section). Within each section the pair of columns on the left represent baseline intake following 0.9% NaCl injection for knife cut (lined column) and sham operated groups (open column). The pair of columns on the right within each section represents intake following 300 mg/kg 2DG. The total height of the columns in total food intake in the 4 hours post injection. The steps within the columns represent cumulative hourly intake. Note the greatly elevated response to 0.9% NaCl injection during the dynamic phase.

DISCUSSION

The critical physiological system disrupted in hypothalamic hyperphagia remains unknown. Knowledge of the presence or absence of any of the known controls of intake such as glucostatic control in the hyperphagic rat would be useful in any explanation of the obesity. There are differences between rats with lesions in the ventromedial hypothalamus and those with parasaggital knife cuts lateral to the ventromedial hypothalamus. The most obvious difference is in the amount of brain tissue damaged. However, both procedures result in hyperphagia and obesity. In both cases the rats pass through a dynamic phase where hyperphagia is most pronounced and then enter a static phase. As this study reveals, the response to glucoprivation by knife cut and lesioned rats is similar when the stage of the obesity and the dose of the glucoprivic agent are considered.

Various lines of evidence suggest that glucoreceptors involved in the control of food intake may be present in the ventromedial hypothalamic area. Production of obesity and lesions in the ventromedial area by goldthioglucose [8] is one line of evidence for glucoreceptors in that area. The protection from goldthioglucose lesions afforded to mice made diabetic by alloxan is more convincing evidence for presence of insulin dependent glucoreceptors [7]. Single unit recordings from both the lateral and ventromedial hypothalamus provide still further evidence of glucose sensitive cells in both areas [1,24].

The question we wished to answer is whether or not the putative glucoreceptors that mediate glucoprivic eating are functional in hypothalamic hyperphagic rats or whether the VMH lesions or parasagittal knife cuts have abolished their ability to respond. In particular we were interested in resolving the inconsistencies in the literature [20, 22, 23].

The results of these experiments indicate that rats hyperphagic and obese as a result of parasagittal knife cuts can respond to glucoprivic challenge just as vigourously as sham operated controls. Their ability to respond to 2DG depends on two factors: the phase of the obesity and the dose of 2DG.

TABLE 1

BLOOD GLUCOSE RESPONSE OF INDIVIDUAL RATS TO 2DG OR SALINE

Surgery	Treatment	Blood Glucose mg/100 ml		
		Preinjection	Postinjection	Δ
Knife cut	2DG 300 mg/kg			
Rat 124		80	195	115
Rat 126		122	235	113
Rat 134		124	282	158
Sham operated	2DG 300 mg/kg			
Rat 129		72	192	120
Knife cut	0.9% NaCl			
Rat 127		83	83	0
Rat 137		90	98	8
Sham operated	0.9% NaCl			
Rat 139		80	72	−8

During the dynamic or rapid weight gain phase baseline intake is already as high as that normally produced by 2DG, and intake is not further increased by 2DG. The capacity of the upper gastrointestinal tract may serve as the rate limiting factor in these dynamic phase obese rats. A neural rate limiting mechanism is also possible.

At high mg/kg doses of 2DG hypothalamic hyperphagic rats did not overeat. This replicates Müller *et al.* [22]. There are three possible explanations: (1) The brain damaged animals may be more sensitive to the central nervous depressant effects of 2DG than are their sham operated controls. Other investigators have noted signs of CNS malfunction in animals treated with very high doses of 2DG. For instance, stupor and ataxia in rats [26]; (2) 2DG may not be distributed in all tissues of the body equally. If 2DG does not enter fat as easily as it does lean body tissue then the obese rat will actually be getting a much higher dose on a mg/kg of lean body mass or a mg/kg of brain basis. Unfortunately, the distribution of 2DG following injection has not been measured. (3) On the basis of metabolic body surface (body wt in kg raised to the 3/4 power) [16] the dose of 2DG for a 500 g lesioned rat should not be twice as much as that for a 250 g rat but only 1.68 times as much. When sham operated and lesioned rats differ in weight by hundreds of grams, care must be taken in calculating equivalent doses. For these or perhaps some other reason obese rats do not respond to 600 mg/kg doses of 2DG. The original report of stimulation of intake in rats by 2DG [26] used a high (750 mg/kg) dose, but that might be considered a maximum rather than a minimum effective dose, for we have shown that doses as low as 150 mg/kg 2DG can reliably stimulate robust food intake response in intact as well as hypothalamic hyperphagic rats.

Since the eating response to 2DG remains intact, it is not surprising that parasagittal knife cuts that produce obesity do not interfere with the hyperglycemic response to 2DG. This is similar to findings in rats lesioned in the ventromedial area [22] and in mice lesioned with goldthioglucose [9].

The non-effect of 2DG in dynamic stage hypothalamic hyperphagic rats is similar to the non-effect of 2DG on gastric secretion in rats with ventromedial hypothalamic lesions [25]. Rats with lesions in the ventromedial hypothalamus have a very high baseline (unstimulated) gastric acid secretion and this secretion is not further increased by 2DG. Likewise, lesioned rats in the dynamic phase have a very high baseline of food intake which is not further increased by 2DG. As baseline intake falls a response to glucoprivic challenge can again be elicited as shown by our results with 2DG and those of Müller *et al.* [22] with insulin.

As discussed elsewhere [13,15] the parasagittal knife cuts that produced obesity appear to sever noradrenergic (NE) fibers as they turn medially from ascending NE bundles to terminate in or near the paraventricular n. Leibowitz [17] finds that the paraventricular n. is also the most effective site for NE induced feeding. We have shown that glucoprivic eating is not mediated by these NE fibers, although it has been demonstrated that glucoprivic eating can be attenuated by chemical blockage of the brain adrenergic system [21].

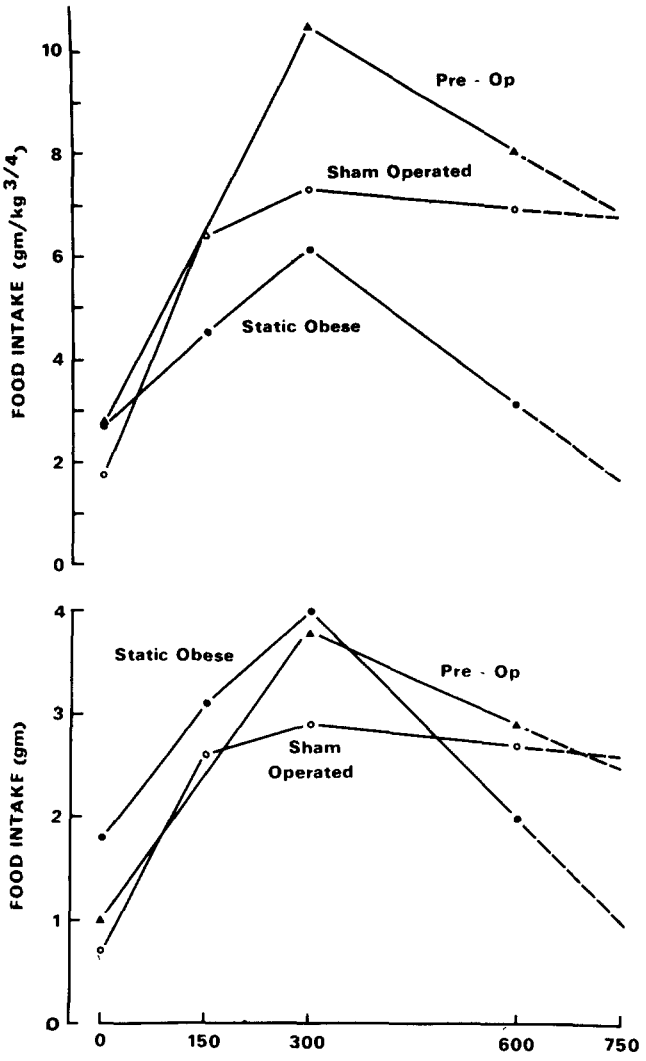

FIG. 3. The dose response curve to 2DG. The food intake response to various doses of 2DG preoperatively (solid triangles) and postoperatively for sham operated (open circles) and static phase knife cut rats (closed circles). The dotted lines represent data which was obtained on another group of knife cut and sham operated animals tested with 750 mg/kg 2DG. These results agree with those of Müller *et al.* [22]. The preoperative point for 750 mg/kg 2DG is that obtained by Smith and Epstein in intact rats [26].

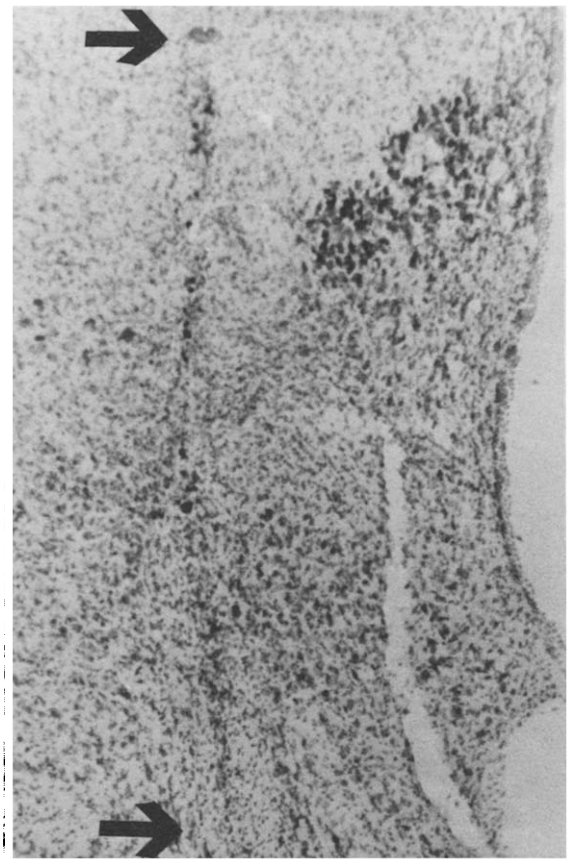

FIG. 4. Horizontal brain section showing the entire 2.7 mm rostro-caudal extent of a typical hyperphagiogenic parasagittal knife cut. Arrows indicate the rostral and caudal ends of the knife cut. The cavity on the right is the third ventricle. The densely staining area in the upper (rostral) right is the paraventricular nucleus. Magnification ×40.

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