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BRIEF COMMUNICATION

Effects of Chronic Diabetes on 2-Deoxy-D-Glucose Induced Feeding and Drinking[1]

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NANCE, D. M. AND R. A. GORSKI. *Effects of chronic diabetes on 2-deoxy-D-glucose induced feeding and drinking.* PHARMAC. BIOCHEM. BEHAV. 1(4) 483–485, 1973.–Chronic diabetes, induced by alloxan treatment, was found to totally attenuate the normal facilatory effects of 2-deoxy-D-glucose (2-DG) on feeding and drinking in rats. Following the administration of eight units of protamine zinc insulin, diabetic animals decreased their daily food and water intake. Since the daily food intake of insulin-treated diabetic rats was increased by 2-DG, it is hypothesized that 2-DG acts upon insulin-dependent receptors to produced feeding.

Diabetes 2-deoxy-D-glucose Feeding and drinking Alloxan Insulin

ACCORDING to Mayer's [4] glucostatic theory, insulin-dependent neurons, presumable in the ventromedial hypothalamus (VMH), regulate short-term satiety. Although it now appears that the VMH regulates long-term feeding behavior instead of short-term satiety [5], the fact that transport of glucose analogs into the VMH depends upon insulin [2], in general, supports Mayer's theory.

Intraperitoneal injections of 2-deoxy-D-glucose (2-DG), a glucose antimetabolite, has been shown to increase feeding, presumably by causing glucoprivation in central glucoreceptors [7]. Since in peripheral tissue the uptake of 2-DG, like glucose, is insulin dependent [9], Panksepp *et al.* [6] tested whether 2-DG induces feeding by blocking glucose utilization in insulin-sensitive neurons. They found that acute diabetes, induced by injections of mannoheptulose, failed to attenuate 2-DG increased feeding or the ability of intragastric glucose to inhibit feeding. However, Booth [1] found that chronic diabetes produced by streptozotocin injections completely attenuated the inhibition of feeding by intragastric glucose loads. Therefore, Experiment 1 tested whether chronic diabetes would also attenuate increased feeding in response to a 2-DG injection.

EXPERIMENT 1

Method

Six male and six female rats, weighing 367.8 and 290.5 g respectively, were used. Animals were from an inbred Sprague-Dawley strain raised in the UCLA animal colony. Animals were housed in individual stainless steel cages and maintained on ad lib powdered Purina Rat Chow and tap water. Lighting was provided 12 hr per day from 7 a.m.–7 p.m. Food and water intakes and body weights were recorded daily at 11:00 a.m. to the nearest 0.1 g or 1.0 ml.

Following a 24-hr fast, one-half of the animals (3 males and 3 females) were made chronically diabetic by a single 150 mg/kg IP injection of alloxan hydrate (CalBiochem). The other 6 animals served as controls. Within 24 hr, all alloxan treated animals showed urinary glucose levels in excess of 2.0% as determined by Lilly Tes-tape. Also, animals treated with alloxan gradually became hyperphagic and polydipsic, and within 2 weeks had stabilized their food and water intakes. Beginning two weeks after the alloxan treatment, additional measurement of food and water intakes were made at noon and 3:00 p.m., thus providing daily 1-, 4- and 24-hr measures of food and water intake.

All animals were given a single IP injection of 350 mg/kg 2-DG dissolved in distilled water at a volume of 1.0 ml/100 g body weight at 11:00 a.m. Effects of 2-DG on feeding were tested by comparing the food and water intakes 1, 4, and 24 hr immediately following the 2–DG injection with both the previous days' intake (pre) and the days' intake following the 2-DG injection (post). Statistical significance was tested by means of a two-tailed *t*-test [3].

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TABLE 1

CUMULATIVE MEAN DAILY FOOD (G) AND WATER (ML) INTAKES ±SE OF DIABETIC AND CONTROL RATS FOR 1, 4 and 24-HR PERIODS ON THE DAY BEFORE (PRE), DAY AFTER (POST) AND DAY OF ADMINISTRATION OF 2-DG

		Controls			Diabetic		
		1 hr	4 hr	24 hr	1 hr	4 hr	24 hr
Pre	Food	0.2 ±0.1	0.6 ±0.2	21.9 ± 2.2	2.0 ± 1.0	4.2 ± 1.2	37.3 ± 3.4
	Water	1.0 ±0.4	2.3 ±0.8	43.0 ± 3.2	9.2 ± 2.6	20.7 ± 4.3	179.2 ± 20.7
2-DG	Food	2.2† ±0.3	6.3‡ ±0.4	20.5 ± 1.1	1.2 ± 0.5	2.3‡ ± 0.6	31.8† ± 2.1
	Water	1.2 ±0.8	7.2‡ ±1.0	42.7 ± 1.7	6.2* ± 1.7	9.8‡ ± 2.2	131.5† ± 13.2
Post	Food	1.1 ±0.5	1.9 ±0.4	21.3 ± 2.2	2.2 ± 0.6	4.4 ± 1.0	37.3 ± 2.7
	Water	2.7 ±0.8	3.8 ±1.1	42.0 ± 2.9	11.2 ± 2.4	21.0 ± 4.9	171.7 ± 20.5

2-DG significantly different from both Pre and Postintakes *$p<0.05$ †$p<0.01$ ‡$p<0.002$

Results

As shown in Table 1, control animals showed a significant increase in food intake one hour following the injection of 2-DG, relative to the pre and postdays' intakes. The diabetic animals showed a significant depression in water intake in response to 2-DG administration during the first hour.

By 4 hours, 2-DG produced a significant increase in food and water intake in the control animals in contrast to the significant depression in food and water intake found with the diabetic group.

Food and water intakes were significantly depressed for the diabetic animals 24 hr after the 2-DG injection.

EXPERIMENT 2

Initial attempts to reinstate 2-DG-induced feeding in diabetic rats by simultaneously administering insulin were without success. For example, a single injection of 8–16 units of an intermediate acting form of insulin (isophane) did not affect the food and water intake of diabetic rats, nor attenuate the depression in food intake induced by 2-DG (Experiment 1). However, 8 units of a long-acting form of insulin (protamine zinc) significantly decreased the food intake of diabetic hyperphagic rats, although primarily at 24 hr. Thus, Experiment 2 compared the effects of 8 units protamine zinc insulin given alone and in combination with 350 mg/kg 2-DG on the 24 hr food and water intake of chronic diabetic rats.

Method

Six rats (3 females and 3 males) were made chronically diabetic and maintained according to the methods of Experiment 1. Food and water intakes were measured daily at 11:00 a.m. The effects of insulin (8 units of protamine zinc insulin, injected SC at the nape of the neck) alone and in combination with 2-DG (350 mg/kg) on food and water intake was tested by comparing the injection-days intakes with the pre and postinjection days intakes. The two test days were separated by 1 week. Results were analyzed by a *t*-test for paired observations [3].

Results

The combined injection of insulin and 2-DG resulted in the death of two animals, one of each sex. Thus, the data in Table 2 represents the mean daily food and water intakes of the surviving four diabetic rats (Table 2).

As shown in Table 2, insulin alone produces a significant decrease in both food and water intakes on the day of injection. When compared to the preinjection days intakes, food intake was back to the hyperphagic level 24 hr later, although water intake was still nonsignificantly depressed.

Insulin + 2-DG resulted in a significant decrease in water intake, as with insulin-alone; however, food intake was not significantly different from the pre and postinjection days (Table 2).

When comparison is made between the insulin-alone and insulin + 2-DG conditions, animals eat significantly more in

response to the 2-DG. Although not statistically significant, the animals also tend to drink more (Table 2).

TABLE 2

DAILY FOOD AND WATER INTAKES (±SE) OF DIABETIC RATS GIVEN 8 UNITS OF PROTAMINE ZINC INSULIN ALONE OR SIMULTANEOUSLY WITH 350 MG/KG 2-DG

		Insulin Alone	Insulin + 2-DG
Pre	Food	35.9 ± 2.3	35.8 ± 2.1
	Water	155.8 ± 21.0	174.3 ± 18.2
Injection	Food	23.9 ± 2.2†	31.0 ± 2.9*
	Water	78.3 ± 12.2†	98.0 ± 19.9
Post	Food	34.3 ± 2.1	34.0 ± 2.0
	Water	108.8 ± 16.5	134.3 ± 34.1

t-test for paired observations
*insulin vs insulin + 2-DG, $p \leq 0.05$
†Pre vs test, $p \leq 0.05$

DISCUSSION

Chronic diabetes induced by alloxan treatment completely attenuated the effects of 2-DG on feeding and drinking. While the control animals showed the expected increase in food and water intakes in response to 2-DG administration, surprisingly, the diabetic animals actually decreased their food and water intakes. Although we presently lack an explanation for the 2-DG depression in food and water intakes of diabetic rats, the failure of 2-DG to facilitate food and water intakes in diabetic rats is consistent with Booth's [1] explanation for the failure of chronic diabetic rats to show glucose-induced satiey. He concluded that "...either an insulin secretory reponse to glucose absorption and circulation or a history of insulin secretion (or both) is necessary for glucose to produce a major part of its postingestive inhibitory effect on feeding." The present authors suggest that a similar conclusion can be made regarding the behavioral response of chronic diabetic rats to a glucose analog such as 2-DG. Suggestive evidence for the intimate relationship between a behavioral response to 2-DG and insulin is the fact that hypothalamic structures necessary for 2-DG to induce feeding and drinking are also essential for insulin to induce eating and drinking [8]. Thus, the opposite behavioral effects of 2-DG on eating and drinking found with chronic diabetic rats in comparison to control animals, as shown in the present experiment, has its counterpart regarding the response of diabetic and control animals to insulin injections. As shown in Experiment 2, 8 units of protamine zinc insulin produced a significant decrease in the food and water intakes of diabetic rats, which is in contrast to the insulin induced increase in food and water intakes found for nondiabetic rats [8].

The demonstration that diabetic animals eat more in response to the administration of insulin + 2-DG than to the administration of insulin alone indicates that the action of insulin can determine the behavioral response of an animal to an injection of 2-DG. Thus, we suggest that 2-DG normally acts upon insulin-dependent receptors, presumably in the brain, to produce a facilitation on eating and drinking behavior. However, an alternate explanation of the data (Experiment 2) is that 2-DG simply may have sustained the diabetic state, and hence depressed the appetite-reducing effects of insulin. Consistent with this alternative, 2-DG can inhibit the appetite suppressing effects of intragastric loads of D-glucose (Panksepp and Nance, unpublished data) results which are the same as found with chronic diabetes [1]. Yet the differences between diabetic and nondiabetic animals remain in that the effects of insulin and 2-DG are additive in normal animals. We have found (unpublished observation) that, unlike diabetic animals (Experiment 2), normal rats overeat more in response to a combination of 2-DG and insulin than to either substance alone.

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