Functional and morphological effects of interleukin-1 β on the perfused rat pancreas

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Summary. We recently reported a potentiating effect of recombinant human interleukin-1 β on glucose-stimulated insulin release from the isolated perfused pancreas. With the aim of determining whether the stimulatory effect of recombinant interleukin-1 β on the B cell in the intact gland was modulated by varying the concentration, time of exposure to recombinant interleukin-1 β or B-cell activity, and to elucidate a possible mechanism of action, we measured in the perfused rat pancreas the release of insulin, glucagon and/or prostaglandin E₂ according to the following three different protocols: (1) perfusion with 20 ng/ml of recombinant interleukin-1 β for 92 min at 5 and 20 mmol/l D-glucose (2) perfusion with varying concentrations of recombinant interleukin-1 β ranging from 0.1×10^{-3} ng/ml to 100 ng/ml at 5 and 20 mmol/l D-glucose (3) perfusion with 20 ng/ml of recombinant interleukin-1 β at 5, 11 or 20 mmol/l D-glucose. Furthermore, in a separate set of experiments we examined the influence of the cytokine on the morphology of the endocrine pancreas. Interleukin-1 β stimulated insulin secretion at 11 and 20 mmol/l D-glucose and potentiated first as well as second phase insulin release in a dose-dependent fashion, with decreasing effect at higher concentrations. Glucagon secretion was also stimulated by recombinant interleukin-1 β ,

irrespective of increasing glucose (5, 11, 20 mmol/l) and insulin concentrations. The potentiating effect of recombinant interleukin-1 β on insulin secretion was evident even after discontinued perfusion with the cytokine, suggesting a priming effect on B-cell function. Furthermore, we did not observe any relation between the recombinant interleukin-1 β mediated insulin and glucagon release and prostaglandin E₂. Electron microscopy of the pancreata perfused with recombinant interleukin-1 β revealed significant B cell and to a lesser extent A-cell lysis as well as induction of cell protrusions ("blebs") in B cells only, accompanied by peripheral degranulation and rearrangement of rough endoplasmatic reticulum. We suggest that in addition to a paracrine effect of locally produced interleukin-1 β systemic interleukin-1 β may have an endocrine effect on A- and B-cell function and viability. Interleukin-1 β should be considered to be a physiological modulator of insulin and glucagon secretion e.g. during the acute phase response, but also as a pathogenetic factor in Type 1 (insulin-dependent) diabetes mellitus.

Key words: Interleukin-1, perfused rat pancreas, insulin, glucagon, Type 1 (insulin-dependent) diabetes mellitus.

Interleukin-1 (IL-1) is a family of 17.3–17.5 kilodalton (kD) peptides mainly produced by activated cells of monocytic/phagocytic lineage (macrophages and natural killer cells), but also by parenchymal cells, such as endothelial cells, renal mesangial cells and astrocytes [1]. The acute phase response seems to be induced primarily by IL-1 and tumour necrosis factor alpha, (TNF) [1]. Recently, it was demonstrated that IL-1 is distributed throughout the peripheral nervous system innervating several organs [2].

Many of the proposed *in vivo* effects of IL-1 have been confirmed by *in vitro* studies. We have reported that recombinant IL-1 (rIL-1), as well as native IL-1, selectively affects pancreatic B-cell activity: stimulation of insulin secretion followed by inhibition and B-cell cytotoxicity [3],

dependent on the period of incubation, dose of rIL-1 and degree of metabolic activity of the B cells [4], and we have hypothesized that IL-1 produced in the islets mediates a paracrine destruction of B cells eventually leading to Type 1 (insulin-dependent) diabetes mellitus [5]. The acute phase response is accompanied by increased levels of systemic IL-1 [1] and an endocrine effect on insulin and glucagon secretion from the pancreas may be possible. However, if physiological and pathophysiological actions of circulating IL-1 on the endocrine pancreas are to be studied, islets in organ culture may not be an ideal model for the endocrine pancreas, since the function of the endocrine cells may be affected by the isolation and culture procedures, and since nutrients and other substances do

not reach the endocrine cells via their physiological route from B to A to D cells [6]. In the islets in the isolated perfused pancreas the endocrine cells can be exposed to substances via the normal vascular supply, and the action of the substance studied without the influence of neuronal or humoral factors.

We have previously reported that recombinant interleukin-1 β (rIL-1 β) potentiates glucose-stimulated insulin release [7]. The aim of this study was to test whether rIL-1 β influences islet cell morphology in the perfused rat pancreas and to achieve further insight into the stimulatory effect of rIL-1 β on insulin, glucagon and prostaglandin E₂ (PGE₂) release in relation to time, dose, glucose concentration and a possible mechanism of action.

Materials and methods

Animals

Male Wistar rats, purchased from Møllegaarden, (Lille Skensved, Denmark), were housed in our laboratory under controlled conditions of light, temperature and humidity for approximately 10 days before the experiments were performed. The rats were given water and standard chow (Altromin, Chr. Petersen A/S, Ringsted, Denmark) ad libitum. All experiments were initiated between 09.00 and 11.00 hours.

Pancreas perfusion

Fed rats (body weight 210–220 g) randomly allocated to perfusion with either rIL-1 β or 0.9% NaCl, were anaesthetized by i.p. injections of sodium pentobarbital (50 mg/kg). The preparation of the isolated pancreas was performed as described by Svenningsen et al. [8]. The *in situ* pancreas was placed in a 37°C prewarmed chamber and was perfused through the celiac trunk and the superior mesenteric artery with a Krebs-Ringer-Bicarbonate buffer (KRB) supplemented with 20 mmol/l Hepes (KRBH) and 4% (weight/volume) bovine serum albumin (fraction V, Miles Laboratories Inc., Elkhart, Ind., USA), filtered, heated to 37°C before perfusion and kept at 37°C in the perfusate reservoirs. The KRBH was gassed with 95% O₂/5% CO₂ before and during the perfusion to maintain pH 7.4 and to supply the organ with oxygen. The venous effluent was collected on ice after a single passage of the pancreas. Sampling was performed at 3-min intervals, except after a change in glucose concentration, where sampling took place at 1-min intervals. The perfusion rate was held constant at 3 ml/min. After isolation of the pancreas, the organ was subjected to an equilibration period of 20 min according to the different experimental protocols. Data from this period were not included in the evaluation of the experiments. Baseline samples were collected from time zero to 12 min. From 12 min to 72 min (except in protocol I) 0.9% NaCl or rIL-1 β was added to the perfusate reservoirs in a blinded design according to the protocols (see below). Perfusion experiments not fulfilling the following technical criteria: ischaemia time less than 3 min, perfusion pressure less than 60 mm Hg, no oedema, continuous gastric secretion or continuous peristalsis of the duodenum, were excluded from the analysis.

Preparation of rIL-1 β

Human rIL-1 β (R-007) was expressed in *Escherichia coli* and corresponded to the amino acid sequence 112–269 (17 kD) of the p17 beta form of the natural IL-1 β [9]. One unit of rIL-1 β corresponded to 0.1 ng of protein. The morphology experiments were performed

with rIL-1 β from two different sources; R-007 and B-44. B-44, supplied by Nordisk Gentofte A/S, (Gentofte, Denmark), was also expressed in *E. coli* and corresponded to the 153 amino acid, pI 7 beta form of the native IL-1 β with the N-terminal amino acid being alanine [10]. The biological activity as estimated in the C3H/HeJ mouse thymocyte co-stimulatory assay was 300 WHO U/ng. Both batches of rIL-1 β were known to be active when tested on isolated islets. From isolated rat islets inhibition of insulin release was obtained with 100 pg/ml of rIL-1 β R-007 and 37 pg/ml of rIL-1 β B-44.

Hormone assay

Rat insulin was determined by a RIA using a guinea pig anti-porcine-insulin serum and ¹²⁵I porcine ¹⁴aminoiodoinsulin as tracer (Nordisk Gentofte A/S). Rabbit anti-guinea pig immunoglobulin (Dakopatts, Denmark) was used to precipitate insulin-anti-insulin complexes, separating bound from free tracer. Rat insulin was used as standard (Novo Industry A/S, Bagsvaerd, Denmark). Non-specific binding was 0.3 $\pm$ 0.06%, *n* = 13. The coefficient of variation of independent replicates was 9.3 $\pm$ 0.5%, *n* = 39. All samples were diluted to a concentration between 0.02 and 0.8 nmol/l, in which interval the standard curve showed linearity to serial dilutions. The detection limit was 0.01 nmol/l.

Glucagon was determined utilizing reagents in a commercially available RIA kit (Novo Biolabs, Bagsvaerd, Denmark). Pork glucagon was used as standard. All samples were analysed undiluted. The coefficient of variation of independent replicates was 8.3 $\pm$ 0.9%, *n* = 9. Non-specific binding was 4.6 $\pm$ 0.31%, *n* = 9. The detection limit of the RIA was 4.0 pmol/l.

PGE₂ was measured by RIA. The RIA previously described [11] and evaluated in control studies by gas chromatography-mass spectrometry [12] included a Sephadex LH-20 chromatographic step which was omitted in the present RIA. Comparison of the original (x) and the modified RIA (y) by regression analysis gave a correlation coefficient (*r*) of 0.995. The detection limit of the present RIA was 20 pg PGE₂/ml of sample. The intra- and interassay coefficients of variation was 1.2–3.7% and 3.6–6.3%, respectively, in the range 0–100 pg/ml, *n* = 16.

Experimental protocols

I The effect of time (Table 1). Previous studies of rIL-1 β in the perfused pancreas employed a perfusion time of 60 min [7]. With the purpose of investigating the effect of prolonging the exposure time to the cytokine to 92 min, pancreata were perfused with rIL-1 β (20 ng/ml, *n* = 5) or 0.9% NaCl (*n* = 3) in the 20 min equilibration period and from 0 to 42 min at 5 mmol/l D-glucose and from 43 to 72 min at 20 mmol/l D-glucose. Each fraction was analysed for insulin.

Table 1. Accumulated insulin release (pmol) from pancreata perfused with recombinant interleukin-1 β (rIL-1 β) (20 ng/ml) or 0.9% NaCl for 92 min

Group <i>n</i>	rIL-1 β 5	0.9% NaCl 3
0–12 min	5.7 $\pm$ 1.4	0.45 $\pm$ 0.05 ^a
13–42 min	1.6 $\pm$ 0.4	0.96 $\pm$ 0.05 ^a
First phase (43–48 min)	31 $\pm$ 8.9	1.7 $\pm$ 0.6 ^a
Second phase (49–72 min)	214 $\pm$ 72.5	36 $\pm$ 16.4 ^a

^a *p* < 0.05

Accumulated insulin release during specified intervals at 5 mmol/l D-glucose (0–42 min) and at 20 mmol/l D-glucose (43–72 min) from pancreata perfused with recombinant interleukin-1 β (20 ng/ml) or 0.9% NaCl during the equilibration period and from 0 to 72 min. All values are mean $\pm$ SEM

Table 2. The effect of varying rIL-1 β concentrations on D-glucose induced insulin release

	Control	rIL-1 0.1 $\times 10^{-3}$ ng/ml	rIL-1 0.1 ng/ml	rIL-1 1.0 ng/ml	rIL-1 10.0 ng/ml	rIL-1 14.0 ng/ml	rIL-1 20 ng/ml	rIL-1 100 ng/ml
<i>n</i>	17	8	6	10	7	6	7	5
0–12 min (nmol/l)	0.02 $\pm$ 0.004	0.01 $\pm$ 0.001	0.02 $\pm$ 0.004	0.04 $\pm$ 0.006	0.05 $\pm$ 0.01	0.03 $\pm$ 0.006	0.01 $\pm$ 0.002	0.03 $\pm$ 0.003
13–24 min (nmol/l)	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.01 $\pm$ 0.001	0.02 $\pm$ 0.004	0.02 $\pm$ 0.002	0.03 $\pm$ 0.006	0.01 $\pm$ 0.0	0.02 $\pm$ 0.003
1st Phase 25–30 min (pmol)	3.4 $\pm$ 0.7	2.8 $\pm$ 1.1	2.5 $\pm$ 0.6	2.8 $\pm$ 1.1	8.6 $\pm$ 3.9	10.2 $\pm$ 2.7	5.6 $\pm$ 1.2	14.1 $\pm$ 4.2
2nd Phase 31–72 min (pmol)	130 $\pm$ 28	63 $\pm$ 19	90 $\pm$ 23	144 $\pm$ 33	290 $\pm$ 86	324 $\pm$ 74	281 $\pm$ 52	222 $\pm$ 71

Release of insulin during specified intervals at 5 mmol/l D-glucose (0–24 min) and at 20 mmol/l D-glucose (25 to 72 min) from pancreata perfused with either 0.9% NaCl (control) or varied concentrations of recombinant interleukin-1 β (rIL-1 β) from 12 to 72 min. First (1st) and second (2nd) phase insulin release in response to 20 mmol/l D-glucose is given as accumulated insulin. All values are mean $\pm$ SEM

II The effect of rIL-1 β concentration (Table 2). Eight groups of pancreata were perfused in the equilibration period and from 0 to 24 min with KRBH containing 5 mmol/l D-glucose and from 25 to 72 min with KRBH containing 20 mmol/l of D-glucose. rIL-1 β (0.1 $\times 10^{-3}$ ($n = 8$), 0.1 ($n = 6$), 1.0 ($n = 10$), 10 ($n = 7$), 14 ($n = 6$), 20 ($n = 7$) or 100 ng/ml ($n = 5$)) or 0.9% NaCl ($n = 17$) was added to the perfusate from 13 to 72 min. Each fraction of effluat was analysed for insulin.

III The effect of glucose concentration (Fig. 1, 2 and 3). Three groups of pancreata were perfused with KRBH containing 5 ($n = 13$), 11 ($n = 20$) or 20 mmol/l of D-glucose ($n = 15$) during the period of equilibration and from 0 to 72 min. In the period from 73 to 84 min the glucose concentration was changed as follows in the three groups: from 5 mmol/l to 11 mmol/l glucose and from 11 or 20 mmol/l D-glucose to 5 mmol/l D-glucose, respectively. The pancreata were perfused with 20 ng/ml of rIL-1 β or 0.9% NaCl from 13 to 72 min. Insulin was assayed in each collected fraction. Glucagon levels were analysed in every second fraction from 0 to 72 min and from 72 to 81 min in each collected fraction. PGE₂ release was analysed in samples collected at 6, 21, 36, 66, and 84 min.

IV Islet morphology at ultrastructural level (Table 3 and Fig. 4 a, b, and c). Nine pancreata were perfused during the equilibration period and from 0 to 42 min with KRBH containing 5 mmol/l of D-glucose and from 43 to 72 min with KRBH containing 20 mmol/l D-glucose. From 12 to 72 min 0.9% NaCl ($n = 4$) or rIL-1 β (20 ng/ml of R-007, $n = 3$ or 15 ng/ml of B-44, $n = 2$) was added to the perfusate reservoirs in a blinded design. At the end of the experimental period the pancreata were perfused with 25 ml 1% glutaraldehyde in sodium cacodylate buffer, pH 7.2. The fixed pancreata were removed, the spleen-near part dissected and cut into 3 mm $\times$ 3 mm pieces while bathed in the buffered glutaraldehyde. After further fixation for 1 h at 4°C in 1% glutaraldehyde, tissue pieces were washed and postfixed with cacodylate buffered OsO₄ (1.5%) for 2 h. Specimens were washed 3 times in cacodylate buffer and were stored in this buffer for a maximum of 10 days. After dehydration with graded

ethanol dilutions the tissue was embedded in epoxy resin according to Spurr [13]. All specimens were sectioned semi-thin and screened for islets after staining with toluidine blue, islet containing tissue was sectioned ultra-thin. For quantitation of lysis events, on micrographs at a final magnification of 5000 $\times$, the number of A and B cells was counted. Lysis was regarded as positive when cytoplasm did not exhibit the usual intermediate contrast but stained opaque. Lysis was additionally confirmed by screening the respective islet cells at higher magnifications for disrupted plasma membrane.

Statistical analysis

Results are expressed as means $\pm$ SEM. In protocol III the hormone release from 12 to 72 min was calculated as the increase in hormone release above mean pre-infusion level (average of baseline level in the particular experiment). The two-tailed Mann-Whitney rank sum test was used to test differences between two groups of rats. The Kruskal-Wallis test of variance was used when comparing more than two groups of unpaired data (protocol II). The Friedman test of variance was used when comparing more than two groups of paired data (protocol III). Chi-squared test was used in the morphology study. The level of significance chosen was 5%.

Results

The effect of 92 min of exposure to rIL-1 β on D-glucose-induced insulin release

rIL-1 β caused a transient increase in insulin release from 0 to 42 min, not observed in control pancreata (data not shown), causing a higher total release of insulin from 0 to

Table 3. Islet and cell count from pancreata perfused with recombinant interleukin-1 β or 0.9% NaCl

Group	No. of experiments	No. of islets	Total no. of cells counted	No. of intact cells			No. of lysed cells (%)		
				A	B	other	A	B	other
NaCl	4	34	1553	315	1012	139	22 (6.5%) $p = 0.04$	64 (6.0%) $p < 0.001$	1 (0.7%) NS
rIL-1 β	5	31	1882	398	1043	119	49 (11.0%)	271 (20.6%)	2 (1.7%)

Nine pancreata were perfused with recombinant interleukin-1 β (15–20 ng/ml, $n = 5$) or 0.9% NaCl from 12 to 72 min at 5 (0–42 min) or 20 mmol/l of D-glucose (43 to 72 min). After termination of the experimental period the pancreata were fixed by perfusion with 1% glutaraldehyde and postfixed in 1.5% OsO₄. Islets, intact and lysed cells were counted in ultrathin slices. A Chi-square test was used

12 min and from 13 to 42 min in the rIL-1 β perfused pancreata (Table 1). Both groups exhibited a biphasic insulin release in response to 20 mmol/l of D-glucose. However, insulin release during first and second phase was significantly higher ($p < 0.05$) in the rIL-1 β perfused group of pancreata compared to the control group.

Dose-response relationship between rIL-1 β concentrations and D-glucose-induced insulin release

All groups showed identical and stable insulin baseline levels from 0 to 24 min and exhibited first as well as second phase insulin release in response to 20 mmol/l of D-glucose (Table 2). The potentiating effect of rIL-1 β on glucose-stimulated insulin release was dependent on the concentration of the cytokine in the perfusate buffer (first phase: $p < 0.005$, second phase: $p < 0.005$, Kruskal Wallis test of variance). However, at concentrations exceeding 14 ng/ml the second phase insulin release declined, indicating a bimodal effect of rIL-1 β on glucose-stimulated insulin release. At the two lowest concentrations of rIL-1 β , first as well as second phase insulin release was decreased. However, this decrease was not statistically significant.

The influence of glucose concentration on the potentiating effect of rIL-1 β on stimulated insulin release

The baseline concentration of insulin in rIL-1 β perfused pancreata and in the control group was identical at 5, 11 and 20 mmol/l of D-glucose. At 5 mmol/l of D-glucose (0 to 72 min) no significant difference in insulin release was observed comparing rIL-1 β ($n = 6$) and 0.9% NaCl ($n = 7$) perfused pancreata. Stimulation with 11 mmol/l D-glucose induced the characteristic first and second phase insulin release in both groups. The amount of insulin released during the first phase (73 to 78 min) was 6.5 ± 1.1 pmol in the rIL-1 β -treated group and 2.2 ± 0.7 pmol in the control group ($p < 0.05$) and during the second phase (79 to 84 min) 9.0 ± 2.2 pmol and 2.5 ± 0.7 pmol, respectively ($p < 0.05$) (Fig. 1 a). At 11 and 20 mmol/l of D-glucose a steady increase in insulin secretion was observed from 12 to 72 min in the control groups ($p < 0.005$, $n = 8$, $p < 0.0005$, $n = 8$). However, rIL-1 β induced a more pronounced release of insulin at both glucose concentrations ($p < 0.0005$, $n = 12$, $p < 0.0005$, $n = 7$, respectively) (Fig. 2 a and 3 a). At 11 mmol/l of D-glucose rIL-1 β induced a significantly higher insulin level in the period from 42 to 72 min ($p < 0.01$ at each time point) and the insulin released above the baseline level was 42 ± 10 pmol compared to 0.4 ± 4.8 pmol in the control group ($p < 0.01$). However, the total amount of insulin released from 13 to 72 min in the two groups did not differ significantly (rIL-1 β : 75.4 ± 11.2 pmol, control: 49.8 ± 12.7 pmol, $p = 0.10$) (Fig. 2 a). At 20 mmol/l of D-glucose the insulin level was significantly higher in the rIL-1 β group only at 66 min ($p < 0.05$). The amount of insulin released above baseline level in the IL-1 β group was 162 ± 45 pmol compared to 83 ± 15 pmol in the control group ($p = 0.06$). The total amount of insulin released in the two groups did not differ,

Fig. 1 a-c. Release of insulin (a), glucagon (b) and prostaglandin E₂ (c) at 5 mmol/l D-glucose – 0 to 72 min – and in response to 11 mmol/l D-glucose – 72 to 84 min – from rat pancreata perfused with 20 ng/ml of recombinant interleukin-1 β ($n = 6$) (●) or 0.9% NaCl ($n = 7$) (▲) from 12 to 72 min (Protocol III)

rIL-1 β : 372 ± 78 pmol, control: 238 ± 38 pmol ($p = 0.15$) (Fig. 3 a). Lowering the D-glucose concentration from 11 or 20 mmol/l caused a rapid, physiological decrease in insulin release in all groups.

At 5 and 11 mmol/l of D-glucose rIL-1 β and 0.9% NaCl perfused pancreata exhibited stable and identical baseline levels of glucagon from 0 to 12 min ($p > 0.05$). At 20 mmol/l of D-glucose the glucagon baseline level in the control group was higher than in the rIL-1 β group ($p < 0.005$). This could be explained by an increased glucagon release secondary to a lower insulin level (Fig. 3 b) [6]. However, the difference in insulin secretion between

Fig. 2 a-c. Release of insulin (a), glucagon (b) and prostaglandin E₂ (c) at 11 mmol/l D-glucose – 0 to 72 min – and at 5 mmol/l D-glucose – 72 to 84 min – from rat pancreata perfused with 20 ng/ml of recombinant interleukin-1 β ($n = 12$) (●) or 0.9% NaCl ($n = 8$) (▲) from 12 to 72 min (Protocol III)

the two groups was not significant ($p = 0.61$). At 5 mmol/l of D-glucose a statistically significant increase in glucagon secretion was observed from 13 to 72 min in both groups (rIL-1 β : $p < 0.005$, control: $p < 0.0005$). Co-perfusion with rIL-1 β induced a more pronounced release of glucagon, which was significant at 54, 60, 66 and 72 min ($p < 0.01$). The shift from 5 to 11 mmol/l D-glucose induced an instant decrease ($p < 0.05$) followed by a rapid increase ($p < 0.05$) in glucagon release in the rIL-1 β group, whereas the glucagon secretion in the control

Fig. 3 a-c. Release of insulin (a), glucagon (b) and prostaglandin E₂ (c) at 20 mmol/l D-glucose – 0 to 72 min – and 5 mmol/l D-glucose – 72 to 84 min – from rat pancreata perfused with 20 ng/ml of recombinant interleukin-1 β ($n = 7$) (●) or 0.9% NaCl ($n = 8$) (▲) from 12 to 72 min (Protocol III)

group was unaffected by the change in glucose concentration (Fig. 1 b). At 11 and 20 mmol/l D-glucose a significant increase in glucagon release was observed exclusively in the rIL-1 β perfused pancreata ($p < 0.0005$), although no difference in glucagon level was observed between the control and the rIL-1 β perfused pancreata at each glucose level ($p > 0.05$) (Fig. 2 b and 3 b). All groups responded with an accentuated glucagon release in re-

sponse to 5 mmol/l D-glucose in the interval from 73 to 84 min.

During perfusion with 5 mmol/l of D-glucose the baseline level of PGE₂ at 6 min was 0.06 ± 0.027 μ g/l in control experiments compared to 0.14 ± 0.015 μ g/l in rIL-1 β perfused pancreata ($p < 0.05$). A marked increase in PGE₂ release was observed in both groups, (rIL-1 β : $p < 0.0005$, control: $p < 0.0005$). The rIL-1 β co-perfused pancreata exhibited at each time point a statistically significant higher level of PGE₂ in the effluate compared to the control group ($0.001 < p < 0.02$) (Fig. 1 c). At 11 and 20 mmol/l of D-glucose the baseline level of PGE₂ in 0.9% NaCl and rIL-1 β perfused pancreata was identical ($p > 0.05$) and all groups exhibited a significant increase in PGE₂ release during the perfusion (Fig. 2 c and 3 c), but no significant differences between the two groups were demonstrable at any given time during perfusion with 11 or 20 mmol/l of D-glucose.

Islet morphology at the ultrastructural level

Electron microscopic examination of islets in situ after rIL-1 β perfusion (5 pancreata) revealed a significant amount of islet cell lysis as compared to 0.9% NaCl perfused control pancreata ($n = 4$) (Table 3). Lysis was evident as disrupted plasma membranes and considerably lower staining of cytoplasm. For quantitation of lysis the number of cells in islet sections was determined and individual cells screened for intactness. Lysed cells still contained typical A- and B-cell granules (Fig. 4 a and b). The lower or absent cytoplasm stain differs from the increased cytoplasm staining regularly seen in necrotic cells [14]. In four 0.9% NaCl perfused glands 34 islets were identified and a total of 1553 cells counted. In five rIL-1 β perfused glands the numbers were 31 islets and 1882 cells, respectively. As seen in Table 3, a small number of A as well as B cells were lysed in the 0.9% NaCl perfused control pancreata. Perfusion with rIL-1 β resulted in increased lysis of all cell types, with a relatively higher increase of B-cell lysis than of A-cell lysis ($p < 0.01$). Still intact B cells of rIL-1 β perfused pancreata often exhibited unique cell protrusions, such a "bleb" was formed by a peripheral part of the B-cell cytoplasm, rarely contained insulin granules and exhibited a typical rearrangement of rough endoplasmic reticulum (rER) membranes (Fig. 4 a and b). "Bleb" formation was never seen on A cells and only on 5 B cells surrounding a capillary in one islet of the 0.9% NaCl perfused pancreata (in 6 of 1012 cells counted).

Discussion

Peripheral hyperinsulinaemia and hyperglucagonaemia are observed after i.v. administration of bacterial endotoxin to rats [15] and insulin secretion from perfused pancreata isolated from rats treated with endotoxins in vivo is enhanced [16]. However, co-perfusion with endotoxin does not affect insulin secretion from the isolated pancreas [17]. These observations imply the existence of a secondary agent, linking endotoxin to pancreatic hypersecretion. Among other effects, endotoxins induce production of IL-1 from macrophages [1], and treatment of rodents in vivo with culture supernatants from macrophages [18] or with rIL-1 [19, 20] induces hyperinsulinaemia and hyperglucagonaemia, as well as enhanced release of the two hormones from the glucose-stimulated isolated pancreas [18, 21]. In the intact perfused rat pancreas we observed a concentration-dependent, stimulatory effect of rIL-1 β on insulin release from the glucose-stimulated B cell, accompanied by stimulation of glucagon secretion, suggesting the existence of an endocrine effect of systemic IL-1 β on A- and B-cell function in addition to the previously hypothesized local effect of IL-1 [3]. The observations of the present study support the hypothesis that hyperinsulinaemia and hyperglucagonaemia observed after endotoxin treatment and during the acute phase response [1] are mediated by an endocrine effect of IL-1 directly on the pancreatic A and B cell, indicating that IL-1 is the link between endotoxin and pancreatic hypersecretion in vivo. The concentration of systemic IL-1 during endotoxin infusions or severe infectious diseases have been reported to vary from 0.500 ng/ml [22] to 1.5 ng/ml [23]. The concentrations in our experiments are high, but because the cytokines adhere to proteins, the fraction of biologically active rIL-1 β reaching the islet cells is unknown.

Our observations on the intact pancreas are identical to described effects of the cytokine on isolated islets during short-term exposure to rIL-1 β (< 6 h) [24]. Inhibition of insulin release as observed with isolated islets exposed to rIL-1 β for more than 6 h [3] was not seen. Prolongation of rIL-1 β exposure time from 60 min, as described in our previous paper [7], to 92 min, did not convert the potentiating effect on glucose-stimulated insulin release to inhibition. However, the dose-dependent stimulatory effect of rIL-1 β exhibited a tendency towards a biphasic effect on second phase insulin release (Table 1). This observation could be explained by an inhibitory effect of rIL-1 β at a high glucose concentration, starting to become apparent after perfusion for a longer period with higher concentrations of the cytokine (> 14 ng/ml).

This shift from stimulation to inhibition of insulin release by rIL-1 β is supported by the morphological findings, which showed that 20% of the B cells were lysed and that the intact B cells were undergoing changes, as indicated by "bleb" formation. "Bleb" formation may be an early stage of cell death, as shown for TNF-induced cytolysis in fibroblast cells, in which the "bleb" formation is caused by destruction of the cytoskeleton, preceding lysis of the plasma membrane [25]. "Bleb" formation was not observed on isolated islets exposed to rIL-1 β in vitro, but dilatation and derangement of the rER and B-cell lysis

Fig. 4 a–c. Electron-microscopic islet morphology from two different recombinant interleukin-1 β (rIL-1 β) perfused pancreata (a and b) and from a 0.9% NaCl solution perfused pancreas (c). rIL-1 β perfused islets revealed preferentially B-cell lysis (IB) and B-cell specific "bleb"-like cytoplasmic protrusions (*) of still intact B cells (a + b). "Blebs" rarely contained granules and showed typical rearrangement of rough endoplasmic reticulum. The control pancreata exhibited a normal morphology. Magnification: $\times 5000$, the bar represents 2 μ m

was found [26, 27]. However, B-cell lysis was more extensive in isolated islets [26]. We suggest that the effect of rIL-1 β on the perfused pancreas is heterogenous, dependent upon the flow through the perfused pancreas influencing the amount of rIL-1 β reaching the islets. Furthermore, the effect is dependent upon the B-cell activity, in that the working cell is more sensitive than the resting cell [28], although very high glucose concentrations (i.e. higher than 22 mmol/l) are protective [3, 27]. This is very pertinent as the B cell population in the pancreas is heterogenous, consisting of a mixed population of resting and active cells [29]. Based on the morphological findings we believe that further prolongation of rIL-1 β perfusion leads to inhibition of insulin release. However, duration of the experiments are limited by the viability of the *in vitro* perfused pancreas and pre-treatment *in vivo* for a longer period may be necessary for induction of inhibited insulin secretion [30].

Glucagon secretion was suppressed at all three D-glucose levels. This observation is not surprising, since the threshold for suppression of glucagon release is <2.5 mmol/l glucose and because maximum suppression of glucagon release is obtained at 6–11 mmol/l of D-glucose [31]. rIL-1 β induced a distinct stimulation of glucagon secretion at 5 mmol/l D-glucose and in spite of increasing insulin and D-glucose concentrations, rIL-1 β induced a statistically significant increase in glucagon secretion at 11 and 20 mmol/l of D-glucose as well ($p < 0.0005$). Furthermore, the rIL-1 β stimulated glucagon secretion at 5 mmol/l of D-glucose could not be suppressed by an elevated glucose concentration from 72 to 84 min, in spite of discontinued rIL-1 β perfusion, indicating that the sensitivity of A cells to glucose is changed by exposure to rIL-1 β , making them unresponsive to an increase in glucose concentration. For comparison, rIL-1 β potentiated first and second phase insulin release in response to a shift in D-glucose concentration from 5 to 11 mmol/l in spite of discontinued rIL-1 β co-perfusion at 72 min, suggesting that the B cells are hyperresponsive after exposure of rIL-1 β to D-glucose, as known for pancreata isolated from endotoxin-treated rats [15] and isolated islets after rIL-1 β perfusion [32]. The stimulatory effect of rIL-1 β on glucagon secretion at 11 and 20 mmol/l D-glucose may be blunted by a counter-acting A-cell inhibitory effect mediated by an increased insulin secretion, since it is known from experiments on the perfused pancreas that the order of islet cellular perfusion and functional interaction is from B to A to D cell [6]. However, these experiments do not rule out a paracrine regulation of islet hormones through the interstitial fluid spaces.

rIL-1 β induces production of PGE₂ from fibroblasts, muscle cells and endothelial cells [1] and rIL-1 β stimulated cortisone release from the perfused adrenal is blocked by the cyclooxygenase inhibitor indomethacin [33]. Furthermore, since arachidonate metabolites – e.g. prostaglandins – may participate in glucose-stimulated insulin release [34, 35] and epinephrine/norepinephrine-stimulated glucagon secretion [36], accordingly a prostaglandin-mediated stimulatory effect of rIL-1 β on insulin and glucagon secretion was possible. However, we observed an increasing PGE₂ release throughout the perfu-

sion period in both 0.9% NaCl and in rIL-1 β perfused pancreata and only at 5 mmol/l D-glucose did co-perfusion with rIL-1 β induce a higher release of the arachidonate metabolite. The major part of the released PGE₂ probably originates from the exocrine tissue and, e.g., endothelial cells and platelets trapped in the tissue preparation.

In conclusion, the acute effect of rIL-1 β on the intact perfused pancreas is characterized by stimulation of insulin and glucagon secretion, dependent upon rIL-1 β concentration and D-glucose level, with a tendency towards a bimodal effect, accompanied by induction of ultrastructural changes predominately in the B cells. We hypothesize that IL-1 β *in vivo* may have an endocrine effect directly on the pancreas influencing A- and B-cell function and structure and should be investigated, not only as a physiological modulator of insulin and glucagon release, e.g. during the acute phase response, but also as a pathogenetic factor in Type 1 (insulin-dependent) diabetes mellitus in predisposed individuals.

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References

1. Dinarello CA (1988) Biology of interleukin-1. *FASEB J* 2: 108–115
2. Schultzberg M, Svenson SB, Undén A, Bartfai T (1987) Interleukin-1-like immunoreactivity in peripheral tissues. *J Neurosci Res* 18: 184–189
3. Mandrup-Poulsen T (1988) On the pathogenesis of insulin dependent diabetes mellitus. *Dan Med Bull* 35: 438–460
4. Spinas GA, Palmer JP, Mandrup-Poulsen T, Andersen H, Nielsen JH, Nerup J (1988) The bimodal effect of interleukin-1 on rat pancreatic beta-cells – stimulation followed by inhibition – depends upon dose, duration of exposure and ambient glucose concentration. *Acta Endocrinol (Copenh)* 118: 307–311
5. Nerup J, Mandrup-Poulsen T, Mølvig J, Helqvist S, Wogensen L, Egeberg J (1988) Mechanisms of pancreatic β -cell destruction in type 1 diabetes. *Diabetes Care* 11 [Suppl. 1]: 16–23
6. Samols E, Stagner JJ, Ewart RBL, Marks V (1988) The order of islet microvascular cellular perfusion is B – A – D in the perfused rat pancreas. *J Clin Invest* 82: 350–353
7. Wogensen LD, Mandrup-Poulsen T, Markholst H, Lernmark Å, Holst JJ, Dinarello CA, Nerup J (1988) Interleukin-1 potentiates glucose stimulated insulin release in the isolated perfused pancreas. *Acta Endocrinol (Copenh)* 117: 302–306
8. Svenningsen A, Dyrberg T, Markholst H, Binder C, Lernmark Å (1986) Insulin release and pancreatic insulin is reduced in young prediabetic BB rats. *Acta Endocrinol* 112: 367–371
9. Dinarello CA, Cannon JG, Mier JW, Bernheim HA, LoPreste G, Lynn DL, Love RN, Webb AC, Auron PE, Reuben RC, Rich A, Wolff SM, Putney SD (1986) Multiple biological activities of human recombinant interleukin-1. *J Clin Invest* 77: 1734–1739
10. Dalbøge H, Bayne S, Christensen T, Heinæs KR (1989) Cloning and expression of an interleukin-1 β precursor and its conversion to interleukin-1 β . *FEBS* 246: 89–93
11. Christensen P, Leyssac PP (1976) A specific radioimmunoassay for PGE₂ using antibody with high specificity and a Sephadex

- LH-20 microcolumn for the separation of prostaglandins. Prostaglandins 11: 399–420
12. Christensen P, Green K, Leyssac PP (1983) The relationship between urinary prostaglandin excretion rates and urine flow in conscious rats. Evaluation of the radioimmunoassay by gas chromatography-mass spectrometry. *Acta Physiol Scand* 117: 41–47
 13. Spurr A (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. *J Ultrastruct Res* 26: 31–43
 14. Farber S (1982) Biology of disease: membrane injury and calcium homeostasis in the pathogenesis of coagulation necrosis. *Lab Invest* 47: 114–123
 15. Cornell RP (1983) Role of the liver in endotoxin-induced hyperinsulinemia and hyperglucagonemia in rats. *Hepatology* 3: 188–192
 16. Yelich MR, Filkins JP (1980) Mechanism of hyperinsulinemia in endotoxemia. *Am J Physiol* 239: E156–E161
 17. Yelich MR, Filkins JP (1984) Role of calcium in the insulin hypersecretory state of the endotoxic rat pancreas. *Circ Shock* 14: 49–62
 18. Filkins JP, Yelich MR (1982) Mechanism of hyperinsulinemia after reticuloendothelial system phagocytosis. *Am J Physiol* 242: E115–E120
 19. del Rey A, Besedovsky H (1987) Interleukin-1 affects glucose homeostasis. *Am J Physiol* 253: R794–R798
 20. Pomposelli JJ, Flores EA, Bistrian BR (1988) Role of biochemical mediators in clinical nutrition and surgical metabolism. *J Parent Ent Nutrition* 12: 212–218
 21. Yelich MR, Umporowicz DM, McCulla EA, Filkins JP (1987) Interleukin-1 (IL-1) in vivo potentiates immunoreactive insulin (IRI) secretion in the in vitro perfused rat pancreas. *FED PROC* 46: 4 (Abstract)
 22. Reuter A, Bernier J, Gysen P, Gevaert Y, Gathy R, Lopez M, Dupont G, Damas P, Franchimont (1988) A RIA for tumor necrosis factor (TNF α) and interleukin 1 β (IL-1 β) and their direct determination in serum. In: Powanda MC (ed) *Monokines and other non-lymphocytic cytokines*. Alan R. Liss, New York, pp 377–381
 23. Girardin E, Grau GE, Dayer JM, Roux-Lombard P, The J5 Study Group, Lambert PH (1988) Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. *N Engl J Med* 319: 397–400
 24. Spinass GA, Palmer JP, Egeberg J, Mandrup-Poulsen T, Andersen HU, Nielsen JH, Nerup J (1988) Differential effects of interleukin-1 on pancreatic alpha and beta cells. *Diabetes* 37 [Suppl. 1]: 202 (Abstract)
 25. Scanlon M, Laster SM, Wood JG, Gooding LR (1989) Cytolysis by tumor necrosis factor is preceded by a rapid and specific dissolution of microfilaments. *Proc Natl Acad Sci USA* 86: 182–186
 26. Mandrup-Poulsen T, Egeberg J, Nerup J, Bendtzen K, Nielsen JH, Dinarello CA (1987) Ultrastructural studies of time course and cellular specificity of interleukin-1 mediated islet cytotoxicity. *Acta Path Microbiol Immunol Scand (C)* 95: 55–63
 27. Sandler S, Bendtzen K, Borg LAH, Eizirik DL, Strandell E, Welsh N (1989) Studies on the mechanisms causing inhibition of insulin secretion in rat pancreatic islets exposed to human interleukin-1 β indicate a perturbation in the mitochondrial function. *Endocrinology* 124: 1492–1501
 28. Palmer J, Helqvist S, Spinass GA, Mølviig J, Mandrup-Poulsen T, Andersen HU and Nerup J (1989) Interaction of beta-cell activity and interleukin-1 concentration and exposure time in isolated rat islets of Langerhans. *Diabetes* 38: 1211–1216
 29. Schuit FC, In'T Veld PA, Pipeleers DG (1988) Glucose stimulates proinsulin biosynthesis by a dose-dependent recruitment of pancreatic beta cells. *Proc Natl Acad Sci USA* 85: 3865–3869
 30. Goodman M, Wang Y, Lumerman J, Sussman KE, Lafferty K, Drazin B (1988) Studies on the mechanism causing the impairment of glucose-stimulated insulin release in rat pancreatic islets exposed to human recombinant interleukin- β . *Diabetes* 37 [Suppl. 1]: 21 (Abstract)
 31. Gerich JE (1983) Glucose in the control of glucagon secretion. In: Lefebvre PJ (ed) *Handbook of experimental pharmacology*, Vol 66: Glucagon II. Springer, Berlin Heidelberg New York, pp 3–18
 32. Zawulich WS, Dierolf B, Zawulich K (1989) Interleukin-1 induces time-dependent potentiation in isolated rat islets: possible involvement of phosphoinositide hydrolysis. *Endocrinology* 124: 720–726
 33. Roh MS, Drzenovich KA, Barbose JJ, Dinarello CA (1987) Direct stimulation of the adrenal cortex by interleukin-1. *Surgery* 102: 140–146
 34. Turk J, Colca JR, Kotagal N, McDaniel ML (1984) Identification and quantitation of lipoxygenase and cyclooxygenase products. I. *Biochim Biophys Acta* 794: 110–114
 35. Turk J, Colca JR, Kotagal N, McDaniel ML (1984) Identification and quantitation of lipoxygenase and cyclooxygenase products. II. *Biochim Biophys Acta* 794: 125–136
 36. Luyckx AS, Lefebvre PJ (1983) Prostaglandins and glucagon secretion. In: Lefebvre PJ (ed) *Handbook of experimental pharmacology*, Vol 66: Glucagon II. Springer, Berlin Heidelberg New York, pp 83–96

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