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Delayed Response to 2-deoxy-D-glucose in Hypothalamic Obese Rats

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KING, B. M., B. A. STAMOUTSOS AND S. P. GROSSMAN. *Delayed response to 2-deoxy-D-glucose in hypothalamic obese rats.* PHARMAC. BIOCHEM. BEHAV. 8(3) 259–262, 1978. – A dose-response relationship for the effects of 2-deoxy-D-glucose (2-DG) (0–400 mg/kg) on food intake was established in normal and obese ventromedial hypothalamic lesioned rats. In normal animals the lowest dose that produced a statistically significant increase over baseline food intake was 100 mg/kg 2-DG. Larger doses produced a progressively greater effect. Most of the increase in food intake occurred during the first hour after the injection of 2-DG, the latency of the first feeding bout being shorter for higher doses of the compound. Obese VMH rats significantly increased their 4-hr food intake after 150, 200, 250, and 400 mg/kg 2-DG, but the increase in feeding was delayed compared to control animals. During the first hour after the injection, the food intake of obese rats was unaffected by doses of 2-DG up to 250 mg/kg, and inhibited by higher doses (300 and 400 mg/kg). The effects of VMH lesions on 2-DG-induced eating are attributed to the elimination of afferents from peripheral glucoreceptors.

2-deoxy-D-glucose Hypothalamic obese rats VMH lesions

IN NORMAL animals the administration of 2-deoxy-D-glucose, a competitive inhibitor of intracellular glucose utilization, produces a prompt and reliable increase in food consumption [17]. Mayer [10] suggested that glucoprivation-induced increases in feeding are mediated by the ventromedial hypothalamus (VMH), an hypothesis which received some support when glucose sensitive cells were found within the VMH [1,15]. Intracarotid infusions of 2-DG decrease the unit activity of VMH cells to 20% of normal while simultaneously increasing the activity of lateral hypothalamic cells [4]. While lesions of the ventromedial hypothalamus do not affect the normal hyperglycemic response to 2-DG [6,13], Müller *et al.* [13] reported that VMH-lesioned rats displayed either no change or a decrease in food intake following the administration of 750 mg/kg 2-DG, a dose which markedly increases feeding in normal animals [9,17]. Houpt and Gold [7] reported similar results for rats with parasagittal hypothalamic knife cuts following 600 mg/kg 2-DG, but observed normal 4-hr increases in feeding by obese VMH rats to lower doses of 2-DG (150 and 300 mg/kg). They suggested that 2-DG administered on a mg/kg basis represents an overdose to obese animals due to differences in metabolic body surface. Recently operated lean VMH rats displayed no increase in food intake to the lower doses, but this was attributed to a masking of the response by the higher baseline intakes.

We [9] recently observed a similar pattern of results with lean and obese VMH-lesioned rats following the administration of a moderate (350 mg/kg) and high (750 mg/kg) dose of 2-DG. Although lean rats failed to increase feeding to either dose, it did not appear to be due to the higher baseline intakes, for control animals ate twice as much as lean VMH rats in the first hour following injection. In fact, the intakes of both lean and obese VMH-lesioned rats were often depressed in the first hour following 2-DG administration. Although they made no mention of it, an inspection of Houpt and Gold's [7] data (Fig. 2) reveals that although their obese rats displayed a normal 4-hr increase in feeding following 300 mg/kg 2-DG, the first hour intakes were severely depressed. Thus in both experiments the increase in feeding by obese rats to 2-DG (300–350 mg/kg) was the result of a delayed response, i.e., an increase during the second-fourth hours following administration. We [9] have found that normal rats display the greatest 6-hr increase in feeding to a large dose of 2-DG (750 mg/kg), but the first hour increase is often suppressed compared to that which follows much smaller doses (350 mg/kg). This suggested to us that 350 mg/kg may be at the upper end of the dose-response curve for animals with VMH lesions. The present experiment examines the response of obese VMH-lesioned rats to very low doses of 2-DG.

METHOD

Animals

Five control and 4 VMH-lesioned adult female Long-

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Evans hooded rats (Simonsen Laboratories, Gilroy, CA) were used. The operated animals were fed ad lib for 40 days prior to the initial injection, at which time the control and VMH-lesioned rats weighed an average of 378.0 (weight gain = 31.7 g) and 678.5 g (weight gain = 324.5 g), respectively. All animals were individually caged in a temperature controlled colony (21–24°C) with a 12 hr light/dark cycle.

Surgery and Histology

Bilateral ventromedial hypothalamic lesions were produced under sodium pentobarbital (Nembutal) anesthesia (50 mg/kg) by passing a 2.0 mA anodal current between the 0.5 mm uninsulated tip of a teflon insulated stainless steel electrode (No. 1 insect pin) and a rectal cathode for 20 sec. With the upper incisor bar positioned 5 mm above the interaural line, the electrodes were stereotaxically positioned 0.8 mm posterior to bregma, 0.7 mm lateral to the midsagittal suture, and 10.0 mm below the surface of the skull.

Upon completion of the experiment, animals with VMH lesions were anesthetized and intracardially perfused with isotonic saline followed by a 10% formol saline solution. Histological analysis was performed by light microscopic examination of cresyl violet stained 80 μ coronal sectoins, cut on a freezing microtome. The atlas of Pellegrino and Cushman [16] was used in estimating the extent of the lesions. All lesioned rats had extensive bilateral damage to the ventromedial hypothalamus, with two animals suffering additional damage to the dorsomedial hypothalamus. There were no apparent differences in the response to 2-DG by rats with lesions restricted to the VMH and those with additional damage to the DMH. All lesions were bordered laterally by the fornix and did not extend beyond the rostral or caudal borders of VMH.

Procedure

Rats with VMH lesions are hyperreactive to handling and we have previously found that even placebo injections can result in a marked decrease in food intake [9]. All animals were therefore given IP injections of 1 ml of sterile water for 4 consecutive days prior to the initial control injection. This resulted in habituation to the experimental procedure (i.e., no depression of food intake after the first control injection). During testing, the animals were given fresh food (Teklad Mouse and Rat Diet, 6% fat, Teklad Inc., Monmouth, IL) ad lib for 1 hr immediately prior to the injection in order to minimize preinjection satiety differences. Injections were given at the same time every other day. Food intake was measured to the nearest 0.1 g after the first, second, and fourth hours by subtracting spillage (collected on paper towels) and uneaten food from the premeasured supply. Latencies to begin eating were recorded to the nearest minute.

Each animal was tested twice with IP injections of 50, 100, 150, 200, 250, 300, and 400 mg/kg 2-DG (Sigma Chemical Co., St. Louis, MO) dissolved in sterile water. Doses were administered in an ascending order. Control injections of equal amounts of sterile water were administered 2 days prior to the first of each dose of 2-DG. The results of the two injections of each of the various doses of 2-DG were averaged and compared to the average intake following all control injections.

RESULTS

The effects of the various doses of 2-DG on food intake and latencies to begin eating are shown in Fig. 1. There were no significant differences between groups on either measure following the administration of sterile water (baseline) (the apparent difference in latencies was due to one control rat which rarely ate within the first 4 hr after injections of sterile water). In control animals, the lowest dose of 2-DG that produced a statistically significant response was 100 mg/kg, reflected in both a decrease from baseline in the latency to begin eating ($p<0.05$) and an increase over baseline in the total ($p<0.01$) and first hour ($p<0.05$) food intake. A reliable feeding response to 100 mg/kg 2-DG has recently been reported by others as well [8]. Further increases in dosage produced a progressively greater increase over baseline in the total 4-hr intake, accompanied by a reliable decrease in the latency to begin eating and a marked increase in feeding over baseline during the first hour following injection.

Obese rats with VMH lesions significantly increased their 4-hr food intake following 150, 200, 250, and 400 mg/kg 2-DG ($p<0.05$). However, injections of 2-DG never produced a significant increase over baseline in first hour food intake, and caused a reliable decrease from baseline in the latency to begin eating only after 150 mg/kg. None of the lesioned animals ate during the first hour following injections of 400 mg/kg 2-DG and only one responded to 300 mg/kg. Four hundred mg/kg 2-DG produced a significant increase from baseline in the latency to begin eating ($p<0.01$). Thus, compared to control animals, the increases in 4-hr intake by obese rats were largely the result of a delayed response, i.e., an increase during the second-fourth hours.

Although doses of 2-DG of 150 mg/kg and greater generally increased food intake in both control and obese VMH rats, the increases over baseline tended to be greater for the unoperated animals. This difference was statistically significant, however, only after injections of 400 mg/kg 2-DG ($p<0.01$). This was not the result of the slightly (but nonsignificantly) greater 4-hr baseline intake by obese rats, for control animals also had a significantly greater absolute food intake than lesioned rats following 300 and 400 mg/kg 2-DG ($p<0.05$).

DISCUSSION

The results of the present experiment demonstrate that while obese VMH-lesioned rats display 2-DG-induced increases in food intake, the response is delayed compared to that of unoperated animals. Control rats displayed a reliable decrease in the latency to begin eating and a marked increase in first hour intake to all doses of 2-DG of 100 mg/kg and greater. Obese rats, on the other hand, did not significantly increase their first hour food intake after any dose of 2-DG. We [9] previously reported a delayed response by obese lesioned rats to 350 mg/kg 2-DG, and a close examination of other experimental data [7,13] reveals a similar pattern of results. The failure of rats with VMH lesions to increase their first hour intake was not due to a slightly (but nonsignificantly) greater baseline intake (1.07 g vs 0.42 g), because the unoperated animals ate at least twice as much as the obese rats during the first hour at all doses of 2-DG of 200 mg/kg or greater. In fact, the first hour intakes by obese rats were significantly depressed following the adminiatration of 300 and 400 mg/kg 2-DG,

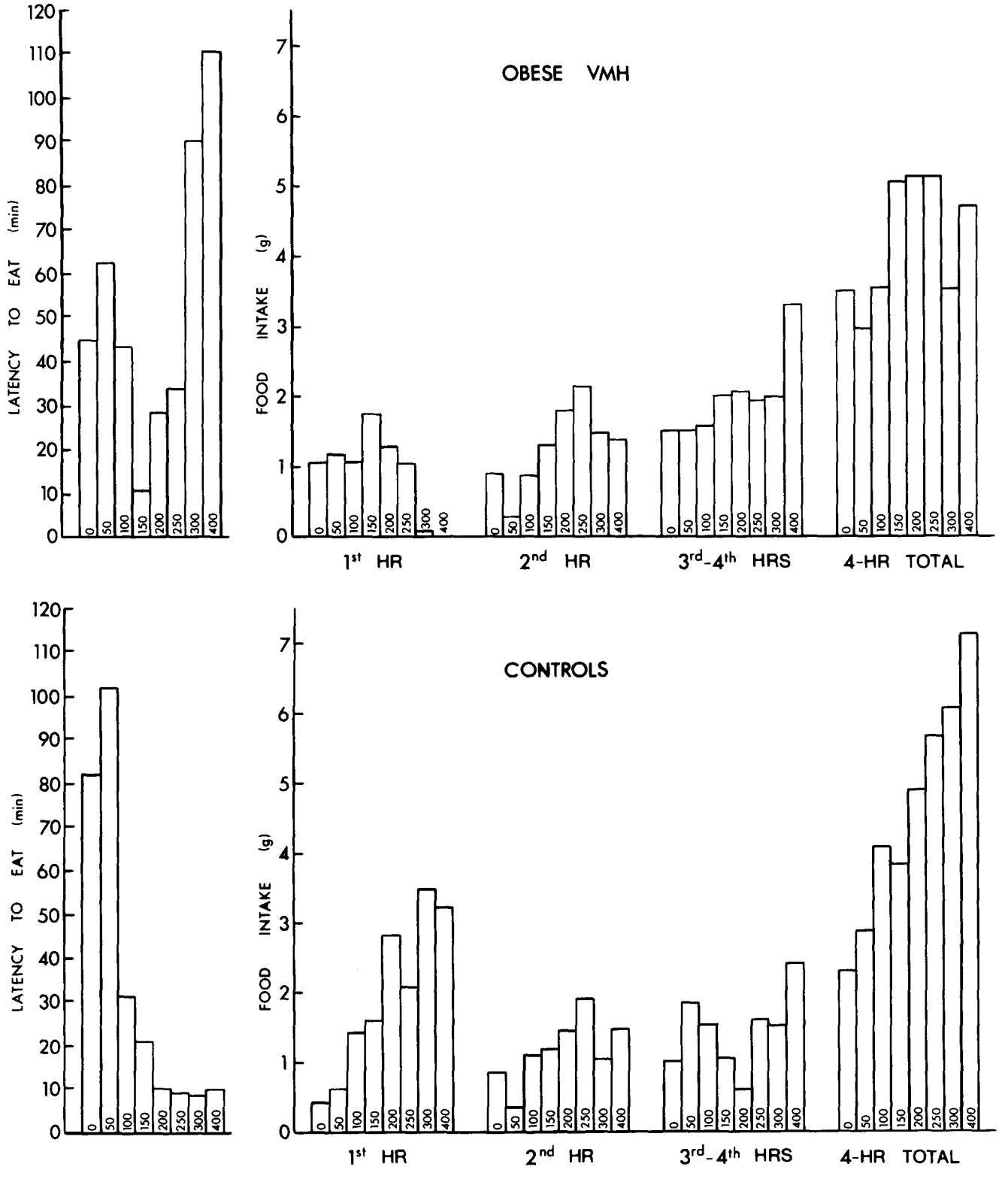

FIG. 1. Mean latencies to first feeding bout, and hourly and total 4-hr food intakes after injections of sterile water (0) or 2-DG (50–400 mg/kg) in unoperated control (bottom) and obese VMH-lesioned rats (top).

with a marked increase in the latency to begin eating. We previously reported a similar depression of first hour intake in lean VMH rats [9].

Houpt and Gold [7] suggested that doses of 2-DG should be determined according to metabolic body surface because injections administered on a mg/kg basis might represent an overdose in obese animals. This explanation, however, does not account for the fact that our obese rats failed to respond within the first hour to 100–250 mg/kg 2-DG, while our control animals displayed reliable first hour increases in feeding to all doses of 2-DG between 100 and 750 mg/kg ([9], present experiment).

Glucoprivation-induced eating can be produced by the administration of either 2-DG or insulin. Insulin promotes glucose uptake into nonneural tissues, thereby causing hypoglycemia. There is evidence that 2-DG affects eating by inhibiting glucose utilization in both brain [11] and hepatic [14] receptors, the latter being apparently critical for the first hour feeding response. Vagotomy delays the glucoprivation-induced feeding following injections of 2-DG much like VMH lesions, but has no effect on feeding after insulin administration [3, 12, 14], suggesting that the effects of insulin on eating may be mediated mainly by central receptors. In fact, there is some evidence that vagotomized rats are hyperresponsive to insulin [12], and we [9] have recently observed a similar supernormal feeding response to insulin in obese VMH-lesioned rats. Anand and Pillai [2] reported electrophysiological evidence that the VMH receives vagal afferents. A possible explanation for the present pattern of results (i.e., delayed response to low doses of 2-DG), therefore, is that VMH lesions may disrupt visceral afferents involved in the initial feeding response to 2-DG while leaving central glucoreceptors intact. The suppressed response to high doses of 2-DG may be the result of an additional lesion-induced super-sensitivity to the depressant effects of 2-DG (e.g., stupor and ataxia) [13].

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