A direct action of insulin on the hypothalamic satiety center

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DEBONS, ALBERT F., ISIDORE KRIMSKY, AND ANNETTE FROM. A direct action of insulin on the hypothalamic satiety center. Am. J. Physiol. 219(4): 938-943. 1970.—Diabetic mice, unlike normal mice, do not develop necrosis of the hypothalamic satiety center after administration of gold thioglucose. In previous studies, the rapid action of intravenously administered insulin in restoring the sensitivity of the satiety center of diabetic mice to gold thioglucose suggested that insulin might act directly on the satiety center. In the present studies, the effect of intrahypothalamic injection of insulin on the restoration of the sensitivity of the center to gold thioglucose necrosis was investigated in diabetic mice. Intrahypothalamic injection of insulin restored the sensitivity of the center. Insulin given by this route does not act by entering the circulatory system, since the effect was obtained in the presence of anti-insulin serum which prevented circulating insulin from acting. It is concluded that insulin can act directly on cells of the satiety center. An important physiological role of insulin in regulation of the satiety center in its control of feeding behavior is indicated.

gold thioglucose-induced obesity; diabetes mellitus; regulation of food intake

MATERIALS AND METHODS

Adult female CBA mice (Cumberland View Farms, Clinton, Tenn.) were used in these experiments. They were fed Purina Laboratory Chow and given tap water freely. The animals were housed in a room maintained at 22.0 ± 0.5 C.

Pork insulin (U-80, Iletin) used in these studies was obtained from Eli Lilly and Co., Indianapolis, Ind. Insulin was inactivated according to De Duve (7) in the following manner. Pork insulin was exposed to 0.1 N NaOH for 3 hr at 37 C, then neutralized with HCl. Inactivation of the insulin was assayed by abolition of its hypoglycemic action in mice. An amount of inactivated insulin 20 times greater than the amount of active insulin needed to lower blood glucose to 30 mg/100 ml had no hypoglycemic effect.

Diabetes mellitus was induced by a single intravenous injection of alloxan monohydrate, 100 mg/kg. It was administered as a 2% aqueous solution into a tail vein. The production of diabetes was ascertained by the presence of glycosuria.

Gold thioglucose, 800 mg/kg, was administered as an 8% aqueous solution in a single intraperitoneal injection.

Intrahypothalamic injections were made with a 10-μl syringe (microliter no. 701, Hamilton Co., Inc., Whittier, Calif.). Mice were lightly anesthetized with ether, and the heads were rigidly positioned horizontally in a stereotaxic instrument. A longitudinal midline incision was made through the skin of the crown of the head. A hole was drilled through the calvaria 0.5 mm from the midline and 18.5 mm from the tip of the snout using a dental drill with a Unitek bit (Unitek Corp., Englewood Cliffs, N. J., catalogue no. 950-101). The syringe needle was introduced vertically through the hole in the calvaria and inserted into the brain until the tip rested on the base of the skull; the opening of the needle was then in the ventromedial portion of the hypothalamus at the level of the median eminence. The location of the needle was verified in every instance by postmortem examination under the microscope. The volume injected was kept at 2 μl.

Normal or anti-insulin serum (0.5 ml) was injected into a tail vein.

THE ADMINISTRATION of gold thioglucose to mice produces hyperphagia and obesity as a result of lesions induced in the ventromedial region (satiety center) of the hypothalamus (8). Diabetes mellitus prevents the gold thioglucose-induced necrosis of the satiety center and the consequent hyperphagia and obesity (4, 5). The administration of insulin restores the susceptibility of the satiety center to gold thioglucose necrosis; restoration of susceptibility was found to occur within 5 min after the intravenous injection of insulin (5). These findings prompted us to investigate the possibility of a direct effect of insulin on the satiety center. We therefore tested the effect of intrahypothalamic injections of insulin on the susceptibility of the satiety center to gold thioglucose necrosis in diabetic mice.

The pathology of the hypothalamic lesion caused by gold thioglucose is readily distinguishable from damage caused by intrahypothalamic needle penetration. The pathology of the fully developed gold thioglucose lesion (20 hr after administration) consists of dissolution of the neuropile, decrease in cell number, and pyknosis of the nuclei of the remaining cells (3). We have confirmed these findings in a separate study (unpublished observations). Needle damage at this time consists only of cell death immediately adjacent to the needle track; the needle track is marked by a mass of disintegrating erythrocytes. These observations permitted us to detect the occurrence of gold thioglucose necrosis in diabetic mice given insulin by intrahypothalamic injection.
When insulin was given intravenously, it was injected into a tail vein in a volume of 0.05 ml.

For histological examination of the hypothalamus, mice were decapitated 20 hr after the administration of gold thioglucose and the brains were fixed in Bouin's fluid. They were imbedded in paraffin, sectioned transversely at 7 μ, and stained with hematoxylin and eosin. Serial sections through the hypothalamus at the level of the median eminence were taken for examination under the light microscope.

Iodine 125-iodinated insulin was obtained from Abbott Laboratories, North Chicago, Ill. The specific activity was approximately 50 mc/mg insulin. Details of the autoradiographic procedures used to localize 125I activity are described in an earlier publication (6).

Guinea pig anti-insulin serum was prepared as described in an earlier report (4).

RESULTS

We first studied the effect of intrahypothalamic injection of saline on the histology of the satiety center in untreated mice and in mice treated with gold thioglucose. In these experiments gold thioglucose was given as a single intraperitoneal injection immediately after the intrahypothalamic injection. The intrahypothalamic injection of saline did not produce necrosis nor did it prevent the necrosis which follows the injection of gold thioglucose (Figs. 1 and 2). These figures are to be compared with sections from animals given no treatment or given gold thioglucose intraperitoneally but no intrahypothalamic injection (Figs. 3 and 4). Therefore, the intrahypothalamic injection of saline does not produce necrosis or prevent the necrosis induced by gold thioglucose.

Diabetic mice in contrast to normal mice given gold

\[1\] All figures are transverse sections through the hypothalamus at the level of the median eminence. Abbreviations; GTG, gold thioglucone; GPNS, guinea pig normal serum; GPAIS, guinea pig anti-insulin serum; iv, intravenously; ip, intraperitoneally; ih, intrahypothalamically.
TABLE 1. Gold thioglucose necrosis of satiety center: inhibition in diabetes; reversal by intrahypothalamic injection of insulin

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>No. of Animals</th>
<th>Intrahypothalamic Injection</th>
<th>Occurrence of Gold Thioglucose Necrosis in Satiety Center, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Normal</td>
<td>15</td>
<td>Saline</td>
<td>100</td>
</tr>
<tr>
<td>Diabetic</td>
<td>26</td>
<td>None</td>
<td>12</td>
</tr>
<tr>
<td>Diabetic</td>
<td>19</td>
<td>Insulin, 6 mU</td>
<td>75*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>11</td>
<td>Insulin, 3 mU</td>
<td>63*</td>
</tr>
<tr>
<td>Diabetic</td>
<td>12</td>
<td>Insulin, 1 mU</td>
<td>50†</td>
</tr>
<tr>
<td>Diabetic</td>
<td>13</td>
<td>Inactivated insulin, 6 mU</td>
<td>0</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5</td>
<td>Saline</td>
<td>0</td>
</tr>
</tbody>
</table>

mU = milliunits. * P < 0.01 (statistical comparison by chi-square analysis was made with diabetic group receiving no treatment). † P < 0.05 (statistical comparison by chi-square analysis was made with diabetic group receiving no treatment).

FIG. 7. Diabetic mouse given saline ih and GTG ip.

FIG. 5. Diabetic mouse given 1 milliunit insulin ih and GTG ip.

FIG. 6. Diabetic mouse given 6 milliunits inactive insulin ih and GTG ip.

TABLE 2. Restoration of susceptibility of satiety center to gold thioglucose-induced necrosis in diabetic mice by insulin injection: prevention of intravenous insulin effect by anti-insulin serum and failure of anti-insulin serum to prevent action of insulin given intrahypothalamically

<table>
<thead>
<tr>
<th>Animal Type</th>
<th>No. of Animals</th>
<th>Treatment*</th>
<th>Gold Thioglucose Necrosis in Satiety Center</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic</td>
<td>36</td>
<td>GTG, GPNS, insulin intravenously</td>
<td>Present 25, Absent 11</td>
</tr>
<tr>
<td>Diabetic</td>
<td>32</td>
<td>GTG, GPAIS, insulin intravenously</td>
<td>Present 4†, Absent 28</td>
</tr>
<tr>
<td>Diabetic</td>
<td>31</td>
<td>GTG, GPNS, insulin intrahypothalamically</td>
<td>Present 20, Absent 11</td>
</tr>
<tr>
<td>Diabetic</td>
<td>32</td>
<td>GTG, GPAIS, insulin intrahypothalamically</td>
<td>Present 22†, Absent 10</td>
</tr>
</tbody>
</table>

GTG = gold thioglucose; GPNS = guinea pig normal serum; GPAIS = guinea pig anti-insulin serum. * GTG (800 mg/kg) was given in a single intraperitoneal injection followed either by GPNS (0.5 ml) or GPAIS (0.5 ml) intravenously 15 min later. At 30 min after GTG administration, 3 milliunits insulin were injected either intravenously or intrahypothalamically. † Difference between the two groups treated intravenously is significant (P < 0.001). † Difference between the two groups treated intrahypothalamically is not significant (P > 0.90).

thioglucose do not develop necrosis of the hypothalamic satiety center (4). The intrahypothalamic administration of insulin, varying in amounts from 6 to 1 milliunit, to diabetic mice significantly restored the susceptibility of the satiety center to gold thioglucose-induced necrosis (Table 1 and Fig. 5). Diabetic mice similarly treated with 6 milliunits of inactivated insulin or saline did not regain their susceptibility to gold thioglucose necrosis (Figs. 6 and 7). The foregoing results are summarized in Table 1.

When the intrahypothalamic injection of insulin was placed more than 2 mm from the midline or 2 mm anteriorly or posteriorly to the median eminence, no effect of injected
insulin was seen, i.e., there was no necrosis after injection of gold thioglucose into diabetic mice.

As a control for the possibility that insulin, when injected intrahypothalamically, restores susceptibility of the satiety center to gold thioglucose necrosis by entering the blood and acting on tissues outside the brain, the following study was made. Gold thioglucose was given intraperitoneally; 15 min later either guinea pig normal serum or guinea pig anti-insulin serum was injected intravenously. Thirty minutes after gold thioglucose was given, 3 milliunits insulin were injected either intravenously or intrahypothalamically. This interval for insulin administration was chosen, since it had been shown in an earlier study (4) that gold thioglucose is at its highest level in the blood during the interval of 20–40 min after intraperitoneal administration. The results of these experiments are shown in Table 2. Insulin given intravenously to diabetic mice treated with guinea pig normal serum restores the sensitivity of the satiety center to gold thioglucose necrosis (Fig. 8). This is in accord with previous work showing that insulin administered by this route restores the susceptibility of the satiety center to gold thioglucose necrosis in diabetic mice (5). Insulin given intravenously to diabetic mice similarly treated with guinea pig anti-insulin serum was prevented from acting (Fig. 9). In contrast, when insulin was given intrahypothalamically, anti-insulin as well as normal serum did not affect the return of susceptibility to gold thioglucose necrosis (Figs. 10 and 11); i.e., the incidence of necrosis was the same as that occurring in animals given normal serum (Table 2) or no serum (Table 1). It is concluded that insulin, when injected intrahypothalamically, did not restore susceptibility to gold thioglucose by getting into the blood, since insulin in the blood was prevented from acting by anti-insulin serum.

In some cases, after intrahypothalamic injection of insulin, gold thioglucose necrosis was present in the contralateral as well as the homolateral satiety center. Apparently, insulin was capable of spreading across the midline from
The guinea pig anti-insulin serum used in this experiment was tested for its ability to produce insulin deficiency in normal mice. After intraperitoneal injection of 1 ml of this serum, glycosuria appeared within 1 hr and was maintained for the next 24 hr.

**DISCUSSION**

Mayer and Thomas (9) postulated the existence of insulin-sensitive glucoreceptor cells in the satiety center. Evidence for insulin sensitivity of the satiety center was provided by the work of Anand et al. (1) and Bach et al. (2). These workers noted that intravenous administration of insulin provoked an increase in the electrical activity of the satiety center. Additional evidence for the insulin sensitivity of the satiety center was provided by Debons et al. (4, 5). It was shown by these workers that gold thioglucose-induced destruction of the satiety center was prevented by diabetes mellitus (4). It was later shown that the sensitivity of the satiety center to gold thioglucose was restored within a few minutes after insulin administration to diabetic animals (5). The rapidity of the response to insulin suggested that insulin might be acting directly on the satiety center. However, these findings did not provide conclusive evidence for a direct action of insulin on the center; insulin might have affected the center indirectly by way of action on other tissues of the organism.
The findings presented in this report demonstrate that insulin can act directly on the hypothalamus in restoring the sensitivity of the satiety center to gold thioglucose. Support for a direct action of circulating insulin on cells of the satiety center is provided by the autoradiograms showing accumulations of insulin-125I in oligodendrocytes of the satiety center after intraperitoneal injection of the insulin. Recently, a histological study including the pathogenesis of the gold thioglucose lesion, autoradiographic localization of gold and insulin dependence of the lesion, provided evidence that oligodendrogliala of the satiety center are glucose-receptor cells (unpublished observations).

Our findings suggest that the well-known hyperphagia of diabetes mellitus is mediated by the satiety center. Normally the satiety center when activated by insulin suppresses food intake, since either destruction of the center or insulin deficiency leads to hyperphagia (4). As shown in this report, insulin can restore the activity of the satiety center in diabetic mice, as measured by sensitivity to gold thioglucose, by a direct action on the center. Therefore, it seems probable that the hyperphagia of diabetes is a result of insulin insufficiency or lack of responsiveness of the center to insulin; this leads to inactivity of the satiety center and consequent hyperphagia.

The authors thank Dr. Sanford C. Spraragen for his interest and encouragement. We also thank Dr. Preston L. Perlman of the Schering Corporation, Bloomfield, N. J., for supplying us with gold d iodoglucose for these studies.

This work was supported, in part, by Public Health Service Grant A. M. 12479.

A preliminary report of this work was presented at the VIIIth International Congress of Nutrition, Prague, August 28-September 5, 1969.

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Received for publication 6 February 1970.

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