Do insulin and the insulin like growth factors (IGFs) stimulate growth of the exocrine pancreas?

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SUMMARY Previous *in vivo* studies have suggested a long term regulatory role for insulin in the exocrine pancreas.¹ Furthermore, we reported that pancreatic acini have specific receptors for IGF I and II,² and, using different techniques (acid washing, trypsinisation, electron microscope autoradiography), that CCK₈ reduces the internalisation of IGF II.^{3,4} To now directly study the long term role for IGF and insulin in the exocrine pancreas we used AR42J cells, a rat cell line that is derived from a transplantable tumour of the acinar pancreas. Hormone binding studies with ¹²⁵I-labelled hormones indicated that those cells have insulin receptors, relatively fewer receptors for IGF II but in contrast with normal acini no detectable IGF I receptors. Insulin at concentrations as low as 1 nM stimulated the growth of AR42J cells, as measured by an increase in cell number, DNA and protein content. At 100 nM insulin had maximal effects stimulating the growth by about 50%. IGF I and II had only very weak growth promoting effects probably due to their interaction with the insulin receptor. Additionally insulin increased amylase synthesis over the same concentration range that it stimulated growth. But immunoprecipitation studies revealed that insulin induced a selective increase of amylase synthesis over general protein synthesis. These studies indicate, therefore, that insulin is a growth promoting hormone for AR42J cells and that additionally it seems to specifically regulate amylase synthesis. The role for the IGFs in the exocrine pancreas, however, still remains to be determined.

The concept of an islet-acinar relationship has a morphological basis on the demonstration of an islet-acinar portal blood system and a functional basis on the appreciation that islet peptides such as insulin show both *in vivo* and *in vitro* regulations of the acinar cell function.⁵ To investigate direct short term effects of insulin on the exocrine pancreas, isolated rat and mouse pancreatic acini have been studied *in vitro*. Isolated acini have specific high affinity receptors for insulin⁶⁻⁸ and insulin, within a few hours, regulates the membrane transport of sugars, the synthesis of protein, and the sensitivity to cholecystokinin.⁹⁻¹² Because of the viability of isolated acini of only up to four hours, long term functions of insulin can not be studied in this system. There are, however, clinical and experimental hints that insulin might have important long term functions for the exocrine pancreas: type I diabetes mellitus in man is associated

with abnormalities of pancreatic exocrine function, pancreatic weight is often decreased and these defects are correlated with the duration and severity of the disease.¹³⁻¹⁷ In diabetic rats pancreatic weight is reduced, the content of enzymes is altered and, in particular, the content of messenger RNA levels for amylase is diminished.¹¹⁸⁻²² Whether long term regulations of insulin are mediated directly by insulin or indirectly by influencing other hormones, however, is still unclear. We, therefore, used AR42J cells, a tumour cell line acinar in origin²³ where potential long term insulin effects could be directly studied.

Methods

CELL CULTURE

AR42J cells, a cell line that was derived from a transplantable tumour of the acinar pancreas,²³ were maintained in subconfluent monolayer culture in Dulbeccos Minimum Essential Medium Eagle

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(DMEM) supplemented with either 5 or 10% fetal calf serum, glutamine, penicillin, streptomycin, amphotericin B and glucose in a humidified incubator at 37 °C at 5% CO₂/95% air.²⁴ For hormone binding studies and measurements of the biological effects of insulin, cells were removed from their flasks by washing with calcium free, phosphate buffered saline and trypsinisation. Cells were then resuspended in culture medium and plated onto 35-mm dishes at a density of $2-4 \times 10^5$ cells/ml.

HORMONE BINDING STUDIES

IGF-I, IGF-II, and insulin were iodinated by a chloramine-T method.^{24, 25} AR42J cells were incubated at 22 °C in 1 ml HEPES-buffered Ringer buffer (HR) and 0.5% (wt/vol) bovine plasma albumin of low insulin like activity. ¹²⁵I-insulin (100 pM), ¹²⁵I-IGF-I (60 pM) or ¹²⁵I-IGF-II (60 pM) and various concentrations of unlabelled ligand were added as indicated. At specified times, incubation media were removed, the cells washed twice with ice cold Tris-saline, scraped off each dish and the radioactivity associated with the cells measured in a gamma scintillation counter.²⁴

BIOSYNTHETIC LABELLING OF AR42J CELLS

AR42J cells were plated onto 35-mm dishes with various factors added as specified. Two days later, cells were washed with methionine free culture medium and then incubated for one hour at 37 °C in methionine free medium in the presence and absence of various hormones plus ³⁵S-methionine (20 µCi/ml). Cells were then lysed by incubation in DMEM H21 containing 1% Triton X100. Incorporation of ³⁵S-methionine into protein was measured using TCA precipitability and counting the radioactivity by liquid scintillation.²⁴

IMMUNOPRECIPITATION

Antibodies were raised in white rabbits against bovine trypsin, bovine alpha-chymotrypsin and rat alpha-amylase.²⁴ Immunopurification of biosynthetically labelled pancreatic enzymes from cell lysates were carried out using the antibodies bound to *Staphylococcus aureus* (IgG Sorb).²⁴

SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

Electrophoresis was done by the method of Laemmli.²⁶ After electrophoresis, the gels were autoradiographed with Kodak X-Omat film and the autoradiographs scanned with a Soft laser scanning densitometer.

ASSAYS

DNA, protein, and amylase were measured as described previously.²⁴

Fig. 1 Competition-inhibition curves for 100 pM ¹²⁵I-insulin binding in AR42J cells. Binding was carried out for 90 min in the presence of various concentrations of insulin, insulin analogues, and unrelated hormones (DOP insulin, desoctapeptide insulin; EGF, epidermal growth factor; PP, bovine pancreatic polypeptide). Data are the mean $\pm$ SD of triplicate samples from a representative of five experiments.

Results

¹²⁵I-LABELLED HORMONE BINDING TO RECEPTORS IN AR42J CELLS

Total ¹²⁵I-insulin binding to AR42J cells at 22 °C was half maximal after 15 minutes and maximal after 60 minutes (data not shown). Non-specific binding was less than 20% of total binding. Competitive inhibition curves revealed that proinsulin was 10% as potent as insulin and desoctapeptide insulin 1% as potent (Fig. 1). The concentration of unlabelled insulin that reduced the binding of ¹²⁵I-insulin by 50% was 4.8 ± 0.4 nM (mean $\pm$ SEM, $n = 5$). ¹²⁵I-insulin like growth factor I (IGF I) showed no saturable binding (Fig. 2, bottom) whereas ¹²⁵I-IGF II bound to AR42J cells in a saturable manner (Fig. 2, top). This binding, however, was lower than that seen in rat pancreatic acini.^{2, 3} Thus, these data provide evidence that AR42J cells have insulin receptors similar to those in normal pancreatic acini, some IGF II receptors but no receptors for IGF I.

EFFECT OF INSULIN AND THE IGFS ON THE GROWTH OF AR42J CELLS

Insulin stimulated the growth of AR42J cells in a dose dependent manner as measured by an increase in DNA and protein content (Figs 3, 4). The insulin effect was detectable at a concentration between 0.3 and 0.7 nM, was one-half maximal at 5 nM, and maximal at 100 nM increasing the DNA-content by about 50% ($46.1 \pm 10.9\%$; mean $\pm$ SEM; $n = 11$) (Fig. 3). The growth promoting effect of insulin was already seen after one day of incubation and was still present after three days (data not shown). Pure

Fig. 2 Time course of ¹²⁵I-IGF-II (top) and ¹²⁵I-IGF-I (bottom) binding to AR42J cells (closed circles). Total ¹²⁵I-hormone binding to AR42J cells is expressed as % total radioactivity bound/mg protein. Open circles: non-specific binding was determined with labelled hormone in the presence of 100 nM semipurified IGF. Open triangles: ¹²⁵I-hormone binding in the presence of 1 μ M (top) and 10 μ M (bottom) insulin. Values are the mean $\pm$ SD of triplicate samples from a representative of three experiments (from ref. 24).

synthetic IGF-I also stimulated growth but this effect was much weaker than that of insulin with a significant effect seen only at 100 nM (Fig. 4). Natural rat IGF-II like IGF-I stimulated growth only at concentrations of 100 nM (data not shown).

EFFECT OF INSULIN ON AMYLASE ACTIVITY AND SYNTHESIS

Insulin increased amylase activity in AR42J cells over the same dose range it stimulated growth. But the stimulatory effect of insulin on amylase activity was greater than its stimulatory activity on growth ($91.0 \pm 15.4\%$ v $46.1 \pm 10.9\%$; $n = 23$) (data not shown). To measure not only insulin effects on amylase activity but on synthesis, cells were grown in the presence of maximal concentrations of insulin for two days and pulsed with ³⁵S-methionine. Insulin treated cells incorporated $51.0 \pm 4.1\%$ (mean $\pm$ SEM, $n = 4$) more ³⁵S-methionine into protein than did control cells. Immunoprecipitation of ³⁵S-methionine labelled amylase, however, revealed that insulin stimulated amylase synthesis by $143 \pm 21\%$ (mean $\pm$ SEM, $n = 3$) (Fig. 5).

Fig. 3 Effect of increasing insulin concentrations on DNA levels in AR42J cells. Cells were plated onto 35-mm dishes. After 24 h, media were removed and fresh media with either no or increasing insulin concentrations were added. After a further 24 h, media and insulin concentrations were replaced and DNA levels were determined the following day (from ref. 24).

Fig. 4 Effect of increasing insulin and IGF I concentrations on protein levels in AR42J-cells. Further details see Fig. 3 (from ref. 24).

Discussion

Insulin belongs to a family of hormones that includes insulin like growth factors I and II.²⁴ IGF I and II at physiological concentrations are potent growth promoting peptides in various tissues.²⁴ Insulin too can stimulate the growth of various tissues but usually at higher than physiologic concentrations.²⁷⁻²⁹ Insulin at high concentrations can interact with the receptor for IGF I, but not IGF II whereas both IGF I and II at high concentrations can interact with the insulin receptor.²³⁰⁻³³ We have now found that insulin stimulates the growth of a pancreas derived cell line and give evidence that this effect is mediated through the insulin receptor itself: the effect of insulin was seen at physiologic concentrations, the same concen-

Fig. 5 Polyacrylamide-SDS electrophoresis of immunoprecipitated amylase. AR42J cells were plated onto 35-mm dishes. After 24 h, the media were changed and fresh media with 100 nM insulin were added. After a further 48 h, media were removed and cells were pulse labelled for 1 h with ^{35}S -methionine. Cells were then lysed with Triton X-100 and the lysate immunoprecipitated and subjected to electrophoresis. An autoradiogram from the resulting fixed dried gel is shown. Lane A, lysate from control cells immunoprecipitated with anti-amylase antiserum; lane B, lysate from insulin treated cells; and lane C, lysate from insulin treated cells immunoprecipitated with non-immune rabbit serum (from ref. 24).

trations where unlabelled insulin displaced ^{125}I -insulin from its receptor; no receptors for IGF-I could be detected in AR42J cells; IGF-I had only a weak potency in stimulating the growth; this weak potency of IGF-I paralleled its potency in displacing ^{125}I -insulin from its receptor; insulin did not displace ^{125}I -IGF-II from its receptor; and finally the weak potency of IGF-II in stimulating the growth could be explained by an interaction of IGF-II with the insulin receptor.

In addition to its growth promoting effect in AR42J cells, insulin selectively increased the content and synthesis of amylase. This effect was much more pronounced than the growth promoting effect but occurred over the same concentration range suggesting that it was also mediated through the insulin receptor. In diabetic rats amylase concentrations are very low as well as mRNA levels for amylase and these levels can be restored after administration of insulin for several days.^{20, 22} From these *in vivo* studies, therefore, rose the possibility that insulin has long term effects on the exocrine pancreas such as increasing amylase synthesis. The present *in vitro* studies with a pancreas derived cell line give support to a potential long term regulatory role of insulin in the exocrine pancreas and that this regulatory function is a direct one.

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