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Feeding induced by intracranial and intravenously administered 2-deoxy-D-glucose

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Intracranial injections of 2-deoxy-D-glucose (2DG) given to free-feeding rabbits induced eating when injected in sites located within the lateral hypothalamic area but not within the ventromedial nucleus. However, greater increases were obtained by the administration of systemic injections of the same agent in greater amounts. Electrolytic lesions of the lateral hypothalamic area did not suppress the facilitatory effects of systemic injections of 2DG on eating in these animals. These results suggest that peripheral glucose receptors may be more important than central glucoreceptors in controlling hunger.

The glucostatic theory of hunger proposes that food intake is primarily controlled by neurons, most likely in the ventromedial nucleus of the hypothalamus (VMH), that meter glucose utilization (Mayer, 1955; Anand, 1967). There is much support for this hypothesis from studies of goldthioglucose-induced hyperphagia associated with VMH damage (Marshall, Barrnett, & Mayer, 1955; Mayer & Marshall, 1956) and from neurophysiological studies showing activation and suppression of VMH and lateral hypothalamic cells, respectively, following glucose infusion (Anand, 1967). However, other results from systemic glucose infusion (Janowitz & Grossman, 1949) or direct VMH infusions (Epstein, 1960) do not support the glucostatic hypothesis. Panksepp and Nance (1972) have recently shown that glucose infusions, while not affecting food intake immediately, did reduce the amount eaten over a 19-h period following the direct application of glucose to the VMH.

The compound 2-deoxy-D-glucose (2DG), which is known to inhibit glucose utilization, has been used in several studies to investigate the glucostatic hypothesis. Smith and Epstein (1969), based upon the results of 2DG infusions in rats and monkeys and comparing it to insulin-induced eating, concluded that it was central rather than peripheral glucoprivation that was the stimulus for eating. Intraventricular infusions, but not intrahypothalamic injections, by Miselis and Epstein (1970) supported this contention.

Balagura and Kanner (1971), using intrahypothalamic injections of minute quantities of 2DG, presented evidence supporting a central locus for glucoreceptors, but found that the lateral hypothalamic area (LHA) was the locus for 2DG-induced eating in free-feeding rats, while the VMH placements did not cause eating. In contrast, a study by Novin, VanderWeele, and Rezek (1973) demonstrated powerful effects of 2DG on eating when injected into the hepatic-portal system compared with similar injections into the jugular vein. This result suggests the existence of peripheral glucoreceptors important in hunger.

The present study compared the effects of intrahypothalamic and intravenous injections of 2DG. In addition, it explored whether lesions made bilaterally in areas responding to inctracranial 2DG would affect the eating produced by intravenous 2DG infusions.

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EXPERIMENT I

The purpose of this experiment was to locate areas of the rabbit's hypothalamus in which intracranial injections of 2DG induced eating. The LHA was explored in view of the positive results obtained in this area of the rat by Balagura and Kanner (1971), while the VMH appeared to be a logical target for exploration because of the widespread belief that this nucleus contained glucose-sensitive cells controlling satiety.

Method

Subjects. The Ss were 20 young female New Zealand rabbits of initial body weight ranging from 2.4 to 3.0 kg. Animals were individually caged and given free access to a diet consisting of Diamond Brand All Purpose Rabbit Pellets and tap water. Consumption of food and water was measured daily. The rabbits were maintained in a 24-h-lighted room with constant temperature set at 21° C.

Surgery. Intracranial guiding cannulas of 25 mm length were manufactured from 21-ga stainless steel tubing. These cannulas allowed the insertion of 28-ga cannulas of different lengths, so that hypothalamic injections could be made at 1-mm intervals in depth from the end of the guiding cannulas. When the outer cannulas were not in use they were kept from clogging by the insertion of insect pins cut to the appropriate length and slightly bent along their shafts. The outer cannulas were individually guided to the target areas while the animal was under Nembutal anesthesia (30 mg/kg). A stereotaxis apparatus and the atlas of Sawyer, Everett, and Green (1954) were used. The tips of the guiding cannulas were aimed at the region just above the VMH or just above the perifornical region of the LHA.

Procedure. After being allowed a recovery period of at least 2 weeks, the rabbits were introduced to a series of experimental

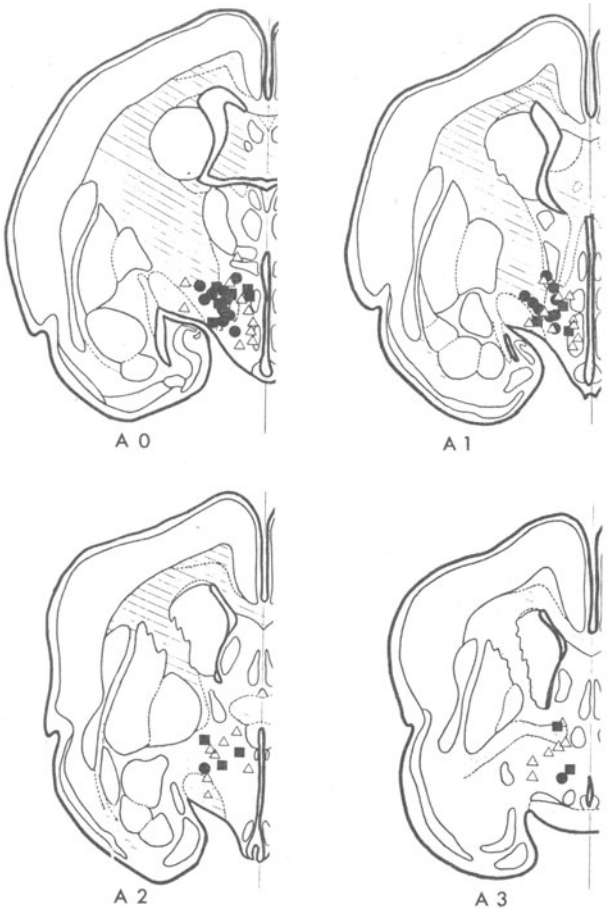

Fig. 1. Schematic representation of loci of intracranial injections. Sites in which 2DG produced increases in eating over control levels are represented by filled symbols (squares = increases of 25%-50%; circles = increases of 51% or over). Sites in which no increases in eating were observed are presented by open triangles.

trials in which 1-microliter injections of 5% solution of 2-deoxy-D-glucose (Cal Biochemicals Co.), 5% solution of glucose, or physiological saline were administered. Injections were given in a random order, and, as an added control, mock injections were also used. Solutions of 2DG were freshly mixed, and all injections were done using aseptic procedures with a minimum infusion duration of 30 sec. At least 3 days were allowed between successive administrations of the experimental drug. Injections were done with the animals in their home cages using a sufficiently long piece of thin polyethylene tubing to connect the injecting cannula and a 10-microliter syringe. This procedure was instituted to minimize disturbance to the rabbits. Food and water containers were measured daily, just prior to and 1 h after the onset of the injection, to determine consumption. Two injections of each solution were done at 1-mm intervals from 1-3 mm from the tip of the guiding cannulas.

Histology. Placement of injections was verified by histological analysis. Under Nembutal anesthesia, rabbits were perfused via a carotid artery with 0.9% NaCl, followed by a 10% Formalin solution, and 80-micron-thick slides were prepared by the freezing technique, which were subsequently mounted and stained with thionin.

Results

After identifying the anatomical placement of injections, the mean was calculated for each locus and classified into one of the three categories: (1) both placements in the LHA, (2) both placements in the VMH, (3) asymmetrical or outside of the hypothalamus.

Figure 1 illustrates the anatomical distribution of symmetrical placements and the effect of 2DG at the inferred site of action. The fact that no significant loci were observed in the VMH suggests that, at least under ad lib conditions, glucoprivation of neurons of this nucleus was not sufficient to induce increases in food consumption. With the exception of a single positive result obtained in the lateral preoptic area, all loci that facilitated eating were located within the bounds of the LHA or the medial forebrain bundle.

Figure 2 depicts the results obtained in the LHA injections. Two-tailed t tests for correlated data revealed that there was a significant difference ($p < .05$) between 2DG and mock-injection scores. Similar comparisons between glucose- or saline- and mock-injection scores did not reach statistical significance. Analyses of results of 2DG, glucose, or saline injections done in the VMH or in nonsymmetrical or nonhypothalamic placements were also not significant. Water-to-food-consumption ratios tended to remain constant, which indicates that drinking probably was a secondary response to eating in this experiment.

EXPERIMENT II

Having demonstrated the presence of hypothalamic sites in the rabbit in which the administration of minute quantities of 2DG produced eating, this experiment was done to compare this response to the one to be obtained from a more general administration of the drug. Also,

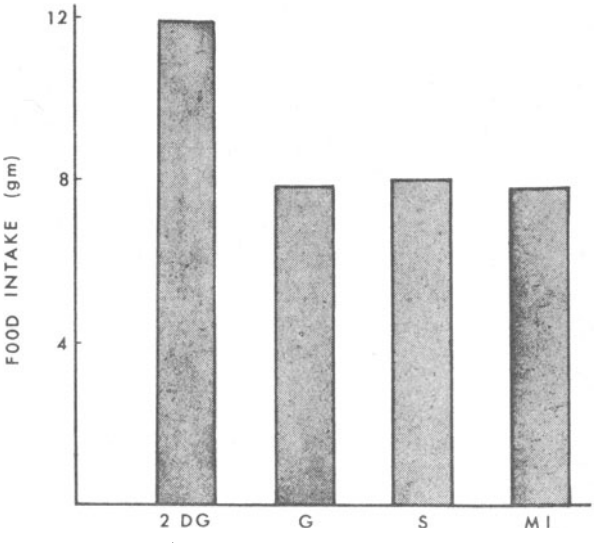

Fig. 2. Mean food consumption for the first hour after LHA injections of 2-deoxy-D-glucose (2DG), glucose (G), and normal saline (S), or after control mock injections (MI).

lesions were done in the LHA of some animals to determine whether this procedure abolished the eating induced by peripheral injections of 2DG.

Method

Subjects. Thirty-two female New Zealand rabbits of initial body weight ranging from 1.9 to 4.8 kg served as Ss. They were individually housed in plastic cages. Attached to each cage was a metal feeding box equipped with a photocell, whose beam, when interrupted by an animal's head, activated an event recorder. All cages, except for those of eight rabbits, had wire floors. Animals had free access to the same diet used in Experiment I, and food and water consumption was measured daily. Cages were kept in a constant-temperature room (21°C) with lights adjusted to a 12-h cycle (on at 0600).

Surgery. Guiding cannulas similar to the ones described in Experiment I were bilaterally implanted in all animals. All implants were aimed at the region just dorsal to the perifornical area of the LHA. The surgical procedure was essentially the same as that described for the previous experiment, with the additional implantation of an intrajugular polyethelene catheter. The construction of this cannula has been described elsewhere (Novin, VanderWeele, & Rezek, in press). It was chronically implanted by inserting no more than 60 mm of the tip of the cannula a few millimeters below the bifurcation of the right jugular vein. In the last six preparations, the insertion was done in the lateral branch above the bifurcation; this procedure seemed to be less traumatic for the animal and provided a faster recovery. The external end of the cannula was cemented to the same assembly that served as anchor for the intracranial cannulas. A volume of 0.5 ml of a solution of 100 units/ml of sodium heparin (Riker Laboratories) was injected daily through these cannulas. Despite this precaution, some cannulas failed before animals reached the proper end of the experiment.

Procedure. After a recovery period of at least 2 weeks, a series of two systemic injections of 2DG (5% solution, 150 mg/kg) was initiated with its saline controls. Dose was adjusted by varying the volume administered to an animal, and infusion rate was also varied in an effort to make each infusion last 6 min. Jugular injections were done with the animals in their cages in a way analogous to the procedure described in Experiment I for intracranial injections. Food and water consumption was measured daily and just prior to the administration of an injection. Hourly measurements were taken for the 3 h that followed the injection. In this experiment, latency was measured, from the beginning of the injection to the initiation of the first meal, from the printed record of the event recorder. A bout of eating was defined to be a meal if the animal stayed in the feeding box for at least 1 min and ate at least 1 g of food.

Once the series of four systemic injections was completed, animals were started in a series of intracranial injections. The procedure used in the previous experiment was used, with the only differences being that consumption was measured hourly for a period of 3 h and that latency measurements were also obtained.

Ten animals whose jugular vein cannulas were still intact after the completion of the intracranial series of injections were selected for the next experimental manipulation. Electrolytic lesions were made in the sites that gave the best response to intracranial 2DG by passing an anodal current of 2.5 mA for 20-30 sec through thin Teflon-coated, stainless steel wires bared 1 mm at the tip. They were inserted into the desired sites through the chronic intracranial cannulas. In three of the animals in which this lesioning procedure did not have any effect on the daily food consumption, the lesion was redone lowering a wire 1 mm longer than the one used to produce the first lesion. After the animal's daily food consumption had stabilized, the animals were retested with peripheral infusions of 2DG and isotonic saline. Four animals that were not lesioned but that still had functional jugular cannulas were also retested with peripheral 2DG.

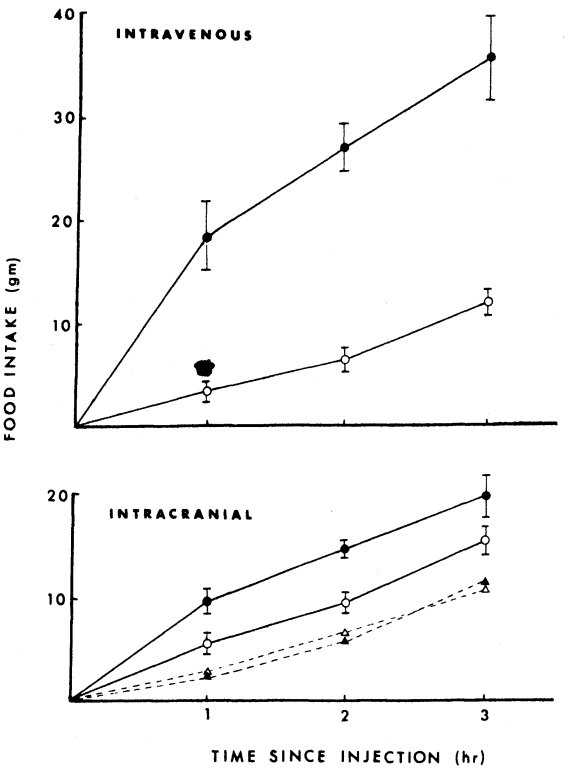

Fig. 3. Cumulative food consumption after intravenous (top graph) and intracranial (bottom graph) administrations of 2DG and normal saline. In the bottom graph, circles represent scores of LHA injections, and triangles scores of nonhypothalamic or nonsymmetrical placements, solid symbols are 2DG, and open symbols are saline in both top and bottom graphs. Bars above and below points indicate the standard error of the mean.

Histology. At the end of the experiment, animals were sacrificed and histology done on their brains for anatomical verification of placements of lesions or injection sites. The procedure described in Experiment I was used.

Results

Intrajugular administration of 2DG produced a sixfold increase of food intake in the first hour and a threefold increase in the cumulative total of the 3 h following the injection, as illustrated in Fig. 3. t tests for correlated data revealed that both the first and the third hour results were statistically significant at the .01 level. Mean latency to first meal after 2DG was 9.3 min, after saline it was 51.5 min. This difference was also statistically significant ($p < .01$).

Intracranial scores were analyzed as described in Experiment I. Scores were grouped into two categories: (1) implants in the LHA, and (2) implants clearly outside of the LHA or nonsymmetrical. Mean scores of food intake for these two groups are presented in Fig. 3. In the case of the LHA injections, despite an almost twofold increase over the first hour and a one-third gain over the 3-h period, the differences were not statistically significant. Latency differences of the LHA group were also not statistically significant. Daily consumption

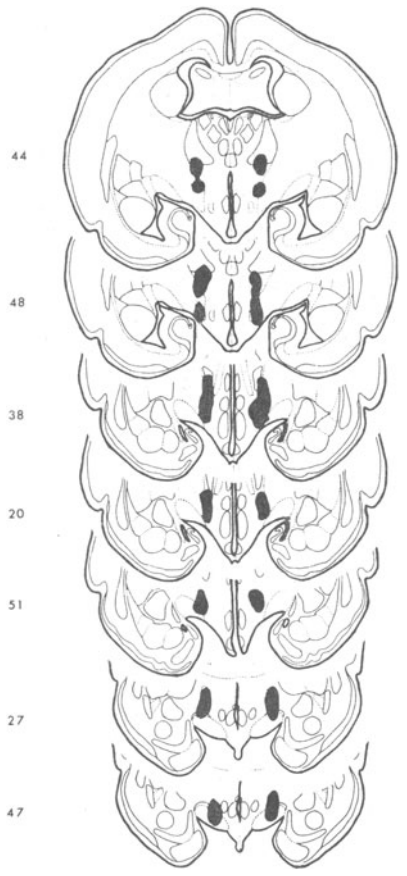

Fig. 4. Reconstruction of the extent of lesions produced in seven rabbits that were retested with systemic injections of 2DG.

scores after 2DG injections, either systemical or intracranial, were not significantly different from scores obtained in control days. Water intake, as in Experiment I, tended to follow food intake.

Data were collected from 7 of the 10 LHA-lesioned animals. A reconstruction of the extent of the lesions is presented in Fig. 4. Food-consumption scores in response to the initial and last series of systemic injections for these 7 animals and their 4 controls are presented in Fig. 5. An analysis of variance for one between-Ss variable (lesion/no lesion) and two within-Ss variables (initial/last series; 2DG/saline) revealed that only the main effect of the 2DG/saline comparison was statistically significant ($p < .01$).

The lesions centered in the lateral hypothalamic area, which in most cases produced a temporary hypophagia lasting 2-14 days, did not alter the effect systemic administration of 2DG had on eating. Food intake in response to IV 2DG was as great following as it was before the lesion.

DISCUSSION

The comparison of the effectiveness of the two routes of administration showed that the more general intravenous injections of 2DG produced a far greater increase in eating than injections given directly into the LHA. In fact, intracranial administration of 2DG in the second experiment, in contrast to the first, failed to show a statistically significant difference in the ability to elicit eating compared to saline. It is possible that this failure to achieve statistical significance despite the greater eating that was shown following 2DG compared to saline could be due to the smaller ratio of positive-to-negative-responding stimulation sites. There was a larger number of implants in the second experiment compared to the first in a more anterior plane where the number of positive responding sites is smaller. It might be thought possible that the prior exposure of the animal to intravenous injections in this second experiment could have produced a desensitization of hypothalamic glucoreceptors. However, this latter explanation is probably not valid, since the positive results obtained in the second series of intravenous 2DG injections would argue against it.

The fact that lateral hypothalamic lesions did not reduce the effect of intravenous 2DG eating is puzzling. The most likely explanation is that the lesions were not extensive enough, since recovery of eating was fairly rapid. However, one would expect that destruction of some of the glucoreceptors in the lateral hypothalamic area would reduce the efficacy of the intravenous 2DG administration. It is also possible that the 2DG-sensitive neurons in the lateral hypothalamic area are very widely scattered, as revealed by the scattering of intracranial

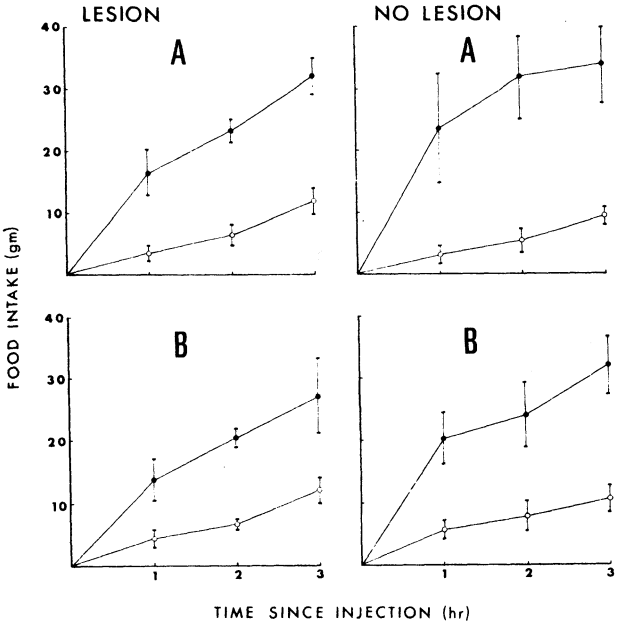

Fig. 5. Cumulative food consumption scores after systemic injections of 2DG (solid circles) and physiological saline (open circles). Top graphs present data obtained in initial series of infusions; lower graphs indicate results obtained after animals were subjected to intracranial injections and electrolytic lesions of LHA (left) or only to intracranial injections (right). Bars above and below points indicate standard error of the mean.

sites where 2DG elicited eating, and thus any one lesion would not eliminate enough of them to reduce the effect. Finally, there is evidence (Novin, VanderWeele, & Rezek, 1973) that there are 2DG-sensitive sites in the periphery, probably in the liver. These latter glucoreceptors might well have a central representation in some area other than the area that is sensitive to direct administration of 2DG.

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