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Direct effect of insulin and insulin-like growth factor-I on the secretory activity of rat pancreatic Beta cells*

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Summary. Purified pancreatic Beta cells were labelled with ^{3}H-tyrosine before studying their secretory activity in perifusion. At 1.4 mmol/l glucose, the cells released similar fractions (0.01% per min) of their contents in preformed and in newly formed insulin. At 20 mmol/l glucose plus 10^{-8} mol/l glucagon, these fractional release rates increased by 16 and 40-fold respectively. The preferential release of newly synthesized as compared to stored insulin is attributable to a heterogeneity in individual cell responses. The secretory responsiveness to glucose plus glucagon was completely suppressed by 10^{-7} mol/l clonidine. Insulin induced a 20% reduction at 10^{-6} mol/l, but remained without effect at 10^{-7} mol/l. Insulin-like growth factor-I provoked a 30% decrease at 5.10^{-9} mol/l. It is concluded that the type-I insulin-like growth factor receptors on pancreatic Beta cells mediate a suppressive action on the insulin release process. Their high affinity for insulin-like growth factor-I allows physiologic levels of this peptide to participate in the regulation of insulin release. Their low affinity for insulin provides the basis for a minor feedback action by this hormone at concentrations exceeding the normal circulating levels.

Key words: Insulin release, Beta cells, Insulin-like growth factor-I.

The insulin release process is kept under tight feed-back control via its exquisite sensitivity to glucose. It is uncertain whether the hormone itself regulates its own release through interaction with the pancreatic Beta cells. In vivo and in vitro studies have suggested an inhibitory action of the hormone [1–11], but it is still unclear whether this effect is mediated through direct or indirect influences on the Beta cell [12–15]. This unclarity is mainly attributable to limitations in the experimental models. Endogenously released insulin may mask effects of the administered hormone [1, 5, 12, 13] until pharmacological doses are reached [1, 3, 5, 6]. The presence of exogenous insulin on the other hand makes it more difficult to precisely monitor any variation in insulin release [1–5]. Finally, the heterogeneous cell composition of the tissues under study does not allow the distinction of direct effects upon Beta cells from indirect influences via other cell types.

The presently used model overcomes several of these obstacles by studying purified Beta cells in the absence of any other cell types and by following the release of endogenously labelled hormone from perifused cells.

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Materials and methods

Preparation of islet Beta cells

Islets of Langerhans were isolated from male adult wistar rats after collagenase digestion of the pancreas [16]. Isolated islets were dissociated in a calcium-free medium containing trypsin (Boehringer, Mannheim, FRG; 25 µg/ml), DNase (Boehringer Mannheim; 2 µg/ml) and 1 mmol/l EGTA [17]. Purified Beta cells were obtained by autofluorescence-activated cell sorting as previously described [16]. After purification, cells were resuspended in HAM-F10 medium supplemented with 5% volume/volume (v/v) heat-inactivated fetal calf serum, plated in a bacteriological culture dish at 5.10^5 cells per 12 ml and reaggregated for 4 h in a Braun shaking incubator at 25 cycles per min. The aggregates were then kept in static culture for 20 h (37 °C-humidified air containing 7.5% CO_2). Cell viability was evaluated routinely by neutral red uptake and always exceeded 90% at the end of the 20 h culture period.

Labelling of islet Beta cells

Cultured Beta cell aggregates were washed in Earle's Hepes (EH) buffer [16] supplemented with 1% Bovine Serum Albumin (BSA; Fraction V, RIA grade, Sigma St. Louis Mo, USA) and then incubated for 120 min in EH containing 10 mmol/l glucose plus 5 µmol/l 3,5 ^{3}H-L-Tyrosine (TRK 200, Amersham, Bucks, U.K.; 50 Ci/mmol). At the end of incubation, the cells were washed in EH supplemented with 1 mmol/l L-Tyrosine and a sample was taken for

extraction in 2 mol/l acetic acid – 0.25% BSA. The remaining cells were used for perifusion.

Perifusion

The technique of cell perifusion has been previously described [18]. A multiple microchamber module (Endotronics, Coon Rapids, Minn, USA) with build-in pump and thermostat was used to conduct one control and one test perifusion in parallel. After labelling, the islet Beta cells were loaded on preformed columns consisting of 1 ml swollen Biogel P2 (Biorad, Richmond, Va, USA) and perifused with EH equilibrated with 95% O_2/5% CO_2. The cells were first exposed to 1.4 mmol/l glucose for 30 min and then to 20 mmol/l glucose plus 10^{-8} mol/l glucagon (porcine, Novo, Copenhagen, Denmark) until the end of the experiment at min 85. From min 50 to 70, test substances were added to one column, using the other column as control. Test conditions consisted of adding bovine insulin (at final concentrations of 10^{-6}, 10^{-7} or 10^{-8} mol/l, purchased from Novo) or IGF-I (recombinant human IGF-I at 5.10^{-9} mol/l final concentration; a kind gift from Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan, to JLVdB) to the high glucose medium. Finally, from min 70 to 85, clonidine (10^{-7} mol/l) was added in order to test the suppressability of the secretory activity. Flow rate was 1 ml/min and 1 ml fractions were collected throughout. At the end of each perifusion, gels were recovered from the columns by suspension in 2 mol/l acetic acid-0.25% BSA and further extracted in this medium. The figures have been corrected for the dead volume of 3 ml.

Analysis of cell extracts

The cellular content in ^{3}H-labelled proteins was measured as trichloroacetic acid (TCA)-precipitable counts in the acetic acid extracts [19]. Before counting, the TCA-pellets were solubilized in 400 µl NaOH (1 mol/l), neutralized with 400 µl HCL (1 mol/l) and suspended in 10 ml Dynagel (Baker, Deventer, The Netherlands).

The cellular content of ^{3}H-labelled insulin immunoreactive material was determined by immunoprecipitation using guinea-pig anti-porcine insulin serum and protein A-sepharose CL4B (Pharmacia, Uppsala, Sweden) [19]. The pellets were resuspended in 2 mol/l acetic acid and their soluble fraction counted in Dynagel. Non-specific binding was obtained by replacing anti-insulin serum with normal guinea-pig serum. (Pro-)insulin biosynthesis was calculated as the difference between both counts. Recovery of HPLC-purified ^{125}I-Tyr-A14-insulin in this procedure was 90%. The insulin content of the cellular extracts was measured by radioimmunoassay [16].

Analysis of effluent

Total radioactivity of each perifusion fraction was determined in 100 µl samples. Aliquots were also taken for measurement of ^{3}H-protein content by TCA-precipitation and ^{3}H-insulin content by immunoprecipitation. This procedure gave a 90% recovery in ^{125}I-Tyr-A14-insulin both in the absence and presence of 10^{-6} mol/l insulin. Insulin immunoreactive content was measured by radioimmunoassay [16].

Statistical analysis

A database was constructed in the Statview 512$^{+\text{TM}}$ program running on Apple Macintosh Plus. Part of the data were further processed according to the Excel 1.0 spreadsheet program. Statistical analysis of differences between experimental and control conditions was performed using the 2-tailed paired Student's *t*-test. Variation of experimental data is expressed as standard error of the mean (SEM) of *n* independent observations.

Results

Labelling of cellular insulin pool

At the end of a 2 h incubation at 10 mmol/l glucose, Beta cell aggregates contained 94.1 ± 0.4% ($n = 30$) of their cellular ^{3}H-thyrosine in the form of TCA-precipitable material. This fraction consisted of 58.5 ± 1.3% ($n = 30$) ^{3}H-insulin immunoreactive proteins. Expression of the ^{3}H-insulin immunoreactivity as a function of total cellular insulin content yields a specific radioactivity of 471 ± 21 cpm/ng insulin ($n = 28$). Considering that each newly synthesized (pro)insulin molecule contains at the most four radioactive tyrosine residues, it can be calculated that 1% or more of the total cellular insulin pool has been labelled.

Release of (^{3}H-)insulin from perifused Beta cells

During perifusion of Beta cell aggregates at 1.4 mmol/l glucose, approximately 0.01% of the cellular insulin pool was released per min (Table 1). Release was increased 16-fold upon exposure to 20 mmol/l glucose plus 10^{-8} mol/l glucagon (Table 1). This stimulatory effect induced the characteristic first and second phases and was completely blocked by clonidine, an alpha-2 adrenergic agonist (Fig. 1). The cells also discharged a fraction of their radioactive protein content according to the same pattern (Fig. 1). At 1.4 mmol/l glucose, this fraction averaged 0.02 ± 0.001% per min ($n = 28$) and consisted of one third ^{3}H-labelled insulin and two thirds of non-insulin ^{3}H-proteins. Under this condition, 0.01% of newly synthesized insulin was released per min; its specific radioactivity in the medium (788 cpm/ng insulin; Table 1) was 70% higher than in the cells (471 cpm/ng insulin).

Stimulation by glucose plus glucagon, increased the discharge of cellular ^{3}H-proteins to 0.28 ± 0.02% per min during the first phase and 0.23 ± 0.01% per min during

Table 1. Secretory activity of perifused pancreatic Beta cells

Perfusion medium	Released insulin/min	Released ^{3}H-insulin/min	
	% of cellular insulin	% of cellular ^{3}H-insulin	cpm/ng released insulin
Glucose 1.4 mmol/l	0.01 ± 0.001	0.01 ± 0.001	788 ± 49
Glucose 20 mmol/l + Glucagon 10^{-8} mol/l			
min 0–5	0.16 ± 0.01	0.42 ± 0.03	1304 ± 91
min 20–35	0.17 ± 0.01	0.39 ± 0.02	1159 ± 57
min 75–85 with 10^{-7} mol/l clonidine	0.02 ± 0.005	0.01 ± 0.001	347 ± 61

Total (left column) and ^{3}H-insulin levels (middle column) were determined in effluent fractions as described in Materials and methods. The release per min is expressed as % of the total or ^{3}H-insulin content that was measured in the Beta cells at start of perifusion. The ^{3}H-insulin radioactivity in the effluent was also expressed as counts per min (cpm) per ng released insulin (right column). Data represent mean ± SEM of 26 independent experiments

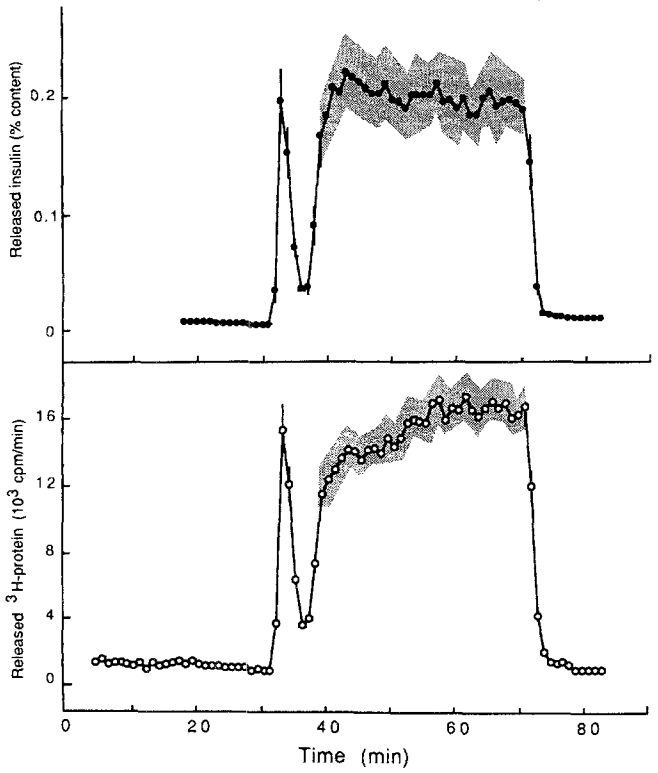

Fig. 1. Secretory activity of perifused Beta cells. Beta cell aggregates (~2.5×10^5 cells/column) were first perifused at 1.4 mmol/l glucose from min 0–30 and then at 20 mmol/l glucose plus 10^{-8} mol/l glucagon from min 30–85. Clonidine (10^{-7} mol/l) was added from min 70 to 85. Effluent fractions were analysed for their content in immunoreactive insulin (top panel) and ^{3}H-protein (lower panel). Data represent mean ± SEM of seven independent experiments

the second phase ($n = 28$). Under stimulatory conditions, the released ^{3}H-proteins corresponded for 90% to ^{3}H-insulin. Compared to the period of low glucose, the release of ^{3}H-labelled insulin had increased 40-fold. The fractional release rate of newly synthesized hormone (0.39% per min) was two to three-fold higher than that of total immunoreactive hormone (0.17% per min). Specific activity of released ^{3}H-insulin was 1159 cpm/ng (Table 1), indicating that at least one out of 50 insulin molecules in the medium corresponded to the newly synthesized hormone.

Addition of clonidine completely suppressed the stimulated release of ^{3}H-labelled proteins (Fig. 1). Release of ^{3}H-insulin was reduced to 0.01% per min, that of immunoreactive insulin to 0.02% per min (Table 1). The specific radioactivity of released ^{3}H-insulin (347 cpm/ng) was now comparable to that measured initially in the cells (471 cpm/ng).

Effect of insulin

The effects of insulin and IGF-I were assessed during the phase of sustained stimulation of insulin release, namely from min 50–70 of perifusion. The peptides were administered in medium containing 20 mmol/l glucose plus 10^{-8} mol/l glucagon.

At a final concentration of 10^{-6} mol/l, insulin decreased the release of ^{3}H-labelled proteins by 18.7 ± 2.2% ($p < 0.01$, Table 2). This inhibitory effect occurred immediately and remained present throughout the period of insulin administration (Fig. 2). It affected the release of ^{3}H-insulin to the same extent (18.9 ± 1.9% inhibition, $p < 0.01$; Table 2). At 10^{-7} mol/l and 10^{-8} mol/l, the hormone no longer exerted a suppressive action upon the release of ^{3}H-insulin (Table 2). In order to assess whether the released hormone exerted an inhibitory effect in itself, cells were perifused with guinea-pig anti-insulin serum at a concentration which was known to bind 10-fold higher insulin concentrations than those present in the eluted fractions. Under these conditions, no difference was measured in the release of ^{3}H-proteins from control and antibody-exposed Beta cells ($n = 3$, data not shown).

Effect of IGF-I

At a final concentration of 5.10^{-9} mol/l, IGF-I inhibited the release of ^{3}H-labelled proteins by 32.0 ± 1.9% ($p < 0.001$; Table 2). This suppressive effect was maintained throughout the period of exposure (Fig. 2). It affected the release of ^{3}H-insulin (31.6 ± 1.7% inhibition, $p < 0.001$; Table 2) and that of immunoreactive insulin to the same extent (35.8 ± 3.8% inhibition, $p < 0.01$ (Fig. 2). The specific radioactivity of the released insulin (1193 ± 85 cpm/ng) was similar to that calculated in control conditions (1136 ± 103 cpm/ng insulin).

Discussion

Purified Beta cell preparations can be used to identify agents that influence insulin release through a direct interaction with the insulin-producing cells. By labelling a fraction of the cellular insulin pool and monitoring the release of ^{3}H-insulin, we examined whether insulin can regulate its own release through direct feedback action on pancreatic Beta cells. The validity of this model ap-

Table 2. Effects of insulin and IGF-I on the secretory activity of perifused pancreatic Beta cells

Perifusion medium	n	Released ^{3}H-protein/min % of cellular ^{3}H-protein	Released ^{3}H-insulin/min % of cellular ^{3}H-insulin
Control	5	0.27 ± 0.03	0.47 ± 0.03
insulin 10^{-6} mol/l	5	0.22 ± 0.03[b]	0.38 ± 0.02[b]
Control	3	0.25 ± 0.03	0.44 ± 0.02
insulin 10^{-7} mol/l	3	0.21 ± 0.02[a]	0.39 ± 0.01[d]
Control	6	0.27 ± 0.02	0.43 ± 0.02
insulin 10^{-8} mol/l	6	0.26 ± 0.02[d]	0.41 ± 0.02[d]
Control	5	0.34 ± 0.01	0.46 ± 0.02
IGF-I 5.10^{-9} mol/l	5	0.23 ± 0.01[c]	0.32 ± 0.02[c]

Effluent fractions of the test period (min 50–70) were pooled and analysed for their ^{3}H-protein and ^{3}H-insulin content. Data represent mean ± SEM of n independent experiments. Paired Student's t-test vs parallel controls: [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$; [d] $p > 0.05$

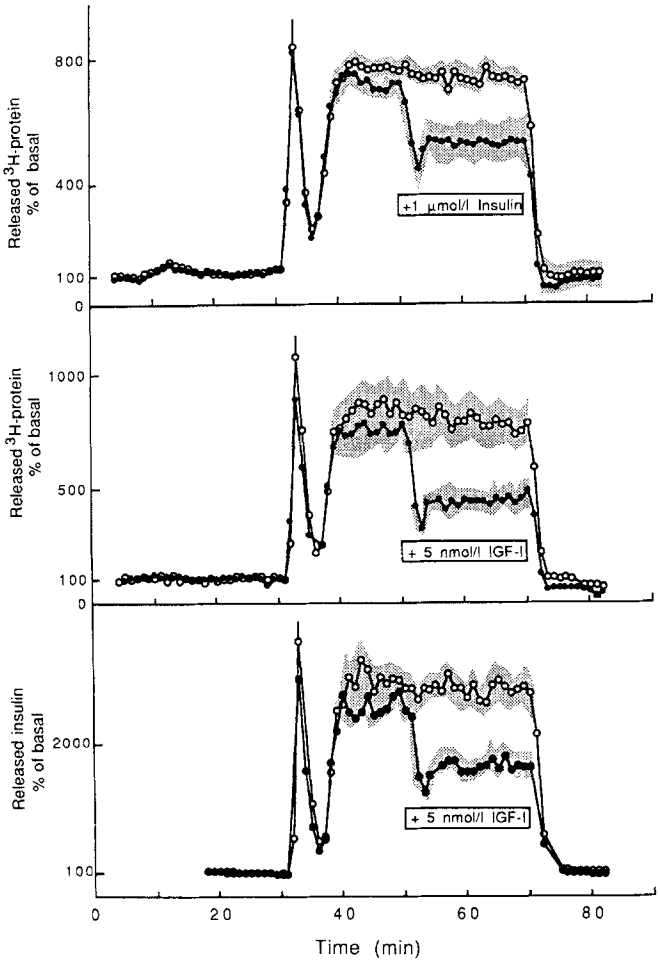

Fig. 2. Effects of insulin and IGF-I upon secretory activity of perifused Beta cells. Beta cell aggregates (~2.5×10^5 cells/column) were perifused as described in Figure 1 and the effluent fractions analysed for their content in ^{3}H-protein (top and middle panel) and immunoreactive insulin (lower panel). Perifusion media contained 1.4 mmol/l glucose from min 0–30 and 20 mmol/l glucose plus 10^{-8} mol/l glucagon from min 30–85. Clonidine at 10^{-7} mol/l was added from min 70–85. Open symbols represent control condition, closed symbols the conditions to which 10^{-6} mol/l insulin (top panel; $n = 9$) or 5.10^{-9} mol/l IGF-I (middle and lower panel; $n = 5$) were added from min 50 to 70. Results are expressed as % of the values measured at 1.4 mmol/l glucose (basal). Data represent mean ± SEM of n experiments

pears from the 40-fold stimulation by glucose plus glucagon and the 40-fold suppression by clonidine, agents which are known to act directly on the pancreatic Beta cells [20, 21]. In the stimulatory condition, the fractional release of ^{3}H-insulin was 2 to 3-fold higher than that of total insulin. Such preferential release of newly synthesized insulin has been previously observed in isolated islets [22, 23]. It was first attributed to an alternative route of granule discharge, but later work made this mechanism unlikely [23]. According to our recent findings [19, 24], the phenomenon of preferential hormone release may well be caused by the unequal contribution of functionally different cells to the secretory response. Isolated pancreatic Beta cells can differ in their individual hormone content [24], as well as in their individual rate of hormone biosynthesis [19]. They may, consequently, differ in their specific radioactivity of newly synthesized hormone. Preferential release of newly synthesized insulin would then merely result from a higher secretory contribution of Beta cells with a higher specific radioactivity. Although compatible with the concept of cellular heterogeneity [19, 24], this mechanism is still to be tested experimentally. It is, meanwhile, necessary to consider the possibility that only a subpopulation of Beta cells is evaluated in experiments where only the release of ^{3}H-labelled hormone is measured. This is, for example, the case in the present study on the effects of insulin.

Insulin was capable of reducing the secretory activity of pancreatic Beta cells but its inhibitory effect was only 20% at 10^{-6} mol/l. This low sensitivity to exogenous insulin is probably not caused by the interference of endogenously released hormone; the latter is not expected to build up during perifusion of small cell aggregates, and addition of anti-insulin serum to the perifusion medium did not alter hormone release from control cells. Rather it supports the idea that the effects of insulin on pancreatic Beta cells are mediated through low affinity receptors for the hormone. We have, so far, been unsuccessful in detecting high affinity binding sites for insulin on pure Beta cell preparations (unpublished observations). It can, however, not be excluded that these negative results are caused by interference of endogenous insulin which is discharged during the static conditions of receptor analysis. On the other hand, a low affinity binding of insulin can occur to the type-I IGF receptors, which have been identified on pancreatic Beta cells [25]. If this binding is responsible for the observed inhibitory effect, one can anticipate that lower concentrations of IGF-I are capable of inducing a similar biological action. This was indeed the case, as IGF-I at 5.10^{-9} mol/l suppressed the release of insulin by 30%. Several other examples exist where high insulin levels can influence cell functions through IGF-I receptors [26–29]. With respect to hormone release, it has been noticed that the secretion of growth hormone can be inhibited by IGF-I as well as by high concentrations of insulin [30].

The present in vitro findings do not support the view that circulating insulin levels exert a feed-back regulation on their own release. Locally released insulin may – to a relatively small extent – influence the secretory activity of neighbouring Beta cells, but such action remains hypothetical as long as the interstitial flow in the islet tissue is not better characterized. Insulin-like growth factor-I should, on the other hand, be considered as a potential regulator of insulin release in view of its inhibitory effect at physiologic concentrations. It thus seems likely that the decline in circulating C-peptide levels after IGF-I administration [31] is mediated, at least in part, by a direct interaction of the growth factor with the insulin producing Beta cells.

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