

*Originals***Human interleukin-1 β induced stimulation of insulin release from rat pancreatic islets is accompanied by an increase in mitochondrial oxidative events**

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Summary. Acute exposure of pancreatic islets to interleukin-1 β results in an increase in insulin release, while an extension of the exposure time induces a functional suppression and eventually, destruction of the B-cells. We have recently suggested that the interleukin-1 β induced inhibition of islet function is mediated through an impairment in oxidative metabolism. The aim of the current study was to investigate if the acute, stimulatory effects of interleukin-1 β on islet function could also be related to changes in the substrate metabolism. For this purpose, rat islets were exposed for 90–120 min to 30 pmol/l human recombinant interleukin-1 β (biological activity of 2.5 U/ml) and their function and metabolism characterized during this period. The cytokine did not increase insulin release in the presence of 1.7 or 5.5 mmol/l glucose but in both the presence of 16.7 mmol/l glucose or 10 mmol/l leucine + 2 mmol/l glutamine there was a 50% increase in insulin release. Interleukin-1 β exposure increased the oxidation of D-[U-¹⁴C]glucose at 5.5 mmol/l glucose by 25% and at 16.7 mmol/l glucose by 60%. Carbohydrate and amino acid metabolism were further examined in the presence of D-[5-³H]glucose, D-[6-¹⁴C]glucose, [1-¹⁴C]pyruvate, L-[U-¹⁴C]glutamine, L-[U-¹⁴C]leucine and L-[1-¹⁴C]leucine. There was no

difference between control islets and interleukin-1 β exposed islets in terms of D-[5-³H]glucose utilization or [1-¹⁴C]pyruvate decarboxylation, but the oxidation of D-[6-¹⁴C]glucose was increased by 64% in the interleukin-1 β exposed islets. There was also an interleukin-1 β induced 45–60% increase in the decarboxylation of L-[1-¹⁴C]leucine and oxidation of L-[U-¹⁴C]leucine and L-[U-¹⁴C]glutamine, all intramitochondrial events. The stimulation of insulin release by interleukin-1 β in the presence of 16.7 mmol/l glucose was abolished in islets incubated in Ca²⁺ depleted medium, but the rate of D-[6-¹⁴C]glucose oxidation remained elevated (47% increase at 16.7 mmol/l glucose). These data indicate an increase in substrate metabolism at the mitochondrial level during acute exposure of rat pancreatic islets to interleukin-1 β . The increase in oxidative events can explain the observed interleukin-1 β induced increase in insulin release during glucose stimulation. Furthermore, these findings raise the possibility that mitochondria are primary targets of interleukin-1 β action in the B-cells.

Key words: Pancreatic islets, insulin release, interleukin-1 β , glucose metabolism, amino acid metabolism.

Interleukin-1 (IL-1) is a polypeptide hormone produced by macrophages and other cell types. IL-1 has several biological effects, ranging from activation of lymphocytes to induction of diverse components of the host acute-phase response to infection and injury [1]. In addition, IL-1 has been found to affect the insulin secreting B-cells of the pancreas. Acute in vitro exposure of pancreatic islets to IL-1 increases the biosynthesis and release of insulin [2–6]. When the exposure time is prolonged, IL-1 induces suppression of insulin release and destruction of B-cells [7–9]. Based on these observations, it has been suggested that IL-1 may be one of the main mediators of the immunologically induced B-cell destruction in Type 1 (insulin-dependent) diabetes mellitus [10, 11].

The mechanisms by which IL-1 initially stimulate and subsequently suppress the B-cells are still unknown. Previous studies in rat islets, dealing with the suppressive phase of interleukin-1 β (IL-1 β) action (48 h of exposure), showed that the cytokine impaired the oxidation of D-[U-¹⁴C]glucose [9, 12]. Subsequent experiments suggested that this impairment was the consequence of a defective mitochondrial oxidation of glucose, while the glycolytic pathway, as evaluated by the utilization of D-[5-³H]glucose, remained intact [13, 14]. After 48 h exposure to IL-1, the modifications in substrate metabolism can, however, be the result of either a direct IL-1 action and/or an adaptative response of the injured B-cells. Indeed, islets maintained in culture after exposure to another B-cell toxin, strep-

Table 1. Influence of interleukin-1 β (rIL-1 β) on the islet insulin release in response to different secretagogues

rIL-1 β (pmol/l)	Secretagogues (2nd h) (mmol/l)	Insulin release (ng · 10 islets ⁻¹ · 60 min ⁻¹)	
		1st h (1.7 mmol/l glucose)	2nd h (different secretagogues)
0	Glucose 16.7	3.2 ± 0.6	19.8 ± 1.4
30	Glucose 16.7	3.9 ± 0.8	29.1 ± 2.8 ^b
0	Leucine 10 + Glutamine 2	2.5 ± 0.4	16.1 ± 2.7
30	Leucine 10 + Glutamine 2	3.4 ± 0.3	23.7 ± 2.2 ^a

Insulin release was measured by incubating islets in groups of 10 (in triplicate) in Krebs-Ringer bicarbonate buffer containing 1.7 mmol/l glucose and, when indicated, 30 pmol/l rIL-1 β . During the 2nd h, the medium was exchanged for medium supplemented with the different secretagogues, with or without the addition of rIL-1 β . Mean values ± SEM are derived from 6 experiments. Significance is given for a chance difference vs the respective control group. ^a $p < 0.05$; ^b $p < 0.01$

Table 2. D-Glucose and pyruvate metabolism in the presence or absence of interleukin-1 β (rIL-1 β ; 30 pmol/l)

Nutrients (mmol/l)	Metabolic rate (pmol · 90 min ⁻¹ · 10 islets ⁻¹)	
	Control	rIL-1 β
D-[U- ¹⁴ C]Glucose (1.7)	78 ± 19	81 ± 16
D-[U- ¹⁴ C]Glucose (5.5)	255 ± 15	319 ± 16 ^a
D-[U- ¹⁴ C]Glucose (16.7)	453 ± 51	705 ± 113 ^b
D-[5- ³ H]Glucose (16.7)	595 ± 120	534 ± 63
D-[6- ¹⁴ C]Glucose (16.7)	223 ± 26	356 ± 24 ^b
[1- ¹⁴ C]Pyruvate (5.0)	544 ± 77	556 ± 98

The oxidation of D-glucose and pyruvate was measured in triplicate groups of 20 islets each. Mean values ± SEM are derived from 5-6 experiments. Significance is given for a chance difference vs the respective control group. ^a $p < 0.05$; ^b $p < 0.01$

tozotocin, also present a selective impairment in the mitochondrial metabolism of glucose [15].

Thus, in order to further explore the mechanisms by which IL-1 affect B-cell function, the current experiments were designed to study the functional and metabolic responses of rat pancreatic islets during the first few hours of exposure to the cytokine.

Materials and methods

Male Sprague-Dawley rats 3-4 months old, belonging to a local stock maintained at the Biomedical Center, Uppsala, Sweden, were utilized for islet isolation. The islets were isolated from the collagenase-digested pancreas with the aid of Ficoll gradients [16]. Groups of 150-200 islets were maintained free-floating in culture medium RPMI 1640 (Flow Laboratories, Irvine, UK) containing 10% (volume/volume) calf serum (National Bacteriological Laboratory, Stockholm, Sweden) antibiotics and 11.1 mmol/l glucose [17]. The culture medium was changed on days 2 and 4. After 5-6 days in culture, 30 pmol/l rIL-1 β was added to the medium and after 15 min the islets were harvested and used for the functional and metabolic studies described below, in the presence of the cytokine. Recombinant IL-1 β (rIL-1 β) was kindly provided by Dr. K. Bendtzen, Laboratory of Medical Immunology, Rigshospitalet, Copenhagen, Denmark. A bi-

ological assay showed that 30 pmol/l of the cytokine had a biological activity of approximately 2.5 U/ml (compared with an interim international standard IL-1 β preparation, NIBSC, London, UK). The activity of the cytokine was confirmed using both the mouse costimulatory assay and the EL 4 murine T cell line [18]. The concentration of rIL-1 β utilized was selected following a previous dose-response study [19], in which rIL-1 β in the concentration range of 3-3000 pmol/l, induced a similar 30-50% increase in insulin release at 20 mmol/l glucose, during acute (2 h) exposure. Furthermore, 30-60 pmol/l rIL-1 β induce a reproducible suppression of β -cell function after 48 h exposure, without causing severe necrotic changes in the islets [13, 20].

Insulin release was studied in triplicate groups of 10 islets, as previously described [9]. The islets were placed in sealed glass vials [21], containing 0.25 ml of Krebs-Ringer bicarbonate buffer [22], supplemented with 10 mmol/l Hepes and 2 mg/ml bovine serum albumin (hereafter designated KRBH buffer) and when indicated 30 pmol/l rIL-1 β . In some experiments the KRBH medium was prepared without the addition of Ca²⁺, and supplemented with 0.25 mmol/l ethyleneglycol-bis-(β -amino-ethyl ether) N,N-tetraacetic acid (EGTA). During the first hour of incubation the medium contained 1.7 mmol/l glucose. The medium was then gently removed and replaced during the second hour by KRBH containing either 5.6 or 16.7 mmol/l glucose or 10 mmol/l leucine + 2 mmol/l glutamine. The insulin concentration in the incubation medium was determined by RIA [23].

For the studies of glucose, pyruvate, leucine and glutamine metabolism radioactive precursors were obtained from the following sources: D-[U-¹⁴C]glucose (270 mCi/mmol), D-[5-³H]glucose (21.1 Ci/mmol), D-[6-¹⁴C]glucose (55 mCi/mmol), L-[U-¹⁴C]glutamine (205 mCi/mmol), L-[U-¹⁴C]leucine (342 mCi/mmol) and L-[1-¹⁴C]leucine (55 mCi/mmol) from Amersham International (Amersham, UK) and [1-¹⁴C]pyruvic acid (13.0 mCi/mmol) from New England Nuclear (Boston, Mass, USA).

In order to study the oxidation of either D-[U-¹⁴C]glucose, D-[6-¹⁴C]glucose, L-[U-¹⁴C]glutamine, L-[U-¹⁴C]leucine, L-[1-¹⁴C]leucine or [1-¹⁴C]pyruvate, triplicate groups of 20 islets each were transferred to sealed glass vials [21] containing the labelled substrates and non-radioactive glucose, pyruvate, glutamine or leucine at the final concentrations given in the tables. The substrate oxidation was measured as previously described [24].

The utilization of glucose was determined as the formation of ³H₂O from D-[5-³H]glucose. Triplicate groups of 20 islets each were incubated in 15 μ l of KRBH buffer and non-radioactive glucose to a final concentration of 16.7 mmol/l glucose, and the glucose utilization measured as described in detail elsewhere [25]. In these and the above described experiments, the mean of the triplicate experiments from one islet isolation was considered as one observation.

Statistical analysis

Data were computed as means ± SEM and compared using Student's *t*-test for paired samples.

Results

Short-term exposure of islets to rIL-1 β did not stimulate insulin release at 1.7 mmol/l glucose (Table 1). However, in the presence of either 16.7 mmol/l glucose or 10 mmol/l leucine + 2 mmol/l glutamine the cytokine augmented the insulin release by approximately 50%. In a separate series of experiments, the insulin release in response to 5.5 mmol/l glucose was also studied. Again there was no stimulation of insulin release by rIL-1 β at 1.7 mmol/l glucose (control, 8.4 ± 0.8 ng · 10 islets⁻¹ · 60 min⁻¹; rIL1 β exposed, 8.4 ± 0.9 ng · 10 islets⁻¹

$\cdot 60 \text{ min}^{-1}$, $n=6$ in both groups). During the second hour of incubation, in the presence of 5.5 mmol/l glucose, there was also no stimulatory effect of the cytokine (control, $7.5 \pm 1.1 \text{ ng} \cdot 10 \text{ islets}^{-1} \cdot 60 \text{ min}^{-1}$; rIL-1 β exposed, $6.3 \pm 1.3 \text{ ng} \cdot 10 \text{ islets}^{-1} \cdot 60 \text{ min}^{-1}$).

In good agreement with the lack of stimulation of insulin release by rIL-1 β at 1.7 mmol/l glucose, the oxidation of D-[U- ^{14}C]glucose at this glucose concentration was unaffected by the cytokine (Table 2). However, at 5.5 mmol/l glucose there was a 25% increase in D-[U- ^{14}C]glucose oxidation and at 16.7 mmol/l glucose there was a 54% increase. Further characterization of the glucose metabolism in the presence of rIL-1 β showed an unchanged D-[5- ^3H]glucose utilization and [1- ^{14}C]pyruvate decarboxylation as compared to control islets, but a 64% increase in the D-[6- ^{14}C] glucose oxidation (Table 2). To test if the observed increase in glucose oxidation induced by rIL-1 β could be a consequence, rather than a cause, of the increase in insulin release, we studied both parameters in a Ca^{2+} free KRBH buffer, supplemented with 0.25 mmol/l EGTA. The depletion of Ca^{2+} abolished the rIL-1 β induced increase in insulin release at 16.7 mmol/l glucose (Table 3). Indeed, both control and rIL-1 β exposed islets failed to increase the insulin release in response to glucose in the absence of Ca^{2+} . There was however, still a 47% increase in D-[6- ^{14}C]glucose oxidation in the presence of rIL-1 β .

The oxidation of L-[U- ^{14}C]glutamine and L-[U- ^{14}C] leucine, as well as the decarboxylation of L-[1- ^{14}C] leucine, were all increased by 45–60% in the presence of rIL-1 β (Table 4).

Discussion

The present results confirm that acute exposure of pancreatic islets to rIL-1 β augments the insulin release in the presence of stimulatory concentrations of glucose, and extend these findings to stimulation of insulin release also in the presence of leucine plus glutamine. At lower concentration of glucose (1.7 and 5.5 mmol/l), rIL-1 β did not induce any increase in insulin release. Previous reports have shown that IL-1 could acutely increase glucose-induced insulin release of isolated islets [2–6] and in the perfused rat pancreas [26]. A similar potentiation by IL-1 was observed in the presence of glyceraldehyde and α -ketoisocaproate [6], but was absent at low glucose concentrations [6, 26]. The combined data suggest that IL-1 potentiates nutrient-induced insulin release, but does not increase the insulin release at non-stimulatory levels of the insulin secretagogues.

The catabolism of glucose and amino acids in the B-cells represents a critical step in the regulation of insulin secretion [27]. An acute stimulation of these metabolic pathways by IL-1 may explain the observed increase in insulin release in response to glucose and leucine. Indeed, in the presence of rIL-1 β there was a significant in-

Table 3. Influence of interleukin-1 β (rIL-1 β ; 30 pmol/l) on islet glucose-induced insulin release and glucose metabolism in Ca^{2+} depleted medium

Variable under study	Glucose (mmol/l)	Control	rIL-1 β
Insulin release ($\text{ng} \cdot 10 \text{ islets}^{-1} \cdot 60 \text{ min}^{-1}$)	1.7	5.4 ± 0.9	3.2 ± 0.4
Insulin release ($\text{ng} \cdot 10 \text{ islets}^{-1} \cdot 60 \text{ min}^{-1}$)	16.7	5.9 ± 1.4	3.7 ± 0.8
D-[6- ^{14}C]glucose oxidation ($\text{pmol} \cdot 90 \text{ min}^{-1} \cdot 10 \text{ islets}^{-1}$)	16.7	122 ± 15	166 ± 17^a

Insulin release and D-[6- ^{14}C] glucose oxidation were measured as described in Tables 1 and 2, respectively, except for the omission of Ca^{2+} in the Krebs-Ringer bicarbonate buffer and addition of 0.25 mmol/l EGTA. Mean values \pm SEM are derived from 6–8 experiments. Significance is given for a chance difference vs the respective control group. ^a $p < 0.05$

Table 4. L-leucine and L-glutamine metabolism in the presence of interleukin-1 β (rIL-1 β ; 30 pmol/l)

Nutrients (mmol/l)	Metabolic rate ($\text{pmol} \cdot 90 \text{ min}^{-1} \cdot 10 \text{ islets}^{-1}$)	
	Control	rIL-1 β
L-[U- ^{14}C]Glutamine (2.0) + Leucine (10)	354 ± 53	498 ± 56^b
L-[U- ^{14}C]Leucine (10) + Glutamine (2.0)	128 ± 11	187 ± 15^a
L-[1- ^{14}C]Leucine (10) + Glutamine (2.0)	218 ± 38	315 ± 33^a

The oxidation of L-glutamine and L-leucine was measured in triplicate groups of 20 islets each. Mean values \pm SEM are derived from 5–8 experiments. Significance is given for a chance difference vs the respective control group. ^a $p < 0.01$; ^b $p < 0.001$

crease in D-[U- ^{14}C]glucose catabolism, at 16.7 mmol/l glucose. This increase was mainly due to an augmented mitochondrial oxidation of glucose, as judged by the increased D-[6- ^{14}C]glucose oxidation in the presence of rIL-1 β . However, there were no changes in either the glycolytic pathway or in the conversion of pyruvate to acetyl-CoA, as evidenced by the D-[5- ^3H]glucose utilization and [1- ^{14}C]pyruvate decarboxylation experiments. It has previously been suggested that B-cells exhibit a dissociated regulation of glycolytic and mitochondrial oxidative events, with a preferential activation of the latter in the presence of stimulatory concentrations of hexoses [15, 28, 29]. The present study reinforces this concept and suggests that rIL-1 β potentiates the selective stimulation of mitochondrial events. It is thus conceivable that the increase in insulin release induced by rIL-1 β can be due to an increased substrate metabolism at the Krebs cycle level.

It is noteworthy that at 5.5 mmol/l glucose there was a dissociation between a minor rIL-1 β -induced stimulation of glucose oxidation and a lack of effect of the cytokine on insulin release. It may be that at this non-stimulatory concentration of glucose, the slight in-

crease in substrate metabolism was not sufficient to trigger insulin secretion. It should also be noted that other factors not directly coupled to substrate metabolism can contribute to the IL-1-induced stimulation of insulin release. Indeed, recent data by Zawulich and co-workers suggest that IL-1 can increase phosphoinositide hydrolysis [6, 30]. However, it is not clear if this process is mediated by the classical inositol triphosphate formation, resulting from a receptor-agonist interaction [31], or is secondary to other cellular events induced by IL-1.

Another interpretation of the current findings must also be considered. The release of insulin is a major energy requiring process in the B-cells, and it has been recently shown that an increased demand of ATP can exert a positive feedback control on the mitochondria [29]. To test this possibility, the effects of rIL-1 β on insulin release and D-[6-¹⁴C]glucose oxidation were studied in a medium depleted of Ca²⁺. In this situation, there was no stimulation of insulin release, and the glucose oxidation was decreased, as compared to data observed in the presence of Ca²⁺. The decrease in D-[6-¹⁴C]glucose oxidation in the absence of Ca²⁺ is in good agreement with recent findings by Malaisse and Sener [29]. Although a primary effect of Ca²⁺ deprivation upon the activity of mitochondrial enzymes can not be excluded, it has been suggested that this finding reflects a decreased energy demand in the B-cell due to decreased insulin release [29]. However, even during incubation in the Ca²⁺ depleted medium rIL-1 β was able to induce an increase in D-[6-¹⁴C]glucose oxidation, as compared to control islets. These findings emphasize the possibility that the observed increase in glucose oxidation was the cause rather than the consequence of the increased insulin release.

The process of leucine-stimulated insulin release depends on an increase in catabolic fluxes in the islets, via both a leucine-stimulated glutamate catabolism [32] and the catabolism of leucine itself [33]. As suggested by the observed increase in [1-¹⁴C]leucine decarboxylation, [U-¹⁴C]leucine oxidation and [U-¹⁴C]glutamine oxidation, rIL-1 β potentiated leucine-induced insulin release by both of the above described processes. The observed increase in [1-¹⁴C]leucine decarboxylation suggests that other intramitochondrial reactions, besides that of the Krebs cycle, may also be stimulated by the cytokine.

When islets are exposed to rIL-1 over a longer time period, there is suppression of insulin release and damage of B-cells [7-9]. This inhibition of insulin release is accompanied by an inhibited mitochondrial metabolism of glucose [13, 14, 20]. The present observations, that an altered mitochondrial handling of substrates is already observed in the first 2 h of exposure to IL-1, raises the intriguing possibility that mitochondria are primary targets of the IL-1 β action in the B-cells. How and why this early phase of stimulation of the mitochondrial metabolism will later proceed to an inhibited state remains, however, to be clarified.

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