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Increased Carbohydrate Consumption by Rats as a Function of 2-Deoxy-D-Glucose Administration[1]

ROBIN B. KANAREK, ROBIN MARKS-KAUFMAN,[2] ROBIN RUTHAZER AND LISA GUALTIERI

Department of Psychology, Tufts University, Medford, MA 02155

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KANAREK, R. B., R. MARKS-KAUFMAN, R. RUTHAZER AND L. GUALTIERI. *Increased carbohydrate consumption by rats as a function of 2-deoxy-D-glucose administration.* PHARMACOL BIOCHEM BEHAV **18**(1) 47–50, 1983.—Dietary self-selection was examined following the administration of the glucoprivic agent, 2-deoxy-D-glucose (2-DG),in adult male rats given access to separate sources of the three macronutrients, protein, fat and carbohydrate. All animals received injections (IP) of saline, 250, 500 and 750 mg/kg 2-DG with nutrient intakes measured at 2, 4, 6 and 24 hrs following injections. Animals consumed significantly more carbohydrate at 4, 6 and 24 hrs after injections of 500 and 750 mg/kg 2-DG than after saline injections. In contrast, fat intake was significantly suppressed by all three doses of 2-DG at 2 hr, by 250 and 750 mg/kg 2-DG at 4 and 6 hrs, and by 750 mg/kg 2-DG at 24 hr after injections. Protein intake was significantly decreased by all three doses of 2-DG at 2 hr after injections. As a result of the increase in carbohydrate intake and complimentary decrease in fat intake following 2-DG injections, total caloric intake of animals given the self-selection regime was not modified as a function of drug administration. In comparison, rats given a single nutritionally complete diet (ground Purina Laboratory Chow) consumed significantly more calories following 2-DG administration than following saline injections. The ability of animals to make appropriate modifications in nutrient selection following regulatory challenges is discussed.

Diet selection   Carbohydrate   Fat   Protein   2-Deoxy-D-glucose

MOST experiments examining the regulation of food intake in experimental animals employ single nutritionally complete diets. While experiments providing animals with a single diet allow conclusions to be drawn about the effects of various manipulations on overall energy intake, they provide no information about the effects of these manipulations on nutrient selection. Recent studies allowing animals access to separate sources of the three macronutrients, protein, fat and carbohydrate have revealed that experimental manipulations which produce similar changes in overall caloric intake can result in different patterns of nutrient selection [4, 5, 7, 8, 10]. For example, destruction of the ventromedial hypothalamus (VMH) and administration of exogenous insulin both lead to elevations in total caloric intake. These two manipulations, however, produce very different patterns of nutrient choice [4,5]. Destruction of the VMH is associated with an increase in either fat or carbohydrate intake and a decrease in protein intake [4], while insulin administration is associated with a selective increase in carbohydrate intake and decrease in fat intake [5]. Experiments employing dietary self-selection regimes thus permit more detailed insights into the control of food intake.

Administration of 2-deoxy-D-glucose (2-DG) stimulates food intake in a variety of species [3, 9, 12, 14, 15, 16]. The effects of 2-DG on food intake have been related to the decreases in cellular glucose utilization which occur in brain and other tissues following the administration of the drug. 2-DG decreases cellular glucose utilization by competitively inhibiting the phosphohexoseisomerase step of glycolysis [2,11]. The selective effect of 2-DG on carbohydrate metabolism suggested that animals might specifically modify carbohydrate intake following administration of the drug. In the present experiment, this suggestion was tested by examining the effects of 2-DG on nutrient choice in rats given separate access to the three macronutrients.

METHOD

Animals and Diets

Fourteen male Sprague-Dawley rats (CD outbred, Charles River, Wilmington MA), weighing an average of 350 g at the beginning of the experiment were used. Animals were housed individually in standard laboratory cages in a temperature-controlled room (21±1°C) maintained on a 12–12 hr light-dark cycle (lights on: 0800–2000 hr).

Animals were divided into two groups matched on the basis of body weight. Animals in the first group (N=6) received Ground Purina Rodent Chow No. 5001 (caloric density = 3.6 kcal/g). Animals in the second group (N=8) had access to three separate dietary rations: a protein ration, a

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[2]Present address: Institute of Human Nutrition, Columbia University, New York, NY.

carbohydrate ration and a fat ration. The protein ration (caloric density=3.8 kcal/g) was composed of 960 g casein (ICN Pharmaceuticals, Cleveland, OH), 40 g minerals (U.S.P. XIV Salt Mixture, ICN Pharmaceuticals), and 22 g vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals). The carbohydrate ration (caloric density=3.8 kcal/g) was composed of 580 g corn starch (Teklad Test Labs, Madison, WI), 280 g dextrin (Teklad Test Labs), 100 g commercial-grade sucrose, 40 g minerals (U.S.P. XIV Salt Mixture) and 22 g vitamins (Vitamin Diet Fortification Mixture). The fat ration (caloric density=7.9 kcal/g) was composed of 912 g hydrogenated fat (Crisco), 48 g safflower oil (Hollywood Health Foods, Los Angeles, CA), 90 g minerals (U.S.P. XIV Salt Mixture) and 49.5 g vitamins (Vitamin Diet Fortification Mixture). Vitamins and minerals were added to the components so that the three dietary rations contained equal amounts of these micronutrients on a per kilocalorie basis. Purina Chow and the protein and carbohydrate rations were provided in Wahmann (Timonium, MD) spill-proof LC 306-A food cups. The fat ration was provided in 75 ml glass cups. All animals received continuous access to water throughout the experiment.

Following a three-week period of adaptation to the dietary regimes, animals were tested for 2-DG induced feeding. On test days, animals received intraperitoneal injections of either 2-DG or physiological saline at 0900 hr. 2-deoxy-D-glucose (Sigma Chemical Corp., St. Louis, MO) was kept frozen, and the required amount mixed with saline to provide a 10% solution immediately preceding its use. Saline and 2-DG were administered according to the following schedule: saline, 500 mg/kg 2-DG, saline, 250 mg/kg 2-DG, saline, 750 mg/kg 2-DG. At least seven days intervened between 2-DG injections. On test days, nutrient intakes were measured to 0.1 g at 2, 4, 6 and 24 hr post-injection.

Data were analyzed using one-way analyses of variance followed by a posteriori multiple comparison tests of within group means using Scheffe's method [13]. As there were no differences in intakes following the three saline injections, mean data for saline injections were used in data analyses.

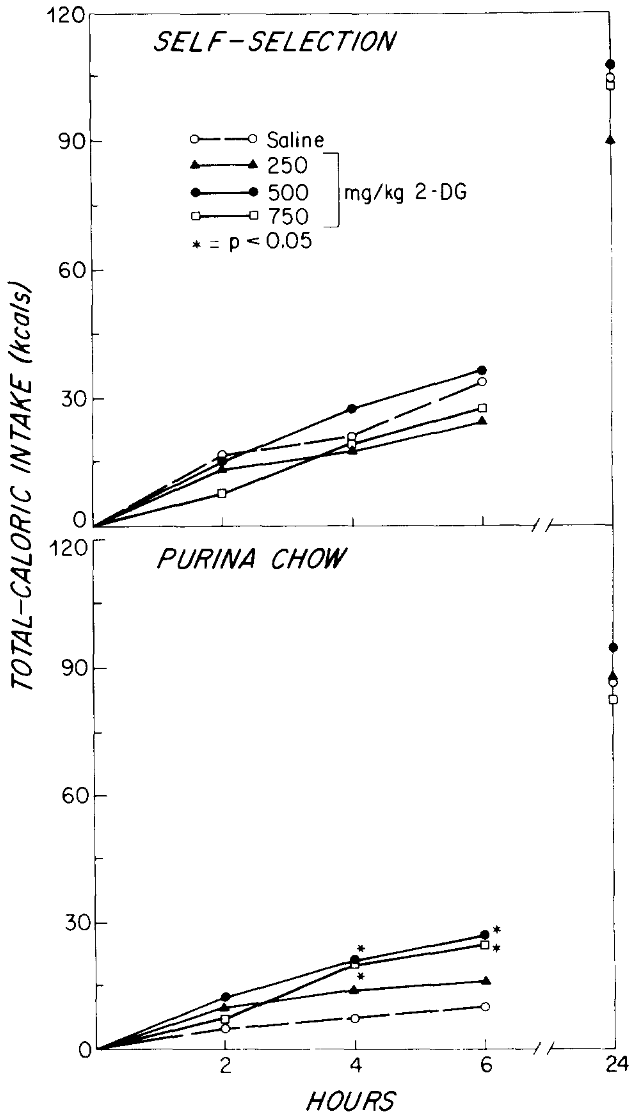

FIG. 1. Cumulative total caloric intakes at 2, 4, 6 and 24 hours following injections of 2-DG or saline for animals maintained on either a dietary self-selection regime (top) or ground Purina Chow (bottom). *Significantly different ($p<0.05$) from saline injections.

RESULTS

Purina Chow Intake

Purina Chow intake increased as a function of 2-DG administration (Fig. 1, bottom). Intake of Purina Chow was significantly greater 4 and 6 hours following injections of 500 and 750 mg/kg 2-DG than following saline injections. No differences in Purina Chow intake were observed as a function of drug administration at 24 hours post-injection.

Individual Macronutrient Intakes for Animals Given the Self-selection Regime

Carbohydrate intake increased as a function of drug administration. (Fig. 2, top). Carbohydrate intake was significantly greater at 4, 6 and 24 hours after injections of 500 and 750 mg/kg 2-DG than after saline injections.

In contrast to carbohydrate intake, fat intake decreased as a function of the administration of the 2-DG (Fig. 2, bottom). All three doses of the drug led to significant reductions in fat intake at 2-hours post-injection. Cumulative fat intake remained significantly suppressed at 4 and 6 hours after injection of 250 mg/kg 2-DG and at 4, 6 and 24 hours after injection of 750 mg/kg 2-DG.

Animals consumed significantly less protein at 2 hours following injections of 500 and 750 mg/kg 2-DG than after saline injections (Fig. 2, middle). No other significant differences were observed in protein intake as a function of 2-DG administration.

As a result of the increase in carbohydrate intake and decreases in fat and protein intakes, no differences in total caloric intake were found as a function of 2-DG administration for animals maintained on the self-selection regime (Fig. 1, top).

DISCUSSION

Rats given access to separate sources of the three macronutrients increased carbohydrate intake and decreased fat intake following the administration of the glucoprivic agent,

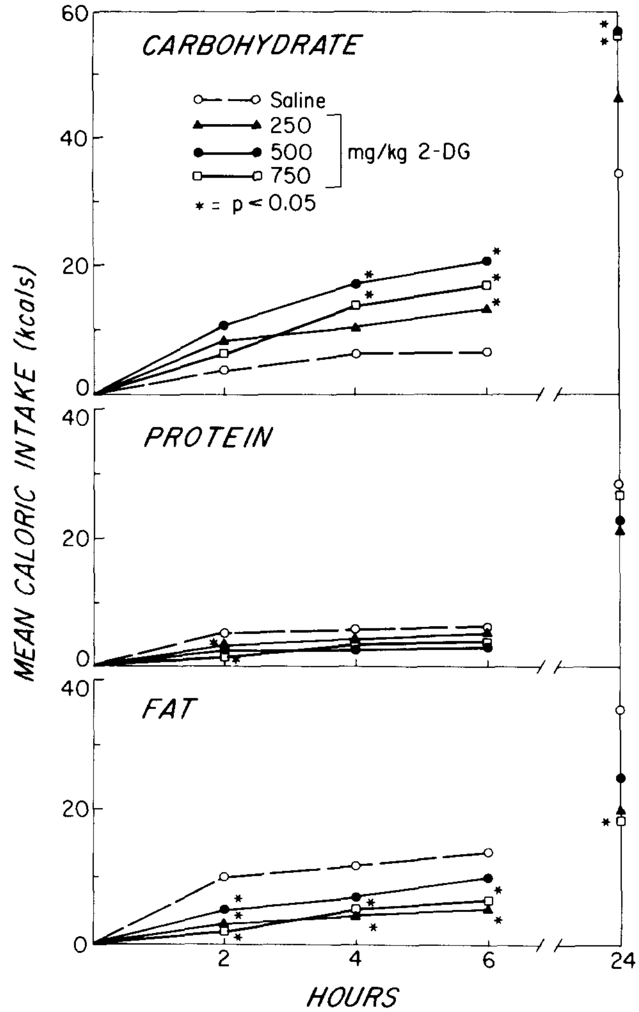

FIG. 2. Cumulative caloric intakes of carbohydrate, protein and fat at 2, 4, 6 and 24 hours following injections of 2-DG or saline for animals maintained on a dietary self-selection regime. *Significantly different ($p<0.05$) from saline injections.

2-deoxy-D-glucose. As a consequence of these alterations in nutrient choice, no differences in total caloric intake were observed as a function of 2-DG administration for rats maintained on the dietary self-selection regime. In contrast, rats given a single nutritionally complete diet significantly elevated caloric intake following 2-DG administration.

The pattern of nutrient choice observed after 2-DG injections was similar to that previously observed following the administration of the other classic glucoprivic agent, insulin [5]. When maintained on the same self-selection regime as that used in the present experiment, rats given daily injections of NPH insulin consumed significantly more carbohydrate and less fat than non-injected animals. As in the present experiment, only minimal changes in protein intake were observed as a function of insulin administration.

Variations in diet palatability among the three dietary rations partially may account for the selective effects of 2-DG and insulin on nutrient intake. Previous experiments have shown that rats maintained on unpalatable diets actually decrease food intake in response to the administration of glucoprivic agents [1, 6, 17]. It might be hypothesized, therefore, that the fat component of the self-selection regime was less palatable than the carbohydrate component and that the relative palatability of these two components may have led to the observed pattern of nutrient intake. However, this seems unlikely as prior to 2-DG injections animals did not appear to have a preference for either fat or carbohydrate consuming 35% of their daily caloric intake as fat and 33% as carbohydrate.

More likely, the alterations found in dietary self-selection patterns following the administration of both 2-DG and insulin reflect the animal's attempt to restore homeostatic balance to its internal milieu. Both 2-DG and insulin lead to decreases in glucose availability at glucoreceptors within the central nervous system (CNS). Feeding during glucoprivation provides exogenous glucose and thereby increases glucose availability to the CNS. Among the three macronutrients, it may be presumed that ingestion of carbohydrate following 2-DG or insulin administration would result in the most rapid elevations in glucose availability.

The increase in food intake typically observed following 2-DG administration, thus may be a consequence of a specific need for carbohydrate rather than a general requirement for energy. The fact that total caloric intake was not elevated for animals maintained on the self-selection regime supports this conclusion. In contrast, rats not allowed to self-select displayed substantial increases in energy intake as a function of 2-DG administration.

The present results, as well as those of previous experiments [4, 5, 7, 8, 10], clearly indicate the advantages of employing dietary self-selection regimes in research on feeding behavior. Experimental procedures which produce similar modifications in energy intake can lead to very different patterns of dietary self-selection. The patterns of nutrient choice observed following different pharmacological and physiological manipulations may help to elucidate the complex mechanisms underlying the control of energy and nutrient intake.

REFERENCES

1. Brandes, J. S. Insulin induced overeating in the rat. *Physiol. Behav.* **18:** 1095–1102, 1977.
2. Brown, J. Effects of 2-deoxyglucose on carbohydrate metabolism. Review of the literature and studies in the rat. *Metabolism* **11:** 1098–1112, 1962.
3. Houpt, T. R. and H. E. Hance. Stimulation of food intake in the rabbit and rat by inhibition of glucose metabolism with 2-deoxy-d-glucose. *J. comp. physiol. Psychol.* **76:** 395–400, 1971.
4. Kanarek, R. B., P. G. Feldman and C. Hanes. Pattern of dietary self-selection in VMH-lesioned rats. *Physiol. Behav.* **27:** 337–343, 1981.
5. Kanarek, R. B., R. Marks-Kaufman and B. J. Lipeles. Increased carbohydrate intake as a function of insulin administration in rats. *Physiol. Behav.* **25:** 779–782, 1980.
6. Kanarek, R. B. and J. Mayer. 2-deoxy-D-glucose induced feeding: relation to diet palatability. *Pharmac. Biochem. Behav.* **8:** 615–617, 1978.
7. Marks-Kaufman, R. and R. B. Kanarek. Modifications of nutrient selection induced by naloxone in rats. *Psychopharmacology* **74:** 321–324, 1981.
8. Marks-Kaufman, R. and R. B. Kanarek. Morphine selectively influences macronutrient intake in the rat. *Pharmac. Biochem. Behav.* **12:** 427–430, 1980.

9. Novin, D., D. A. VanderWeele and M. Rezek. Infusion of 2-deoxy-D-glucose into the hepatic-portal system causes eating: evidence for peripheral glucoreceptors. *Science* **181:** 858–860, 1973.
10. Orthen-Gambill, N. and R. B. Kanarek. Differential effects of amphetamine and fenfluramine on dietary self-selection in rats. *Pharmac. Biochem. Behav.* **16:** 303–309, 1982.
11. Rezek, R. and E. A. Kroeger. Glucose antimetabolites and hunger. *J. Nutr.* **106:** 641–645, 1976.
12. Rowland, N. Glucoregulatory feeding in cats. *Physiol. Behav.* **26:** 901–903, 1981.
13. Scheffé, H. *The Analysis of Variance.* New York: Wiley, 1959.
14. 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.
15. Smith, G. P., J. Gibbs, A. J. Strohmayer and P. E. Stokes. Threshold doses of 2-deoxy-D-glucose for hyperglycemia and feeding in rats and monkeys. *Am. J. Physiol.* **222:** 77–81, 1972.
16. Thompson, D. A. and R. G. Campbell. Hunger in humans induced by 2-deoxy-D-glucose: glucoprivic control of taste preference and food intake. *Science* **198:** 1065–1067, 1977.
17. Vasselli, J. R. and A. Sclafani. Hyperreactivity to aversive diets in rats produced by injections of insulin or Tolbutamide, but not by food deprivation. *Physiol. Behav.* **23:** 557–567, 1979.