Insulin-like growth factor I does not inhibit insulin secretion in adult human pancreatic islets in tissue culture

Décio L Eizirik, Anna Skottner¹ and Claes Hellerström

Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden; Department of Pharmacology, Pharmacia Kabi Peptide Hormones, Stockholm, Sweden


Insulin-like growth factor I (IGF-I) has been found to increase insulin sensitivity and suppress insulin secretion, thereby having a potential interest as a therapeutic agent for non-insulin-dependent diabetes mellitus (NIDDM). The aim of the present study was to investigate the direct actions of IGF-I (400 ng/ml) on human pancreatic islets, or on rat pancreatic islets, during a 48 h period in tissue culture. Insulin-like growth factor I did not affect medium insulin accumulation, DNA or insulin content or short-term glucose-induced insulin release of human islets. However, in rat islets the peptide induced a significant decrease in the insulin increase ratio in response to 16.7 mmol/l glucose. In conclusion, the present data suggest that IGF-I does not directly affect the function of human pancreatic β-cells. If this in vitro data can be extrapolated to the in vivo situation, it suggests that the observed inhibitory effects of IGF-I on serum insulin levels may be secondary to peripheral effects of the peptide.

Décio L Eizirik, Department of Medical Cell Biology, Biomedical, PO Box 571, S-751 23 Uppsala, Sweden

Insulin-like growth factor I has been shown to increase glucose disposal and suppress insulin secretion in control subjects (1–3) and in patients with non-insulin-dependent diabetes mellitus (NIDDM) (1, 2, 4); these effects are beneficial in states associated with insulin resistance (1, 2). It remains to be determined if the suppressive actions of IGF-I on insulin release are effected directly at the β-cell level. In favour of this hypothesis, it has been shown previously that rodent β-cells possess IGF-I receptors (5), and that IGF-I acutely (10–20 min of exposure) suppresses glucose-induced insulin release either from adult rat β-cells (6) or from perfused rat pancreas (7). It is noteworthy that longer (3 days) exposure of rat islets to IGF-I in the presence of 2.8 mmol/l glucose leads to increased insulin accumulation in the culture medium (8). However, no such studies have been performed in human β-cells. Considering that any eventual clinical use of IGF-I will require chronic administration of the peptide, it is of relevance to test the effects of IGF-I on human islets over long periods of tissue culture. For this purpose, in the present study we investigated the effects of a 48 h exposure of human or rat islets to IGF-I on islet function.

Material and methods

Six human pancreata were excised from organ donors and transported to the Central Unit of the β-Cell Transplant, Brussels, and the islets isolated as described previously (9). The age of the donors was 39 ± 5 years (mean ± SEM) (range 25–57). Aliquots of the islet-enriched fraction were examined by electron microscopy (N = 6), revealing 5 ± 1% dead cells and 2 ± 1% acinar cells. The prevalence of insulin-positive and glucagon-positive cells was evaluated by light microscopic examination of immunocytochemically stained islets (10), indicating 50 ± 6% insulin-positive cells and 9 ± 1% glucagon-positive cells. Isolation of islets from adult Sprague-Dawley rats in Uppsala was performed as described previously (11).

Following isolation, human islets were cultured in Ham’s F-10 medium containing 6.1 mmol/l glucose and supplemented with 0.5% bovine serum albumin. The culture period was 3–8 days (5.5 ± 0.9 days) and the islets were subsequently sent by air to Uppsala, Sweden (for a detailed description of human islet transport and culture, see Ref. 9). The islets were then cultured free-floating in groups of 100–150 per dish in medium containing 5.6 mmol/l glucose and 10% (v/v) fetal calf serum (FCS), with medium changed every 2 days (9, 11). Rat islets isolated in Uppsala were cultured similarly, except for the presence of 11 mmol/l glucose in the RPMI medium (11). We have shown previously that functional preservation in RPMI 1640 is optimal at 5.6 mmol/l for human islets (9, 12) and at 11 mmol/l for rodent islets (11, 12).

After 5–7 days in culture, human or rodent islets in groups of 70–100 were transferred to RPMI 1640 (same glucose concentrations as above) + 1% FCS.
without or with the addition of 400 ng/ml recombinant human IGF-I. Islet exposure to IGF-I was performed at 1% FCS in order to decrease eventual interferences by growth factors present in the FCS on IGF-I actions. Note that culture at 1% FCS may increase basal insulin release at 1.7 mmol/l glucose and consequently decrease the insulin increase ratio (16.7/1.7 mmol/l glucose) (Ezirik, unpubl. data). The IGF-I (batch 71706-A51) was produced at KabiPharmacia Peptide Hormones, Stockholm, Sweden, and the peptide activity was assessed both by radioimmunoassay (13) and radioreceptor assay (14). Following the experiments performed in Uppsala, aliquots of the IGF-I batch utilized were sent back to Stockholm and tested again, confirming the original activity (data not shown). Following addition of IGF-I, islets were cultured for 48 h and samples of the culture medium were collected for measurement of insulin accumulation. After this period, islet glucose-stimulated insulin release, islet DNA and insulin contents were determined as described previously (9, 12).

For statistical analysis, values are expressed as means ± SEM and groups of data were compared using Student’s paired or unpaired t-test. In all experimental series, each islet preparation (i.e. islets obtained from one donor) was considered as one observation. When experiments were performed in duplicate or triplicate, a mean was calculated and considered as one observation.

Results

Culture of human islets for 48 h in the presence of 400 ng/ml IGF-I did not affect islet DNA or insulin content (Table 1). Insulin accumulation in the culture

Table 1. Deoxyribonucleic acid and insulin content, and insulin release of isolated human pancreatic islets cultured for 48 h without (control) or with 400 ng/ml IGF-I.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IGF-I</th>
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<tbody>
<tr>
<td>Insulin release at 1.7 mmol/l glucose (pmol/10 islets x 1 h)</td>
<td>1.7 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Insulin release at 8.3 mmol/l glucose (pmol/10 islets x 1 h)</td>
<td>3.7 ± 0.6</td>
<td>5.1 ± 1.5</td>
</tr>
<tr>
<td>Insulin release at 16.7 mmol/l glucose (pmol/10 islets x 1 h)</td>
<td>4.6 ± 0.9</td>
<td>5.6 ± 1.4</td>
</tr>
<tr>
<td>Increase ratio (16.7/1.7)</td>
<td>3.1 ± 0.4</td>
<td>4.1 ± 1.2</td>
</tr>
<tr>
<td>Medium insulin accumulation (pmol/10 islets x 48 h)</td>
<td>181 ± 44</td>
<td>221 ± 96</td>
</tr>
<tr>
<td>Insulin content (pmol/10 islets)</td>
<td>108 ± 23</td>
<td>139 ± 40</td>
</tr>
<tr>
<td>DNA content (ng/10 islets)</td>
<td>200 ± 43</td>
<td>186 ± 26</td>
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</table>

*Groups of 70–100 human islets were cultured for 48 h in RPMI 1640 medium containing 5.6 mmol/l glucose and 1% fetal calf serum. For insulin release experiments, islets were incubated for 60 min in duplicate groups of 10 in KRBH buffer containing different glucose concentrations. The increase ratio was calculated by dividing the insulin release observed at 16.7 mmol/l glucose by that observed at 1.7 mmol/l in each individual experiment. The results are means ± SEM of six independent observations.

medium was also similar between controls and islets exposed to IGF-I. When glucose-stimulated insulin release was examined after culture, control islets showed a clear increase in insulin release at 8.3 or 16.7 mmol/l glucose, as compared to the insulin release observed at 1.7 mmol/l (Table 1). Similar responses to glucose were observed in human islets cultured in the presence of IGF-I. Thus, IGF-I failed to affect insulin production and release in human pancreatic islets over a 48 h period of culture.

As observed in human islets, rat islet insulin and DNA contents and medium insulin accumulation were not affected by IGF-I (Table 2). However, the peptide induced a significant increase in insulin release at 1.7 mmol/l glucose and a trend towards lower insulin release at 16.7 mmol/l. This was reflected in a significant decrease in the insulin increase ratio (16.7/1.7 mmol/l glucose) of rat islets exposed to IGF-I.

Discussion

The present observations suggest that human islets cultured in medium containing IGF-I subsequently release insulin in a similar way as do islets cultured in the absence of IGF I. The concentration of IGF-I tested (400 ng/ml) was higher than previous dosages shown to acutely (10–20 min) inhibit glucose-induced insulin release in rodent pancreatic islets (6, 7). It was ascertained that IGF-I utilized in the present experiments was active, as judged by radioimmunoassay, radioreceptor and biological assays performed both before and after the current experiments. We observed a mild inhibition by IGF-I of rat islet insulin release in

Table 2. Deoxyribonucleic acid and insulin content, and insulin release of isolated rat pancreatic islets cultured for 48 h without (control) or with 400 ng/ml IGF-I.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin release at 1.7 mmol/l glucose (pmol/10 islets x 1 h)</td>
<td>3.6 ± 0.5</td>
<td>4.8 ± 0.4**</td>
</tr>
<tr>
<td>Insulin release at 8.3 mmol/l glucose (pmol/10 islets x 1 h)</td>
<td>10.0 ± 2.5</td>
<td>9.8 ± 1.1</td>
</tr>
<tr>
<td>Insulin release at 16.7 mmol/l glucose (pmol/10 islets x 1 h)</td>
<td>21.0 ± 3.3</td>
<td>15.1 ± 1.5</td>
</tr>
<tr>
<td>Increase ratio (16.7/1.7)</td>
<td>5.8 ± 0.4</td>
<td>3.2 ± 0.3*</td>
</tr>
<tr>
<td>Medium insulin accumulation (pmol/10 islets x 48 h)</td>
<td>253 ± 38</td>
<td>246 ± 12</td>
</tr>
<tr>
<td>Insulin content (pmol/10 islets)</td>
<td>118 ± 7</td>
<td>107 ± 9</td>
</tr>
<tr>
<td>DNA content (ng/10 islets)</td>
<td>179 ± 20</td>
<td>186 ± 25</td>
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</table>

*Groups of 70–100 rat islets were cultured for 48 h in RPMI 1640 medium containing 11 mmol/l glucose and 1% fetal calf serum. For insulin release experiments, islets were incubated for 60 min in duplicate groups of 10 in KRBH buffer containing different glucose concentrations. The increase ratio was calculated by dividing the insulin release observed at 16.7 mmol/l glucose by that observed at 1.7 mmol/l in each individual experiment. The results are means ± SEM of four independent observations. *p < 0.05 and **p < 0.01 when compared to respective controls (paired t-test).
response to glucose but no effect on human islets, suggesting differences between the two species in the response to IGF-I. In line with this possibility, human islets seem to present a different pattern of response to different suppressive agents, as compared to rodent islets. Thus, while human islets are more susceptible to the deleterious effects of high glucose (9), they are less sensitive than rat or mouse islets to inhibition by cytokines (15, 16), alloxan, streptozotocin or sodium nitroprusside (12).

It has been suggested previously that IGF-I directly suppresses insulin release in humans (3, 4). Our present in vitro observations raise the possibility that the in vivo effects of IGF-I on insulin secretion might be secondary to improved insulin sensitivity and/or other as yet unknown actions of the peptide. Alternatively, it may be that the in vitro conditions do not allow full expression of IGF-I actions. One potential problem in our system could be that islet isolation would induce loss of surface IGF-I receptors. Against this possibility, it has been shown that purified rat β-cells preserve IGF-I receptors in vitro (5). Furthermore, human islet preparations similar to those used in the present series respond to the stimulatory effects of interleukin-1β (15), an effect abrogated by an IL-1 receptor antagonist protein (15), and are suppressed by combinations of three or four cytokines (16), suggesting that at least surface receptors to different cytokines are not affected by human islet isolation and culture.

In conclusion, under in vitro conditions IGF-I fails to inhibit human islet insulin release. In light of these observations, further studies are needed to clarify the inhibitory effects of IGF-I on circulating insulin concentrations in vivo.

Acknowledgments. We thank Professor D Pipeleers, coordinator of the β-Cell Transplant, for providing human islet preparations and detailed information regarding the cell composition of the human islet preparations. We are grateful for the excellent technical assistance by L-B Hullgren, E Tornelius, A Nordin and M Engkvist. This study made use of human pancreatic islets prepared by the Central Unit of the β-Cell Transplant, Brussels, Belgium, with the financial support of a concerted action in Medical and Health Research of the European Community. This study was supported by grants from the Swedish Medical Research Council (12X-9237–connected to the β-Cell Transplant, European Concerted Action for the Treatment of Diabetes; 12X-109; 12X-9886), the Juvenile Diabetes Foundation International, the Swedish Diabetes Association, the Swedish CNF ("Forskningsanslag för alternativa metoder"), the Novo-Nordisk Insulin Fund and the Family Ernfors Fund.

References

Received January 12th, 1995
Accepted April 24th, 1995