Insulin Storage and Release in Rats Bearing Growth Hormone Secreting Tumors

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SUMMARY

The effects of chronic hypersomatotropism on pancreatic islet function were studied in rats bearing the growth hormone secreting tumor MtTW15. These rats did not become hyperglycemic and minimally hyperinsulinemic in the fed state following prolonged massive elevations of serum growth hormone. Islets from tumor-bearing rats were grossly larger and beta cells more granulated than in control rats by light microscopy. Electron microscopy confirmed the increase in cell cytoplasm and increased number of beta granules per cell. Isolated islet preparations from tumor-bearing rats contained four times the insulin found in control rats. Fed control and tumor-bearing rats had comparable serum glucose and immunoreactive insulin concentrations after glucose intragastrically and tolbutamide intravenously. After a forty-eight-hour fast the tumor-bearing rats had higher serum immunoreactive insulin than controls and their insulin levels were higher after glucose and tolbutamide than controls. Some of the tumor-bearing rats became hypoglycemic during the forty-eight-hour fast. The increased stored insulin in the tumor-bearing rats was mobilized by a combination of the individually potent insulinogenic stimuli theophylline and glucagon. DIABETES 18:619-24, September, 1969.

We have previously reported on greatly elevated levels of immunos assayable plasma growth hormone attained in rats bearing Furth's transplantable pituitary tumor MtTW15. This tumor has been shown to produce large amounts of growth hormone and prolactin by bioassay, but has no other detectable hormonal secretions.

Despite this evidence of hypersomatotropism these animals are not overtly diabetic. This is not surprising, for the rat is particularly resistant to many hormonal treatments which induce diabetes in other species. If growth hormone is administered to rats after subtotal pancreatectomy, however, diabetes develops. Similarly, Bates, Scow and Lacy have induced diabetes in rats following subtotal pancreatectomy by transplantation of the Furth tumor MtTF4, a tumor which secretes not only growth hormone but also prolactin and corticotropin.

Since the lack of a diabetogenic effect from growth hormone treatment in rats could be due to a large pancreatic insulin secretory reserve, we were of the view that a study of rats bearing a tumor secreting the animal's native growth hormone would provide important information concerning islet function and insulin reserve. In the present paper we will describe changes in serum insulin and glucose following a number of provocative stimuli in rats bearing the growth hormone secreting tumor MtTW15.

MATERIAL AND METHODS

The pituitary tumor MtTW15, obtained from Dr. Bruce Sells, Memphis, Tenn., had previously been passed for many successive generations. Eighty to 120 gm. female Wistar-Furth rats were obtained from A. R. Schmidt Co., Madison, Wis. The rats were housed in groups of six to eight per cage and fed Purina Laboratory Chow and water ad libitum. The tumor was transplanted as a mince injected subcutaneously into the posterior cervical region of rats weighing 80 to 100 gm. The tumor has grown in all of the Wistar-Furth rats into which it has been injected. The experimental studies performed on the tumor-bearing rats reported here were done when the tumor was greater than 1.5 cm. in diameter and the plasma growth hormone was between 2.5 and 45 μg/ml. At this time, six to eight weeks post-transplantation, the rats appeared healthy, were eating well, and gaining weight at a greater than normal rate. Control rats of the same age as the tumor-bearing rats were obtained from the supplier at the same time as the tumor-injected animals and treated in...
INSULIN STORAGE AND RELEASE IN RATS BEARING GROWTH HORMONE SECRETING TUMORS

Glucose was measured by a ferricyanide method requiring 50 to 100 µl of serum with the Technicon AutoAnalyzer. Serum insulin was determined by a radioimmunoassay as described by Morgan and Lazarow. Serum growth hormone was determined by the radioimmunoassay for rat growth hormone previously described by us. The mean (± S.E.M.) plasma growth hormone concentration in control female Wistar-Furth rats was 60 µg./ml. (± 10).

For histologic studies the animals were anesthetized with pentobarbital and the pancreas was removed and fixed in Bouin’s solution for light microscopy. For electron microscopy, tissues were fixed by immersion in cold 3 per cent gluteraldehyde buffered with 0.67 M. S-collidine, pH 7.4, and postfixed in 2 per cent osmium tetroxide in the same buffer. Paraffin sections were stained with hematoxylin and eosin and aldehyde-fuchsin for identification of granules in beta cells by light microscopy. Tissues for electron microscopy were embedded in araldite and stained after sectioning with alcohol in uranyl acetate and lead citrate.

Individual islets were prepared by the method of Lacy and Kostianovsky. Four pools of ten islets were obtained from each pancreas. Each pool was independently extracted with acid alcohol and insulin was determined directly on a small aliquot from each pool.

Glucose (2.5 gm./kg.) was administered by stomach tube to rats anesthetized with pentobarbital. A polyethylene catheter was placed through the jugular vein into the superior vena cava for blood sampling. Tolbutamide (30 mg./100 gm.) was administered intravenously through the catheter. Theophylline (5 mg./100 gm.) and glucagon (2 µg./rat) were given alone to both control and tumor-bearing rats. When combined the dose of theophylline was 15 mg./rat and glucagon 2 µg./rat.

RESULTS

Histologic studies: Pancreatic tissue sections from twelve tumor-bearing and five control rats were examined by the light microscope. Although no apparent difference was observed in the number of islets of Langerhans per unit area, the islets from rats bearing growth hormone secreting tumors were two to three times larger in diameter than islets from the normal rats (figures 1 and 2). This increase seemed on visual inspection to be due both to hypertrophy and hyperplasia of the islet cell, since there appeared to be an increase in the number of nuclei per islet and an increase in the cytoplasmic area of each cell. An increase in the number of beta granules of islets from tumor-bearing rats was visible by both phase microscopy and aldehyde-fuchsin staining. These changes were equally apparent in electron micrographs (figures 3 and 4). The cytoplasmic area of the beta cells from tumor-bearing rats was increased and a marked increase in the number of granules in the beta cells was observed. The increase in granularity as evidenced by phase microscopy, special staining, and electron microscopy could reflect the increase in the amount of cytoplasm per cell and not a
FIG. 3. Electron micrograph of an islet from a normal female Wistar-Furth rat. Part of a cell nucleus can be seen. Membrane-bound beta granules are scattered throughout the cell cytoplasm. Final magnification was 23,000 X. Reduction 50 per cent.

FIG. 4. Electron micrograph of an islet from a female Wistar-Furth rat bearing growth hormone secreting tumor MtTW15. There is an apparent increase in the number of cytoplasmic granules of this cell when compared to figure 3. Final magnification was 23,000 X. Reduction 50 per cent.

real increase of granules per unit area of cytoplasm. The beta granules in the two sets of rats did not differ qualitatively in regard to size or structure.

**Insulin content of isolated islets:** Immunoreactive insulin content of the acid alcohol extracted isolated islets in six control Wistar-Furth rats was $5.7 \pm 0.15^* \text{ mU.}$ per islet. The mean insulin content of a single islet from six tumor-bearing rats was $20.6 \pm 0.17^* \text{ mU.}$, or four times the amount found in control rats. This difference was significant at the 0.1 per cent level. This study confirms the histologic impression of a major increase in beta granulation in tumor-bearing rats.

**Glucose tolerance and insulin release:** The concentrations of serum glucose and insulin of fed control and tumor-bearing rats are shown in figure 5. These values represent different groups of animals bled at each time period. No significant differences were observed. Also, when these rats fasted, the serum glucose levels at the twenty-four-hour and forty-eight-hour periods were not significantly different although the mean level was slightly lower in the tumor-bearing rats. However, a difference in serum insulin was evident at the forty-eight-hour period. In the control rats the concentration of serum insulin fell markedly but there was little change in the concentration of serum insulin in the tumor-bearing rats.

No significant differences in serum insulin were observed between fed control and fed tumor-bearing rats given glucose intragastrically. After a forty-eight-hour fast the serum insulin was higher in tumor-bearing rats before and after glucose intragastrically (figure 6). In both control and tumor rats the peak glucose rise was delayed. This delayed glucose absorption was presumably due to the experimental method (pentobarbital anesthesia and gastric intubation) since it was seen in
INSULIN STORAGE AND RELEASE IN RATS BEARING GROWTH HORMONE SECRETING TUMORS

Tolbutamide given intravenously to fed control and tumor-bearing rats resulted in comparable rises in serum insulin and a fall in serum glucose (data not shown). After a forty-eight-hour fast the control rats had lower serum insulin and responded minimally to tolbutamide intravenously (figure 7). However, the tumor-bearing rats had a higher fasting insulin and serum insulin increased significantly after tolbutamide. The fasting glucose values in these rats were low. Serum glucose did not fall significantly in either fasted control or tumor-bearing rats despite a rise in insulin. This may indicate some degree of resistance to endogenous insulin in fasted rats.

Insulin response to theophylline and glucagon (table 1): Theophylline given intravenously to fed tumor rats resulted in a higher and more rapid rise in serum insulin than in control rats. Similarly, glucagon given intravenously to fed rats resulted in a greater serum insulin concentration in tumor-bearing rats than in control rats. Also, it should be noted that the baseline insulin concentrations were lower in control than tumor-bearing rats in these experiments. In these particular animals it would appear that tumor-bearing rats were

FIG. 5. Fall in serum glucose and immunoreactive insulin (IRI) in control and tumor-bearing rats during a 48 hr. fast. Each circle represents thirty-two determinations at 0 time, thirteen to sixteen determinations at 24 hrs., and twenty-seven determinations at 48 hrs. Vertical lines are standard error of mean. Only at 48 hrs. was a significant difference noted in insulin levels (p < 0.01).

FIG. 6. Serum glucose and immunoreactive insulin (IRI) in nine Wistar-Furth rats bearing MtTW15 tumor and seven control Wistar-Furth rats following a 48 hr. fast and given glucose intragastrically. Glucose levels were comparable in the two groups of rats. However, serum insulin is elevated throughout the test in the tumor-bearing rats. Values are mean ± S.E.M.

TOLBUTAMIDE TOLERANCE TEST (2 DAY FAST)

FIG. 7. Glucose and immunoreactive insulin (IRI) levels following intravenous injections of tolbutamide in seven tumor-bearing and five control rats fasted 48 hrs. Serum glucose was significantly lower (p < 0.01) in tumor-bearing than in control rats throughout the test. A fall in glucose did not occur in the tumor rats after intravenous injection of tolbutamide despite a rise in serum insulin (p < 0.05 for -10 vs. 15 min.). The insulin rise in the control rats was not statistically significant. The tumor rats had higher serum insulin (p < 0.01) than control only at 15 min. Values are mean ± S.E.M.
Table 1

<table>
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<th>Treatment</th>
<th>Time (min.)</th>
<th>0</th>
<th>1</th>
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<tr>
<td>Glucagon (2 μg./rat)</td>
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<td>MtTW15 (N = 5)</td>
<td>119(21)</td>
<td>272(55)</td>
<td>304(54)</td>
<td>354(42)</td>
<td>289(68)</td>
<td>269(83)</td>
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<td>44(3)</td>
<td>59(13)</td>
<td>132(34)</td>
<td>167(30)</td>
<td>189(33)</td>
<td>231(18)</td>
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<td>Glucagon (2 μg./rat) + theophylline</td>
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<td>MtTW15 (N = 6)</td>
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<td>185(16)</td>
<td>176(20)</td>
<td>188(21)</td>
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<td>79(20)</td>
<td>116(16)</td>
<td>98(27)</td>
<td>68(21)</td>
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<td>370(69)</td>
<td>381(71)</td>
<td>418(71)</td>
<td>340(57)</td>
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<td>78(26)</td>
<td>115(22)</td>
<td>133(31)</td>
<td>120(34)</td>
<td>172(34)</td>
<td>257(37)</td>
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Serum insulin concentrations in MtTW15 bearing and control rats. All values are mean μU./ml. serum (± S.E.M.).

Theophylline (5 mg./100 g.) and glucagon (2 μg./rat) were given intravenously to rat groups in order to study the response of insulin secretion in tumor-bearing rats. Theophylline increased insulin secretion more than glucagon, with a peak response at 20 minutes. The combined effect of theophylline and glucagon resulted in a greater increase in insulin secretion, with a peak response at 10 minutes.

DISCUSSION

These studies have demonstrated surprisingly little alteration in serum glucose concentrations in rats bearing tumors secreting growth hormone. Hyperinsulinemia was also not a prominent feature in fed tumor-bearing rats. Although hyperinsulinemia was seen in some groups of tumor-bearing rats when compared to the controls in a particular experiment, pooling of the data for insulin in all feeding control and fed tumor rats resulted in no significant difference. In fact, under normal circumstances of feeding there was neither the hyperglycemia nor hyperinsulinemia which is characteristic of human acromegaly. Only after prolonged fasting or when insulin secretion was stimulated by theophylline and glucagon was it possible to demonstrate markedly elevated circulating levels of insulin in peripheral serum. While these tumors are known to secrete prolactin, the contribution of this hormone to the observed effects must remain conjectural. Although there is little evidence that our tumor-bearing rats developed a prominent resistance to insulin, the presence of elevated plasma insulin concentrations and normal glucose concentrations in forty-eight-hour fasted rats could be interpreted as evidence for some degree of insulin resistance.

Despite the fact that evidence of increased insulin secretion in response to peripheral insulin resistance was relatively slight in tumor-bearing rats, there was an increase in the size and beta cell content of the islets and a four-fold increase in insulin content per islet. This suggests an effect of growth hormone on beta cell hyperplasia and insulin storage. Our findings extend previous microscopic studies of Ogilvie, who found a stimulation of growth of islet cells in normal and diabetic rabbits given growth hormone injections. Further support of the trophic action of growth hormone on the pancreatic beta cell has been provided by the studies of Kipnis and Stein and of Merimee, Burgess and Rabinowitz. These authors have found that hypopituitary subjects have a decreased ability to secrete insulin in response to a glucose load. This deficiency can be restored by growth hormone administration.

While this manuscript was in preparation Martin et al. reported similar studies of rats bearing tumor MtTW15. In their experiments tumor-bearing rats pair fed to control rats were euglycemic and had increased plasma insulin before and after glucose administration. In their pair fed tumor-bearing rats, islet volume determined by a histologic technic was estimated to be two to three times that of control rats. Insulin secretion in vitro by pancreatic slices and by isolated islets was found to be two to three times greater than control rats. Islet insulin content per mg. of islet protein was also increased. The fact that our studies, which were conducted with ad libitum fed rats, are in substantial agreement with those of Martin et al. indicates that dietary factors are not instrumental in the development of islet hyper trophy and the abnormalities of islet function which we have observed.

The increased stored insulin in our rats with experimental acromegaly was not easily mobilized by glucose or tolbutamide administration. In order to provide a more potent stimulus for insulin release we have utilized the intravenous administration of theophylline and glu-
cagon. By itself, glucagon is a well-recognized releaser of insulin both in vivo and in vitro. The effects of glucagon were potentiated by the addition of theophylline. This agent inhibits the specific phosphodiesterase which degrades 3',5' cyclic adenosine monophosphate and allows higher concentrations of this nucleotide to accumulate in islet tissue resulting in increased insulin release. The combination of provocative agents clearly demonstrated the increased insulin secretory potential of the tumor-bearing rats.

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REFERENCES