EFFECTS OF GROWTH HORMONE AND OF HYPOPHYSECTOMY ON THE RELEASE OF INSULIN FROM RAT PANCREAS IN VITRO

By
P. R. Bouman and R. S. Bosboom

ABSTRACT

The release of insulin from ligated rat pancreas in vitro was measured after pre-incubation of the tissue at either high or low glucose concentrations. Insulin release was estimated by determining the glucose uptake of rat hemidiaphragms after joint incubation with the pancreatic tissue. Addition of bovine growth hormone (GH) to the pre-incubation medium in a concentration of 0.1 mg/ml significantly increased the response to glucose stimulation. Pancreatic tissue of hypophysectomized rats failed to respond to GH in vitro, although previous hypophysectomy was shown to reduce the response to glucose stimulation. Pretreatment of hypophysectomized rats with GH in doses which restored the growth rate to normal, caused the reappearance of the effect of GH in vitro. The glucose sensitivity of pancreatic tissue was also increased by pretreatment with GH in vivo alone.

It is concluded from these findings that GH may increase the sensitivity of the β-cells to glucose by direct action. Since hypophysectomy exerts an opposite effect, it is suggested that physiological levels of endogenous GH may be involved in the regulation of insulin release.

Growth hormone induced β-cell damage with concurrent diabetes is a well established phenomenon. On the other hand, various data suggest that growth hormone (GH) also exerts a pancreatrophic action under certain conditions and may even stimulate insulin release prior to impairing the β-cell function (reviewed by Ketterer et al. 1957). This concept is supported in particular by

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studies on plasma insulin-like activity in hypopituitary and acromegalic patients and in cats and rats subjected to hypophysectomy or treatment with GH (Randle 1954 a, 1954 b; Randle & Young 1956). Nevertheless, the available evidence for a direct stimulatory action of GH on insulin release has remained inconclusive (Young 1963) and direct measurements of the release of insulin from isolated pancreatic tissue have failed to demonstrate such an effect (Grodsky et al. 1962; Coore & Randle 1964). Recently, however, it has been reported by Pfeiffer (1965) that within minutes after intravenous injection of human GH into dogs, a substantial increase occurred in the insulin content of the pancreatic venous effluent, as determined by biological and immunological assay procedures.

In the present investigation the effect of bovine GH on insulin release was studied in an in vitro system at two different glucose concentrations, using isolated pancreatic tissue of the rat. As has been previously shown, insulin release in this system is stimulated by high concentrations of glucose in the incubation medium and by the addition of sulphonylurea drugs in vitro (Bouman 1960; Bouman & Gaarenstroom 1961; Pensuwan et al. 1963). In contrast to our earlier studies, ligated pancreatic tissue was used in the present experiments in order to exclude various disturbing influences related to the presence of exocrine parenchyma.

MATERIALS AND METHODS

The technique used to measure the release of insulin in vitro has been amply described on previous occasions (Bouman 1960; Bouman & Gaarenstroom 1961). Essentially the experimental design is as follows. Pancreatic tissue is pre-incubated at a specific glucose concentration for 1 hour and subsequently jointly incubated with a hemidiaphragm for 30 minutes in the absence of glucose. Insulin released during the latter phase adheres to the diaphragm and is allowed to exert its action during a third incubation period of 1 hour, in which the hemidiaphragm is incubated alone at a glucose concentration of 2 mg/ml. The uptake of glucose over this period is measured. Release of insulin is indicated by a positive difference in glucose uptake between the test diaphragm and a control diaphragm incubated in the absence of pancreatic tissue.

Glucose was estimated with anthrone reagent according to Seifter et al. (1950). The incubations were carried out in a Dubnoff metabolic incubator at 37°C in Krebs-Ringer bicarbonate solution saturated with 95% (v/v) and 5% (v/v) CO₂. Bovine GH* (lot nr. 19693, prepared by N.V. Organon according to Wilhelmi et al. 1948), with a tibia test potency of 1.6 USP units per mg, was dissolved in saline (pH 8.5) and diluted with Krebs-Ringer solution containing the appropriate amount of glucose. To avoid direct exposure of the test diaphragm to GH, the addition of GH was confined to the preincubation period. Consequently, any effect on glucose uptake during

* We are indebted to Dr. J. D. H. Homan of N.V. Organon, Oss, for generously supplying this GH preparation.

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the third incubation period should be accounted for in terms of insulin release. Furthermore, control experiments have revealed that GH when added directly to incubated diaphragms, failed to affect glucose uptake either in the presence or absence of insulin.

Pancreatic tissue was obtained from female rats of 140–160 g body weight. Degeneration of the exocrine parenchyma had been induced by ligating the caudal portion of the pancreas 4 weeks previously (Miahle & Meyer 1961). The tissue added per vessel consisted of 4 pieces with a total weight of 20 mg. Tissue of a single rat was divided over 2 or 4 vessels, depending on the number of different preincubation media used in a particular experiment. Diaphragms were obtained from female rats of 120–125 g body weight, which had been fasted overnight. Pairs of test and control diaphragms were derived from the same rats.

The results were tested for statistical significance by means of Student’s t-test. The level of significance was chosen at a bilateral tail probability \( P \leq 0.05. \)

RESULTS

In Table 1 the results are shown of a series of experiments in which ligated pancreatic tissue of normal rats was preincubated in a medium containing 0.1 mg of GH per ml. The effect of GH was studied simultaneously in the presence of high and low concentrations of glucose. At a glucose concentration of 1 mg/ml, the addition of GH did not affect the release of insulin. When, however, the glucose concentration was increased to 3 mg/ml the presence of GH caused a significant increase in insulin release. Apparently high concentrations of glucose are required for the occurrence of a GH hormone effect. An alternative possibility becomes evident, however, when the response to elevated glucose levels is calculated. As shown in Table 1 this response was markedly

Table 1.
Effect of GH in vitro on the release of insulin from ligated pancreatic tissue of normal rats.

<table>
<thead>
<tr>
<th>Preincubation medium</th>
<th>Glucose uptake diaphragm mg/g/h</th>
<th>difference ± s. e. m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glucose mg/ml</td>
<td>test diaphragm</td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
<td>3.62 (16)</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>4.11 (15)</td>
</tr>
<tr>
<td>GH 0.1 mg/ml</td>
<td>1.0</td>
<td>3.63 (16)</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>4.78 (16)</td>
</tr>
<tr>
<td>Effect of increase in glucose concentration</td>
<td>GH</td>
<td>0.34 ( \pm 0.16 )</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>1.02 ( \pm 0.17 )</td>
</tr>
</tbody>
</table>
Table 2.
Effect of hypophysectomy on the release of insulin from ligated pancreatic tissue in vitro.

<table>
<thead>
<tr>
<th>Ligated pancreas</th>
<th>Glucose concentr. mg/ml</th>
<th>Glucose uptake diaphragm mg/g/h</th>
<th>difference ± s. e. m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>test diaphragm</td>
<td>control diaphragm</td>
</tr>
<tr>
<td>Normal</td>
<td>1.0</td>
<td>4.38 (16)</td>
<td>3.45 (16)</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>5.25 (16)</td>
<td>3.35 (16)</td>
</tr>
<tr>
<td>Hypex (3 weeks)</td>
<td>1.0</td>
<td>4.92 (16)</td>
<td>3.97 (16)</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>4.69 (16)</td>
<td>3.43 (16)</td>
</tr>
</tbody>
</table>

Effect of increase in glucose concentration
- Normal: 0.97 ± 0.19
- Hypex: 0.31 ± 0.16

Increased by the presence of GH in the pre-incubation medium. Addition of an indifferent protein like serum albumin did not affect insulin release.

Data concerning the effect of hypophysectomy on insulin release in vitro are presented in Table 2. Hypophysectomy was performed 3 weeks prior to the actual experiment. When pre-incubated at a glucose concentration of 4 mg/ml, ligated pancreas of hypophysectomized rats appeared to release significantly less insulin than tissue of intact rats. At a glucose concentration of 1 mg/ml such a difference was not detectable. Thus, the stimulating effect of high glucose concentrations appeared to be diminished by previous hypophysectomy.

Table 3.
Effect of GH in vitro on the release of insulin from ligated pancreatic tissue of hypophysectomized rats.

<table>
<thead>
<tr>
<th>Preincubation medium</th>
<th>Glucose uptake diaphragm mg/g/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>test diaphragm</td>
</tr>
<tr>
<td>glucose mg/ml</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>GH</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1 mg/ml</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Effect of increase in glucose concentration
- GH control: 0.61 ± 0.28
- NS

0.59 ± 0.24
Gain in body weight induced by subcutaneous administration of bovine GH for 3 days to hypophysectomized rats.

Table 3 gives the results of a series of experiments in which pancreatic tissue of hypophysectomized rats was exposed to GH in vitro. The release of insulin was not influenced by the addition of GH to the medium, either at high or at low glucose concentrations. It would appear, therefore, that hypophysectomy

Table 4.
Effect of GH in vitro on the release of insulin from ligated pancreatic tissue of hypophysectomized rats pretreated with 0.2 mg of GH for 3 days.

<table>
<thead>
<tr>
<th>Preincubation medium</th>
<th>Glucose uptake diaphragm mg/g/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glucose mg/ml</td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>GH</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1 mg/ml</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Effect of increase in glucose concentration

- control: 0.41 ± 0.20  
- GH: 0.88 ± 0.16
rendered the pancreas refractory to the effect of GH, previously observed in tissue of normal rats.

Effects of GH added in vitro may be considered as more or less acute and their occurrence might depend on the previous functional state of the β-cells. For this reason the in vitro effect of GH on pancreatic tissue of hypophysectomized rats was re-investigated after pretreating the animals subcutaneously with 0.2 mg GH per day for three consecutive days. As is shown by the data in Fig. 1 this dosage of GH caused a significant increase in body weight. The weight gain of 6.6 ± 0.9 g over this period roughly equals the normal growth rate of intact rats of similar sex and age. The effect of GH in vitro on pancreas of pretreated animals is summarized in Table 4. Addition of GH to a preincubation medium containing 4 mg/ml of glucose caused a significant increase in the release of insulin. At a glucose level of 1 mg/ml, GH was not active in vitro. The calculated response to glucose stimulation seemed to be enhanced by the presence of GH in the incubation fluid, but full statistical significance was not reached. In general, however, it can be stated that by pretreating hypophysectomized rats with GH in vivo, the response to GH in vitro was restored.

The in vivo administration of 0.2 mg of GH alone failed to affect insulin release from pancreatic tissue of hypophysectomized rats. On raising the daily dose to 0.5 mg, however, insulin release was increased when tested at a glucose concentration of 4 mg/ml (Table 5). A slight effect was also observed at a glucose level of 1 mg/ml. The calculated response to an increase in glucose concentration tended to be higher in GH pretreated tissue, but the difference

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### Table 5.

Effect of pretreatment of hypophysectomized rats with 0.5 mg of GH for 3 days on the release of insulin from ligated pancreatic tissue in vitro.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose concentr. mg/ml</th>
<th>Glucose uptake diaphragm mg/g/h</th>
<th>difference ± s. e. m.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>test diaphragm</td>
<td>control diaphragm</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>4.16 (20)</td>
<td>3.35 (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.60 (20)</td>
<td>3.33 (20)</td>
</tr>
<tr>
<td>GH 0.5 mg/day s. c.</td>
<td>1.0</td>
<td>4.48 (19)</td>
<td>3.25 (19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.98 (19)</td>
<td>3.05 (19)</td>
</tr>
</tbody>
</table>

Effect of increase in glucose concentration

<table>
<thead>
<tr>
<th></th>
<th>Glucose uptake diaphragm mg/g/h</th>
<th>difference ± s. e. m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>0.46 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>GH</td>
<td>0.70 ± 0.21</td>
<td>NS</td>
</tr>
</tbody>
</table>

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did not reach statistical significance. As is shown by Fig. 1 the daily administration of 0.5 mg of GH stimulated the growth rate to supra-normal values.

**DISCUSSION**

Before considering the results of the present investigation, attention should be drawn to various problems concerning the method used for the study of insulin release in *vitro*.

Insulin released from pancreatic tissue represents only a minor fraction of the total insulin in store. Recent estimations of the insulin content in rat pancreas by Davidson *et al.* (1963) yielded values of 2.8 U per gram of tissue. In contrast, plasma concentrations of insulin as detectable by immuno-assay procedures are extremely low, 100 ìU per ml representing a peak value. The half-time for the disappearance of tracer-insulin from the peripheral circulation in rats has been estimated to approximate 20 minutes (Goodner & Freinkel 1960; Ricketts *et al.* 1963). Assuming a 50% removal of insulin from portal venous blood by the liver (Madison *et al.* 1959; Mortimore & Tietze 1959), it may be calculated that in medium weight rats the release rate needed to maintain a plasma level of 100 ìU per ml, will amount to about 220 mU per 24 hours, representing 10% of the insulin stored in the pancreas. It will be clear, therefore, that even the most potent stimuli for the release of insulin must operate for many hours in order to induce a demonstrable fall in pancreatic insulin content. In fact, the earliest manifest decrease in pancreatic insulin induced by sulphonylurea drugs has been reported to occur between 3 and 12 hours after the beginning of treatment (Pfeiffer *et al.* 1957; Root 1957; Grossky & Peng 1959; Dulin & Miller 1959). These considerations suggest that any change in insulin release in *vitro*, induced by the short-term exposure to a substance added in *vitro*, does not occur secondarily to a change in the insulin content.

*In vitro* studies using pancreatic tissue of hypophysectomized rats are complicated by the fact that hypophysectomy causes marked atrophy of the exocrine parenchyma, which in turn causes an increased density of islets in the pancreatic tissue (Griffiths 1941; Bryans *et al.* 1952; Kinash *et al.* 1953). Since the total insulin content of the rat pancreas is not appreciably altered by hypophysectomy (Haist 1940; Fraenkel-Conrat *et al.* 1941; Griffiths 1941; Haist 1944), the insulin content per unit of weight may actually increase. This may explain why in preliminary experiments with non-ligated pancreatic tissue of long-term hypophysectomized rats, the release of insulin in *vitro* was found to be increased, whereas a normal release was observed one week after hypophysectomy. To exclude such pseudo-effects due to changes in the islet-acinar
ratio, ligation of the caudal part of the pancreas causing a complete degeneration of all acinar tissue, was carried out as a routine in the present series of experiments.

The use of ligated pancreatic tissue also serves another purpose. During incubation of intact rat pancreas, enzyme material is released into the medium which is capable of degrading and inactivating insulin. This material may be related to the active pancreatic protein (APP) recently described by Bingle & Czerkawski (1964). Experiments with $^{131}$I-labelled insulin indicate that very little degrading activity is released from ligated pancreatic tissue in amounts used at present (Bouman & Bakker, unpublished results). For these reasons and in view of the fact that enzyme protein synthesis in the acinar tissue is decreased after hypophysectomy (Barrett et al. 1955; Baker et al. 1956) and increased by hormonal replacement including GH (Baker et al. 1961), the use of ligated pancreatic tissue seemed a prerequisite in this study.

Our results indicate that in the presence of high glucose concentrations, bovine GH is capable of enhancing the release of insulin by a direct action at the level of the $\beta$-cells. Since at low glucose concentrations GH when added in vitro fails to affect insulin release, it may be concluded that under these conditions the slope of the concentration-effect curve of glucose is increased. Thus, GH would appear to potentiate the response of the $\beta$-cells to the stimulus of high glucose concentrations. It should be mentioned, however, that treatment of hypophysectomized animals with 0.5 mg of GH was found to exert some effect at low glucose levels too. Apparently, sensitization in the sense of a shift to the left of the glucose concentration-effect curve may follow the administration of GH in vivo. In addition, the possibility cannot be excluded that a dose-dependent response to GH is enhanced by glucose in vitro. Further experiments will be necessary to clarify these points.

The action of GH observed in the present experiment is at variance with results obtained by other workers using isolated pancreas preparations. Perfusion of isolated rat pancreas with rat anterior pituitary extracts and bovine GH was reported to inhibit insulin release induced by high glucose concentrations in the perfusate (Anderson & Long 1947). In these studies the blood sugar response of adrenomedullated-diabetic-hypophysectomized rats was used for the bio-assay of insulin. This procedure seems less dependable in view of the fact that a hyperglycaemic substance other than glucagon is released from the pancreas under the influence of GH (Fod & Galansino 1962). This objection does not apply to the use of the radio-immunoassay of insulin in perfusion studies with isolated rat pancreas as conducted by Grodsky et al. (1962), where no effect of GH on insulin release was observed. In the latter experiments, however, GH was not tested in the presence of high glucose concentrations (Grodsky, personal communication). Coore & Randle (1964) adding GH to rabbit pancreas slices in vitro, were unable to detect any stimu-
lating effect of bovine GH, either at high or at low glucose concentrations. In fact, human GH even caused some depression of the release at high glucose levels. There is no explanation for the latter discrepancies, but differences in experimental conditions may be responsible, since rat pancreas failed to give consistent release of insulin in the in vitro system used by these authors (Randle 1964). In contrast to these negative findings, Pfeiffer (1965) recently reported that the release of insulin from dog pancreas in situ rapidly increased after intravenous administration of human GH. The stimulation was even more intense than that exerted by glucose or tolbutamide under similar conditions.

The concentration of GH effective in stimulating the release of insulin in vitro undoubtedly exceeds physiological levels. Concentrations of 0.01 mg per ml were not found to be active. On the other hand, certain bovine GH preparations are known to affect the transport of amino acids into rat diaphragm in vitro even in concentrations of 0.001 mg per ml (Kostyo & Engel 1960). This raises the question whether GH might be actually involved in the regulation of insulin release in vivo. In this connection it should be stressed, however, that hypophysectomy caused definite effects on the release of insulin in vitro, which could be alleviated by administering bovine GH either in vivo or by joint administration in vivo and in vitro. Hypophysectomy was found to diminish the sensitivity of the tissue to glucose stimulation, an effect opposite to that of GH in vitro on tissue of intact animals. Secondly, the decrease in insulin release in the presence of high glucose levels as induced by hypophysectomy appeared to be reversed by pretreatment with GH in vivo, although relatively large amounts of hormone were needed (0.5 mg per rat per day). Finally, hypophysectomy abolished the response to GH in vitro whereas pretreatment with 0.2 mg of GH per day induced its reappearance. Since this dosage normalized the growth rate it can be regarded as roughly equivalent to the daily output of endogenous GH.

The evidence as outlined above suggests the existence of a certain degree of hypophyseal control over insulin release by means of a GH-mediated mechanism, which operates by increasing the glucose sensitivity of the β-cell above a certain GH-independent basal level. The short latency of the effect of GH suggests that its action is directed at the level of the actual mechanism of insulin release rather than at the process of synthesis. This view is substantiated by considerations presented previously in this discussion with regard to the interpretation of effects on insulin release in vitro.

Additional support for an action of GH as presently postulated, may be derived from a number of recent clinical reports. In patients with active acromegaly the increase in plasma insulin following glucose loading is often strikingly enhanced in the presence of a normal or slightly decreased glucose tolerance (Karam et al. 1963; Cerasi & Luft 1963, 1964). Likewise, the administration of human GH to hypopituitary patients was found to result in a
remarkable enhancement of the plasma insulin increase after glucose loading (Stein et al. 1962; Kipnis & Stein 1964; Luft & Cerasi 1964). The latter phenomenon could not be attributed to excessive hyperglycaemic stimulation due to the concomitant decrease in glucose tolerance, since induction of an identical degree of hyperglycaemia one week after the end of the GH hormone administration gave a normal plasma insulin response (Luft & Cerasi 1964). These data, therefore, seem to indicate that primary elevation of the endogenous GH level also enhances the sensitivity of the β-cell to glucose stimulation in vivo. It remains uncertain at present whether similar effects might be brought about by a secondary elevation of the plasma GH level as induced by certain metabolic stimuli (Roth et al. 1963, 1964).

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