Inhibition of gold thioglucose-induced hypothalamic obesity by glucose analogues

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Female CBA mice were used to determine the effect of the glucose inhibitors, 2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose on gold thioglucose-induced hypothalamic obesity. A marked inhibition in the incidence of obesity resulted when gold thioglucose was administered to mice receiving 2-deoxy-D-glucose (1 g/kg body wt) or 2-amino-2-deoxy-D-glucose (3 g/kg body wt). Gold deposits at the site of the hypothalamic lesion were readily observed, after neutron activation using autoradiographic techniques, in all gold thioglucose-treated animals. These deposits were more extensive in obese than in nonobese mice. The results indicated that 2-deoxy-D-glucose and its amino analogue interact with cells in the “satiety center” to inhibit gold thioglucose-induced hypothalamic damage and obesity. In a separate study, a pronounced hyperphagic response was noted while 2-deoxy-D-glucose was administered to control (untreated) animals. This finding tends to further support the contention that 2-deoxy-D-glucose interacts with cells in the satiety center.

glucose inhibitors; food intake regulation; autoradiography

The syndrome of hyperphagia and obesity in mice after the administration of gold thioglucose was first reported in 1949 (6). Marshall et al. (23) later established that gold thioglucose damaged hypothalamic neurons known to be associated with satiety in normal feeding behavior. The administration of other gold compounds, such as gold thiogalactose and gold thiomalate, failed to induce comparable hypothalamic damage and obesity (24, 25). These observations support the glucostatic theory of the regulation of food intake (25). In the earlier study, Debons et al. (8) obtained further evidence in support of this theory by demonstrating, using neutron activation and autoradiographic techniques, the full extent of gold deposition at the site of the hypothalamic lesion.

It is well established that 2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose inhibit the intracellular transfer and oxidation of glucose in peripheral and central nervous tissue (16, 17, 31, 32, 33) and elevate blood glucose levels (7, 16, 17). The amount of 2-amino-2-deoxy-D-glucose needed to inhibit glucose metabolism has been shown in vivo studies to be three to five times that required of 2-deoxy-D-glucose (3, 32, 33). This may be attributed to steric factors which are known to play an important role in the rates of entry and metabolism of these glucose analogues (11, 18, 26). Phosphorylated derivatives of these glucose antimetabolites are unable to serve as metabolic substrates for either the phosphohexose-isomerase or glucose 6-phosphate dehydrogenase reaction (29, 34).

In the work to be reported we investigated the effect of 2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose on gold thioglucose-induced hypothalamic hyperphagia and obesity in mice.

MATERIALS AND METHODS

Animal studies. Animals used in the following experiments were 7- to 9-week-old female CBA mice weighing 20 to 25 g. They were fed Purina laboratory chow ad lib. and given tap water. Room temperature, which could not be controlled, varied from 20 to 27 C under the experimental conditions reported. Temperature was recorded daily by means of a Taylor thermograph.

The effect of glucose inhibitors, 2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose, on hypothalamic hyper-
phagia and obesity induced by gold thioglucose was investigated in three separate experiments (experiments 1–3). Mice were assigned at random to six groups: 1) control, 2) gold thioglucose, 3) 2-deoxy-d-glucose, 4) 2-deoxy-d-glucose and gold thioglucose, 5) 2-amino-2-deoxy-D-glucose, and 6) 2-amino-2-deoxy-D-glucose and gold thioglucose. Each cage contained seven or eight animals. Control and gold thioglucose-treated mice were given four intraperitoneal injections of demineralized water at 3-hr intervals, followed by four additional injections at 6-hr intervals. Groups treated with glucose inhibitor and glucose inhibitor plus gold thioglucose were given these materials dissolved in a minimum amount of water on the same schedule. In each of the eight injections, 2-deoxy-D-glucose\(^5\) was given at 1 g/kg body wt. The glucose inhibitor 2-amino-2-deoxy-D-glucose\(^6\) was given at 2 g/kg body wt in experiment 1, and at 3 g/kg body wt in experiments 2 and 3. Animals which received gold thioglucose (800 mg/kg body wt) were given this drug intraperitoneally in a single injection 2 hr after the third injection of water or inhibitor.

The mice were maintained on trial for 10 weeks during which time gain in body weight, food intake, and mortality were recorded. Gold thioglucose and glucose inhibitor plus gold thioglucose-treated mice that had a gain in weight exceeding the range of weight gained by mice in control and glucose inhibitor-treated (control) groups were designated as obese. During the last 2 weeks on trial obese and nonobese animals were separated from the treated groups. Four to five animals were housed per cage and daily food intake of obese and nonobese animals, respectively, were recorded. Daily food intake consumed by individual animals was calculated from the pooled food intakes.

At the completion of each experiment, four obese and four nonobese animals were randomly selected from each of the control and treated groups for quantitation of oxygen and 14C incorporation of the induced activity was detected by a 4 x 5-inch neutron activation. Neutron activation of the brains selected was carried out in the low-intensity test reactor at the Oak Ridge National Laboratory. The irradiation bottle (polyethylene) containing tissues and standards was exposed to a thermal flux of about 10\(^{14}\) neutrons/cm\(^3\) per sec for 24 hr. Tissue damage due to heat was avoided by maintaining the temperature of the brains during irradiation below 50 C. After irradiation, the samples were allowed to decay for 48 hr to reduce the activity of short half-life radionuclides, principally \(^{24}\)Na, which would otherwise interfere with the subsequent gamma ray analysis. The gamma emission resulting from the decay of the induced activity was detected by a 4 x 5-inch system.

**TABLE 1. Obesity and weight gain of mice treated with gold thioglucose, inhibitor, and inhibitor plus gold thioglucose**

| Treatment | Animals in Trial | Surviving Animals | T* | Weight Gain, g
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<tr>
<td></td>
<td></td>
<td>Obese</td>
<td>Nonobese</td>
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<tr>
<td>Control</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>GTG, 800 mg/kg</td>
<td>30</td>
<td>15</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>a-DG, 1 g/kg</td>
<td>15</td>
<td>15</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>a-DG + GTG</td>
<td>30</td>
<td>7</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>2-ADG, 2 g/kg</td>
<td>15</td>
<td>13</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>2-ADG + GTG</td>
<td>30</td>
<td>18</td>
<td>11</td>
<td>14</td>
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<td>a-DG + GTG</td>
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<td>2-ADG, 3 g/kg</td>
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<td>2-ADG + GTG</td>
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<td>7</td>
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<td>a-DG, 1 g/kg</td>
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<td>13</td>
<td>15</td>
</tr>
<tr>
<td>a-DG + GTG</td>
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<td>12</td>
<td>25</td>
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<tr>
<td>2-ADG, 3 g/kg</td>
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<td>15</td>
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<tr>
<td>2-ADG + GTG</td>
<td>60</td>
<td>17</td>
<td>12</td>
<td>19</td>
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Abbreviations: GTG, gold thioglucose; a-DG, 2-deoxy-D-glucose; 2-ADG, 2-amino-2-deoxy-D-glucose. * Statistical comparisons based on chi-square analysis. † Mean total gain in weight ± SE per mouse during the 10-week experimental period.

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5 2-Deoxy-D-glucose, supplied by Calbiochem, Los Angeles, Calif.
NaI (TI) crystal and analyzed by a 400-channel analyzer. The presence of $^{198}$Au was easily detected by its characteristic 0.411 Mev gamma ray emission. The gold content of brains was determined after correction for $^{20}$Na activity in the region of the $^{198}$Au photopeak. This was accomplished by comparing the activity in the photoplate observed for the brains to that of standards containing a known amount of gold.

**Autoradiography.** Following quantitation of induced $^{198}$Au activity by gamma by spectroscopy, the brains were embedded in paraffin blocks and transverse serial sections 5 $\mu$ in thickness were prepared for histologic and autoradiographic study. Approximately 10 days following neutron irradiation the deparaffinized brain sections, mounted on glass slides, were dipped in photographic liquid emulsion (Kodak, NTB-2). Details of the autoradiographic and subsequent staining procedures are described in an earlier publication (8). Under the conditions of these experiments, a 5-day exposure of tissue to photographic emulsion was necessary for satisfactory resolution of induced $^{198}$Au activity.

Analysis of variance, accompanied by the t test, was routinely used to test the numerical data statistically (13).

**RESULTS**

**Effects of inhibitor treatment on obesity induced by gold thioglucose.** Results of experiments 1–3, designed to test the effects of 2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose on gold thioglucose-induced hyperphagia and obesity, are given in Tables 1 and 2 and shown in Fig. 1.

**Early effects of treatment.** Mice generally lost weight during the 33-hr treatment period, except for the 2-deoxy-D-glucose treated group. Mice administered 2-deoxy-D-glucose gained an average of 1–2 g.

**Incidence of obesity.** In experiments 1–3, the incidence of obesity induced by gold thioglucose in untreated control animals varied from 52 to 100%. The variability in percent obesity appeared to be dependent on ambient temperature at the time of gold thioglucose administration. It is noteworthy that at the ambient temperature of 21 C (exp. 3) all untreated control animals given gold thioglucose became obese. In further studies (to be reported separately) related specifically to the incidence of obesity induced by gold thioglucose at controlled ambient temperature, we have noted that all normal CBA mice treated with gold thioglucose at a controlled ambient temperature of 21 ± 2 C developed hyperphagia and obesity. Furthermore, the incidence of obesity was less than 100% at temperatures above and below 21 ± 2 C.

In experiment 1, treatment of animals with 2-deoxy-D-glucose (1 g/kg body wt) appeared to lower the incidence of obesity following gold thioglucose. The administration of 2-amino-2-deoxy-D-glucose (2 g/kg body wt) failed to reduce the incidence of gold thioglucose induced obesity. Although the response of 2-deoxy-D-glucose on the reduction of obesity in this experiment was found not to be statistically significant on chi-square analysis, the finding was of sufficient biological interest to pursue these studies further under conditions where the variability in production of obesity by gold thioglucose could be controlled. In experiment 2, and of particular interest, experiment 3, under conditions in which the variability in response of untreated control animals to gold thioglucose was controlled (100% obesity), animals which received 2-deoxy-D-glucose (1 g/kg body wt) or 2-amino-2-deoxy-D-glucose, at 3 g/kg body wt, exhibited a significant reduction in the incidence of obesity induced by gold thioglucose. We have also noted (unpublished data) that, under the conditions of experiment 3, 2-deoxy-D-glucose (1 g/kg) or 2-amino-2-deoxy-D-glucose (3 g/kg) given in a single intraperitoneal injection 3/2-hr prior to gold thioglucose administration did not lower the incidence of obesity in surviving mice.

The body weight gains were significantly ($P < 0.05$) greater in all obese compared to nonobese mice in both the gold thioglucose and glucose inhibitor plus gold thioglucose-treated groups. It is noteworthy that in experiment 3, 2-deoxy-D-glucose (1 g/kg) or 2-amino-2-deoxy-D-glucose (3 g/kg) given in a single intraperitoneal injection 3/2-hr prior to gold thioglucose administration did not lower the incidence of obesity in surviving mice.

**Content of gold in brain.** The total gold content of brains taken from obese mice given gold thioglucose alone was not significantly ($P > 0.05$) higher than that of similarly treated nonobese mice (Table 2). Although in certain instances the total gold content of brains from obese animals treated with glucose inhibitor plus gold thioglucose was significantly higher ($P < 0.05$) than that of brains from nonobese animals, this effect was not consistently reproducible. Compared to gold thioglucose-treated groups of obese and nonobese animals, the brains of mice treated with 2-deoxy-D-glucose and gold thioglucose consisted had significantly ($P < 0.05$) less gold. The obese and nonobese groups of mice treated with 2-amino-2-deoxy-D-glucose (3 g/kg) and gold thioglucose did not have less gold in the brain than respective groups of animals treated with gold thioglucose alone.

**Autoradiographic findings.** Autoradiographs illustrating gold deposits at the site of the hypothalamic lesion are shown in Fig. 1. All the brains from mice given gold thioglucose, both with and without a glucose inhibitor, had gold deposits at the site of the hypothalamic lesion. In all instances, the accumulation of gold at this site was less in nonobese than in obese mice. Hypothalamic gold deposits in nonobese mice that had been treated with 2-deoxy-D-glucose and gold thioglucose were scarcely detectable. The amount of gold deposits at the site of the hypothalamic lesion varied among obese groups on the different treatments as well as among nonobese groups. Compared to those treated with gold thioglucose alone, the mice given 2-deoxy-D-glucose and gold thioglucose and those given 2-amino-2-deoxy-D-glucose (3 g/kg)
DISCUSSION

and gold thioglucose appeared to have diminished gold deposits at the site of the hypothalamic lesion. Since the groups given 2-deoxy-D-glucose plus gold thioglucose and those given 2-amino-2-deoxy-D-glucose (3 g/kg) plus gold thioglucose contained a larger proportion of nonobese mice than the gold thioglucose group, it is apparent that administration of glucose inhibitor decreased gold deposition at the site of the hypothalamic lesion.

Mortality. The mortality following the administration of either gold thioglucose at 800 mg/kg or 2-deoxy-D-glucose at 1 g/kg averaged 7% (Table 1). Mortality after administration of 2-amino-2-deoxy-D-glucose at 2 g/kg and 3 g/kg averaged 13%. When 2-deoxy-D-glucose-treated animals received gold thioglucose the average mortality rose to 29%. The highest mortality, which averaged 52%, was found when gold thioglucose was given to animals receiving 2-amino-2-deoxy-D-glucose (3 g/kg). It is important to note that all mice surviving the inhibitor and inhibitor plus gold thioglucose treatments were observed to be in normal health and indistinguishable from control animals.

Blood glucose. Average blood glucose concentrations at the time of gold thioglucose administration for 2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose at 2 g and 3 g/kg body wt were, respectively, 38%, 50%, and 71% above control values as determined by the method of Somogyi and 25%, 27%, and 51% above control values as determined by the glucose oxidase method.

Body weight and food intake responses to inhibitor treatment. Results of experiments designed to test the effect of 2-deoxy-D-glucose on food intake and gain in weight during the time of inhibitor administration are shown in Figs. 2 and 3. During the first day on trial, food intake of mice given 2-deoxy-D-glucose was significantly (P < 0.01) greater than that of the control and 2-amino-2-deoxy-D-glucose groups (Fig. 2). When observed, the 2-deoxy-D-glucose-treated animals were found to be continuously eating as opposed to intermittent feeding behavior on the part of control and 2-amino-2-deoxy-D-glucose-treated animals. This hyperphagia response was accompanied with a significant (P < 0.01) gain in weight above controls (Fig. 3) similar to that noted earlier in experiments 1-3. Subsequently, the mice given 2-deoxy-D-glucose exhibited an anorexia accompanied by a loss in weight before returning to a food intake and weight gain equal to the control groups.

DISCUSSION

In the present study, the administration of 2-deoxy-D-glucose (1 g/kg) and 2-amino-2-deoxy-D-glucose (3 g/kg) 

FIG. 1. Typical autoradiographs illustrating the localization of 14C-labeled gold thioglucose in brain of control, gold thioglucose-treated, 2-deoxy-D-glucose-treated, 2-amino-2-deoxy-D-glucose-treated and gold thioglucose-treated obese. Magnification 80 X.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>µg Gold/g Brain Tissue, Wet Wt*</th>
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<tbody>
<tr>
<td></td>
<td>Obese animals</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.72±0.136</td>
</tr>
<tr>
<td>2-DG, 1 g/kg</td>
<td>0.40±0.017</td>
</tr>
<tr>
<td>2-DG + GTG</td>
<td>0.53±0.034</td>
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<tr>
<td><strong>Experiment 2</strong></td>
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<tr>
<td>Control</td>
<td>0.74±0.044</td>
</tr>
<tr>
<td>2-DG, 1 g/kg</td>
<td>0.51±0.061</td>
</tr>
<tr>
<td>2-DG + GTG</td>
<td>0.77±0.122</td>
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<tr>
<td><strong>Experiment 3</strong></td>
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<tr>
<td>Control</td>
<td>1.25±0.084</td>
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<td>2-DG, 1 g/kg</td>
<td>0.48±0.014</td>
</tr>
<tr>
<td>2-DG + GTG</td>
<td>1.15±0.086</td>
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</table>

Abbreviations as in Table 1. Values are means ± SE of four animals selected at random from each group. * Gold content was assayed by 400-channel pulse-height analyzer, after thermal neutron activation of tissue samples in the low-intensity test reactor at the Oak Ridge National Laboratory.

to CBA mice markedly inhibited the incidence of obesity and diminished gold deposition at the site of the hypothalamic lesion resulting from gold thioglucose. In accord with the known inhibitory effects of these inhibitors on cellular entry and oxidation of glucose (16, 17, 31-33), these results may be attributed to competitive inhibition between the glucose inhibitor and gold thioglucose for attachment at hypothalamic glucoreceptor cell sites (23, 25). Alternatively, the glucose inhibitors may be phosphorylated at the intracellular level (14, 29, 34), thereby inhibiting further utilization of either glucose or gold thioglucose or both by these cells. The hyperphagia and increased gain in weight noted while 2-deoxy-D-glucose was administered to control (untreated) animals supports the contention that this inhibitor may be exerting a direct intracellular effect on the hypothalamic cells of the "satiety center." Such an effect would diminish the metabolic activity of these cells, thereby reducing the physiological inhibitory control normally exerted on the more lateral hypothalamic "feeding center." Furthermore, the data would seem to suggest that the hypothalamic cells of the satiety center possess a high affinity 7

7 By the term satiety center, we are referring to a specific group of brain cells which limit food intake by inhibiting neuronal activity stemming from the lateral hypothalamus or feeding center. These cells are not necessarily concentrated as a single cell type within a sharp anatomical focus.
for the glucose antimetabolite, 2-deoxy-D-glucose, since if cells of both the satiety and feeding centers had an equally high affinity for the inhibitor, one would have expected an aphagic response as has been observed after stereotactically induced lesions in both these areas (1).

The role of hyperglycemia in hypothalamic gold uptake and hyperphagia in gold thioglucose-treated mice has recently received some attention by other workers (10) and should be considered. In our studies, all mice given a glucose inhibitor had a mild hyperglycemic response. In contrast to animals given 2-amino-2-deoxy-D-glucose, 2-deoxy-D-glucose-treated animals exhibited a significantly lower total brain uptake of gold after gold thioglucose administration. Despite these differences, diminished gold deposits at the site of the hypothalamic lesion were noted in the majority of hyperglycemic mice given 2-deoxy-D-glucose and 2-amino-2-deoxy-D-glucose after gold thioglucose. In a recent publication, Edelman et al. (10) studied the effect of hyperglycemia induced by the administration of glucose on hypothalamic gold uptake and hyperphagia in gold thioglucose-treated mice. They found that the amount of hypothalamic gold deposition and the degree of resultant hyperphagia varied directly with the blood glucose concentration at the time of gold thioglucose administration. Hyperglycemia was found to be associated with enhanced gold uptake throughout the brain. Failure of hyperglycemia to competitively inhibit gold deposition in the hypothalamic area as reported by these workers cannot be regarded as evidence against the glucoreceptor satiety mechanism. Although our data on brain and hypothalamic gold uptake differ from those reported by Edelman and co-workers, they are not inconsistent. Our findings and those of Anand et al. (2) do not support the contention by Edelman and co-workers (10) that hyperglycemic levels account for the increased hypothalamic uptake of gold in gold thioglucose-treated hyperglycemic animals. The increased hypothalamic uptake of gold noted by these investigators may represent an example of enhanced hypothalamic activity resulting from elevated arteriovenous differences associated with induced hyperglycemia.

When viewed in this manner their data remains consistent with the concept of the glucoreceptor governing the satiety mechanism.

In contrast to our earlier studies, we found gold deposits at the site of the gold thioglucose-induced hypothalamic lesion both in obese and nonobese animals. The accumulation and distribution of gold at the site of the hypothalamic lesion was more extensive in gold thioglucose and glucose inhibitor-gold thioglucose-treated obese than in nonobese mice. Brecher et al. (5), using Swiss albino mice (NIH strain), found hypothalamic lesions in nearly all animals treated with gold thioglucose despite a limited incidence of obesity. They were led to conclude that obesity was correlated with the extent of the lesion, rather than with their presence. Since the ventromedial nuclei (satiety center) were found to be largely spared in gold thioglucose-treated hyperglycemic animals. The in- 

FIG. 2. Average daily food intake (±SE) of controls, 2-deoxy-D-glucose (1 g/kg body wt) and 2-amino-2-deoxy-D-glucose (3 g/kg body wt)-treated animals. Each bar represents the mean daily food intake of mice from five cages of animals, each cage housing four or five animals.
REFERENCES


