**Bimodal Effect of 2-Deoxy-D-Glucose on Feeding**

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Perfusion of 2-deoxy-D-glucose (2-DG) into the IIIrd ventricle (i.c.v.) or intraperitoneal (i.p.) injections produced changes in feeding and other overt behavior, as well as indicative changes in electroencephalograms (EEG). Applications of 2-DG, either i.p. or i.c.v., induced hyperphagia within 4 hr which was then followed by hypophagia for at least 96 hr. EEGs evinced low frequency patterns during the lethargy and ataxia symptoms which were present after i.p. injection. After i.c.v. injections, the low frequency EEG during the lethargy and ataxia were not evident. Present results in connection with prior reports indicate that 2-DG has a long term bimodal effect on feeding which may be mediated through central neurons. Hypophagia after peripheral application of 2-DG appeared to be caused at least as much by concomitant traumatism as by effects on neural control of feeding.

(Key Words: 2-Deoxy-D-glucose, Feeding, Hypophagia, Hyperphagia, EEG)

**INTRODUCTION**

Administration of 2-deoxy-D-glucose (2-DG), a non-metabolizable analogue of glucose, to a variety of animals, produces marked intracellular functional glucopenia as a result of competitive inhibition of phosphohexose isomerase, an essential enzyme in the metabolism of glucose. The functional lack of glucose activates the sympathicoadrenomedullary system and elicits generalized hyperglycemia and hyperlipacidemia (3, 6, 31, 32). It has been reported that this cellular glucoprivation induces feeding in a variety of animals (4, 7, 26, 27). Feeding is believed to be initiated by glucose sensitive cells in the lateral hypothalamic area (LHA) (10, 11). The author has studied the relationship between gastric acid secretion and its hypothalamic control, including determination of secretion related LHA neurons, their characteristics, and the implications of their relations with other functions (9, 14, 15, 16, 17, 18, 19, 24, 25, 28, 29). It has also been confirmed that the conduction pathway of these specific LHA neurons, which are either concerned with or directly control gastric acid secretion, projects to specific neurons of the medulla oblongata and then goes via the vagus, to oxyntic cells of gastric glands (20). Hyperphagia has been reported to occur sometime within the first 6 hr following 2-DG administration in many species (4, 7, 26, 27), but except for two reports (13, 30) food intake data have usually been limited to periods lasting no more than 6 hr (30).

The present study analyzes additional characteristics of 2-DG effects on feeding for observation periods up to 96 hr.
MATERIALS AND METHODS

Approximately 80 male JCL-Wistar rats, weighing between 300-450 g, were used in this investigation. Prior to the experiments, the animals were individually housed in a temperature-controlled room maintained at 22 ± 1°C, and food and water were freely available.

For at least 6 days prior to intraperitoneal injection of 2-DG, rats were housed in their usual individual living cages with free access to food and water, but illumination was continuous throughout 24 hr. After 6 days of adaptation to continuous illumination, rats were injected intraperitoneally with 2-DG and returned to their cages. The average food and water consumption for all animals for the day prior to injection was used as the base line, and saline injected rats were used as controls. After injection, food and water consumption were measured every 6 hr for a total of 96 hr. Consumption was measured by subtracting food or water remaining in the receptacles, plus spilled food, from the initial quantity of food or water supplied. Food was a standard rat chow (JCL-C2, Japan Clea) and consumption was measured as cumulative g/rat.

Electroencephalograms (EEG) were recorded through electrodes implanted in 4 rats which received intraperitoneal injections, and 4 rats which received intra-IIIrd ventricle infusion (Fig. 1). Stainless-steel, 3 mm long, 0.7 mm OD silver-coated cortical electrodes were placed 3 mm anterior (frontal, F), 2 mm posterior (central, C), 5 mm posterior (parietal, P) and 10 mm posterior (occipital, O) from the bregma. The electrodes were screwed into the skull and extended through an opening cut in the dura matter to the surface of the neocortex. Implanted bipolar or monopolar electrodes used for hypothalamus activity recording were 0.25 mm diameter stainless-steel wires with cashew varnish coating except for 0.1 mm at the tip. Bipolar electrode separation was 1.0 mm. Stereotaxic positions were: LHA--P3.5, L 2.0, V 8.0 mm; ventromedial nucleus (VMH)--P3.5, L 0.7, V 9.5 mm from the bregma. After positioning all electrodes, each one was soldered to one pin of an 8 pin connector (8 × 6 × 17 mm). The connector was then fixed to the surface of the skull with dental cement. During recovery from the operation, the animals were given routine postoperative care with antibiotics and general chemotherapy. After complete recovery from the operation (ca. 10 days), experiments were begun. The animals were connected to an electro-cannular slip ring (AIRFLYTE ELECTRONICS, NJ, U.S.A.) with a 20 gage cannula swivel, which permitted free movement. EEGs were recorded in the 24 hr period before 2-DG administration and periodically after administration. Solutions of 2-DG (SIGMA or WAKO Pure Chemical Industries, Osaka) were prepared with sterile 153.8 mM NaCl.
RESULTS

1) Effects of 2-DG on feeding

Feeding was enhanced in the first 24 hr after injection and suppressed from that time until 96 hr after injection. The effect of systemic (intraperitoneal) administration of 2-DG on feeding is presented in Table 1 and Fig. 2 from observations over 4 days.

Table 1 summarizes food consumption after injection of either 2-DG or saline control into 20 rats. Food consumption of the saline control and each of the 2-DG treated groups was calculated at the end of each of the first two 6 hr periods and at 12 hr intervals thereafter. The values shown are the average cumulative consumption per rat up to 96 hr. The food consumption of all 2-DG groups (200, 500 and 750mg/kg) was significantly different statistically (p<0.01), from the saline control consumption, except at 24 hr after administration of 750mg/kg and 36 hr after administration of 200 and 500mg/kg. These results agree with the i.c.v. data (15) which show that feeding suppression occurred after the initial hyperphagia. Apparently the administration route and dose of 2-DG made little difference in its effects on feeding behavior.

Fig. 2 shows the effects of various doses of 2-DG on food intake com-
pared to the saline control. The average consumption by the saline controls was taken as 100% at each measuring period. The data for plotting the graph was taken from Table 1.

Table 1  Cumulative food consumption from time of 2-DG or saline injection to time after administration indicated at the top of each column.

<table>
<thead>
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<tr>
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<td>6</td>
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<tr>
<td>Saline (n = 5)</td>
<td>1.52 ± 0.34 13.49 ± 1.57 24.76 ± 3.11 34.04 ± 3.02 45.31 ± 4.08 55.08 ± 4.21 66.30 ± 5.08 77.05 ± 6.98 85.99 ± 6.85</td>
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<tr>
<td>2-DG 200mg/kg (n = 5)</td>
<td>5.57 ± 0.91 31.96 ± 6.26 45.85 ± 7.20 54.81 ± 5.04 64.46 ± 3.48 71.07 ± 8.15 79.01 ± 9.26 85.98 ± 10.17 93.05 ± 8.46</td>
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<tr>
<td>500mg/kg (n = 5)</td>
<td>6.32 ± 0.86 46.91 ± 5.33 63.82 ± 5.54 72.23 ± 8.26 80.51 ± 6.33 86.48 ± 4.56 93.18 ± 9.42 98.34 ± 5.98 103.64 ± 10.14</td>
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<tr>
<td>750mg/kg (n = 5)</td>
<td>3.32 ± 0.34 19.94 ± 4.00 36.56 ± 5.28 42.95 ± 6.20 47.74 ± 4.23 52.04 ± 6.18 55.62 ± 5.14 59.47 ± 6.88 63.98 ± 8.25</td>
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*average cumulative food intake in grams per rat, standard deviation of mean.

Fig. 2  Effects of three different doses of 2-DG on food intake compared to effect of saline control. Dotted line: average consumption by the saline controls (taken as 100% at each measuring period). Abscissa: time after injection (note break in time scale between 0 and 6 hr). Ordinate: food intake as percentage of saline control intake. Curve for each dose of 2-DG indicated by legend at upper right.
2) Effects of 2-DG on EEG

Observation of overt behavior of animals for up to 96 hr after intraperitoneal injection or i.c.v. infusion of 2-DG indicated lethargy as a possible factor contributing to the depression of feeding. Chronic EEGs (Fig. 1) were recorded to see if there was some association between overall brain activity, lethargy and feeding behavior. Recordings were made after systemic (750mg/kg i.p.) and after central (10μg/rat i.c.v.) administration of 2-DG. Fig. 3 shows specimen examples of EEG recordings. Comparison of the EEG data with behavior observations indicated that the timing of EEG

![ EEG recordings](image)

Fig. 3 EEG before and after i.p. administration of 750mg/kg 2-DG in “chronic” rat as shown in Fig. 1. Note substantial increase in amplitude of slow waves for all except P-O recording. Slight evidence of remission begins to appear at 48 and 72 hr. These effects on EEG slow waves were much less evident after i.c.v. infusion (not shown). F, frontal cortex; C, central; P, parietal; O, occipital; LHA, lateral hypothalamic area; VMH, ventromedial nucleus of hypothalamus; mp, monopolar recording; bp, bipolar recording. Ordinates: 100μV calibration. Abscissa: time, 1 sec calibration.
slow waves coincided with the lethargy and the depression of feeding behavior. The depressed feeding can thus be associated with lethargy, but was probably not due to illness.

The i.c.v. administration of 2-DG, on the other hand, produced no such decrease in EEG during the time in which lethargy or activity depression occurred. This was true for all of the recording regions observed (not shown).

DISCUSSION

The entire long term effect could be due to the responses of specific hypothalamic neurons (or neuron groups) to 2-DG. This does not seem unreasonable, since the reaction of LHA neurons to 2-DG appears as an increase in the unit activity of some and as a decrease for others (23). It has been reported that 2-DG lingers in the system for more than 72 hr (31), but LHA unit activity was not measured for that long.

It has been established that 2-DG induces food intake when applied either centrally or peripherally. This has been verified for the transient effect, but observation of feeding behavior for several days indicates that the stimulatory effects reverses to depression of food intake after 24 hr.

Since 1969, it has been well known that the glucose analogue, 2-DG, induces a sudden increase in food consumption. This hyperphagia has been reported to occur sometime during the first 6 hr after either i.p. or i.c.v. administration in several species, including rabbits (4), mice (7), rats (26), and monkeys (26, 27). Food intake data have usually been reported for periods lasting no more than 6 hr except in two instances (13, 30), probably as a result of the knowledge that hyperphagia normally coincides with the relatively brief period of glucoprivation induced by 2-DG. Ritter et al., (12) reported that hyperphagia can be delayed until after the hyperglycemic response to 2-DG has spontaneously subsided by withholding food for the first 6 or 8 postinjection hours, but even using this procedure the increase in food intake appears to subside after 10 hr following the injection. Thompson et al. reported (30) a decrease in 24 hr food intake following injection of 750mg/kg i.p. of 2-DG, but they said that rather than simply balancing the increased food intake by a compensatory reduction in feeding, the rats actually significantly reduced below normal their total 24 hr food consumption.It was not clear why the reduction in feeding which follows the initial hyperphagia produced by 2-DG should more than balance the initial hyperphagia. Feeding suppression only has been reported by Jalowiec et al. who observed dose dependent decreases in food intake in cats (5).

Various types of neuron responses to electroosmotic application of 2-DG in the LHA have been reported (23). The types which exhibited suppression and long term facilitation followed by suppression, were especially unique and interesting. The present, and prior studies have revealed important evidence which, when considered together, might contribute to explanations of some observed phenomena; 1) discovery of 2-DG responsive neurons in the so-called feeding center, the LHA, which might be concerned either directly or indirectly with feeding (9, 14-25, 28, 29); 2) the loca-
tion and concentration of these cells coincides with glucose sensitive neurons and neurons reported to be related to feeding behavior (9, 14-25, 28, 29); 3) 2-DG had different effects on different neurons which might explain initial hyperphagia and subsequent suppression of long term feeding behavior (4 days or more) by either i.p. or i.c.v. administration if sufficiently extended unit recordings could be carried out; 4) EEG recordings showed unusually slow activity at the neocortex and hypothalamic regions after i.p. injection of 2-DG. This continued for 3 to 4 days in both the day and night periods and was accompanied by some behavioral changes (symptoms) such as stupor, ataxia, retching and hypothermia (21, 22). After i.c.v. administration of 10μg 2-DG, although the hyper-hypophagia sequence still occurred (13), the slow activity of the EEG during the other symptoms did not appear. It thus seems that the animals were not able to eat after systemic application of 2-DG and did not want to take food after i.c.v. administration of 2-DG. It is suggested that some specific LHA neurons might contribute to this suppression phenomenon (23).

Reports of the feeding effects of 2-DG administration to the hypothalamus have been contradictory. Balagura and Kanner (1) reported elicitation of feeding when 2-DG was administered to the LHA, whereas in a study by Miselis and Epstein (8), they failed to obtain feeding. More recently, Berthoud and Mogenson (2) reported that food intake was initiated by 0.6, 1.2, and 2.4 mg 2-DG when administered i.c.v. but not when it was administered by microinjection into the VMH, the LHA, or other forebrain sites. The present paper and others (13) have shown the importance of central administration of 2-DG to induce feeding, but injection of 2-DG into the hypothalamus for the purpose of observing its effects on feeding by that route is yet to be studied.

Smith et al. (27) reported the threshold dose of 2-DG to induce hyperglycemia in the rat to be 100mg/kg, and the threshold dose to induce feeding to be 400mg/kg, although feeding effects were observed here at 200mg/kg. In any case, the hyperglycemia system is at least 2 times as sensitive as the feeding system to the glucoprivation produced by 2-DG, if glucoprivation is the actual cause of the 2-DG effect on feeding. In this feeding study, 200 to 750mg/kg were used for systemic administration and ca. 20 to 200μg (0.15-15μM) for i.c.v. administration of 2-DG to obtain similar feeding results. These seem to be reasonable doses although the physiological significance of a foreign substance like 2-DG is very difficult to estimate. Nevertheless, a reasonable conclusion appears to be that the primary 2-DG effect on feeding behavior is centrally mediated while the peripheral symptoms produced by 2-DG, although superficially contributory, are coincidental. The present study describes a bimodal effect of 2-DG on feeding behavior after i.p. or i.c.v. administration. This is presumed to mean that 2-DG has two effects on feeding behavior. The reason for the dual effect might be associated with, among other things, the various types of LHA response to 2-DG electroosmotic application (28), but this is certainly not clear at this time. The complete reason for the observed feeding behavior, its initial facilitation followed by depression, is not yet considered to be known.
REFERENCES
Bimodal Effect of 2-DG on Feeding


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