Interleukin-1 induced impairment in pancreatic islet oxidative metabolism of glucose is potentiated by tumor necrosis factor

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Abstract. Recent observations suggest that human interleukin-1 (IL-1) causes functional impairment and death of pancreatic B-cells. This action seems to be potentiated by another cytokine, tumor necrosis factor (TNF). In the present investigation, the effects of recombinant human (r) IL-1 (10, 30 and 150 pmol/l), and a combination of rIL-1 and human rTNF (25 μg/l), on islet glucose metabolism were examined in the presence of D-[3-3H] and D-[6-14C] glucose. The utilization of glucose was not affected by rIL-1 or rIL-1 plus rTNF. However, rIL-1 induced a 40% decrease in glucose oxidation, which was further potentiated by the addition of rTNF. rTNF alone did not impair islet glucose utilization or oxidation. It is concluded that rIL-1 induces a perturbation of islet glucose handling, mainly at the mitochondrial level. This impairment in the oxidative metabolism of glucose is further increased by the addition of rTNF.

There is increasing evidence suggesting that in insulin-dependent diabetes mellitus (IDDM) the B-cells are damaged during the course of a chronic autoimmune process (Eisenbarth 1986). The molecular mechanisms underlying the B-cell destruction are still unclear. It has been recently proposed that cytokines, mainly interleukin-1 (IL-1), may cause functional impairment and lysis of pancreatic B-cells (Mandrup-Poulsen et al. 1986; Bendtzen et al. 1986; Sandler et al. 1987; Eizirik et al. 1988c). This effect seems to be potentiated by tumor necrosis factor (TNF) (Mandrup-Poulsen et al. 1987; Pukel et al. 1988), another cytokine released mainly from macrophages.

A common finding in all studies dealing with IL-1 effects on B-cell is a clear inhibitory effect of the cytokine on glucose-stimulated insulin release (Zawalich & Diaz 1986; Comens et al. 1987; Sand¬ler et al. 1987; Eizirik et al. 1988c). The facts that theophylline is able partially to restore this impaired response to glucose (Eizirik et al. 1988c), and that the oxidation of uniformly labelled glucose is reduced after IL-1 exposure (Sandler et al. 1987; Eizirik et al. 1988c) suggest that IL-1 can affect the coupling between glucose metabolism and insulin release in its early steps. Furthermore, recent data suggest that this impaired glucose metabolism occurs at the mitochondrial level (Sandler et al. 1988). However, little is known about the metabolic effects of exposure of the islets to IL-1 in the presence of TNF.

Therefore, the aim of this study was to investigate to what extent the TNF induced potentiation of IL-1 action is related to a further deterioration in the mitochondrial handling of glucose in the affected islets.

Material and Methods

Islet isolation and culture

Male Sprague-Dawley rats 3–4 months old, belonging to a local stock bred at the Biomedical Centre, were utilized for islet isolation. The islets were isolated with the aid of Ficoll gradients (Lernmark et al. 1976), from collagenase-digested pancreas (Howell & Taylor 1968). Groups of 150–200 islets were maintained free floating in RPMI 1640 culture medium (Flow Laboratories, Irvine, UK) containing 10% (vol/vol) calf serum (National Bacteriological Laboratory, Stockholm, Sweden) and...
11.1 mmol/l glucose, according to Andersson (1978). The culture medium was changed on days 2 and 4. After 5 days, islets in groups of 75–100 were transferred to new culture dishes and maintained for the 48-h experimental period. Islets allocated to the experimental groups were cultured as described above, with the addition to the medium of either human recombinant TNF (rTNF, 25 µg/l), recombinant rIL-1 (rIL-1) β (10, 30 or 150 pmol/l) or a combination of rTNF (25 µg/l) plus rIL-1 β (same concentrations as above). In all experiments, immediately after the 48-h experimental period, the islets were subjected to histological processing or metabolic studies, as described below.

In the interleukin preparation, 150 pmol/l IL-1 β, kindly provided by Dr S. Gillis, Immunex, Seattle, WA, had a biological activity of approximately 12.5 U/ml (compared with an interim international standard II-1 β preparation (NIBSC, London, UK)). The activity of the cytokine was confirmed using both the mouse thymocyte costimulatory assay and the EL 4 murine T cell line (Svenson & Bendtzén 1988). Human rTNF was provided by Dr G. R. Adolf, Boehringer Institute, Vienna, Austria, and contained approximately 4 × 10^5 U/10 µg when tested on the LM fibroblast assay (Aggarwal et al. 1984).

The exposure of islets to IL-1 in the presence of 10% calf serum, as utilized in the present study, induces a similar effect on islet function and morphology as observed in the presence of 0.5 to 1.0% human serum (Eizirik, unpublished data), the culture condition utilized in previous studies by our group (Sandler et al. 1987; Eizirik et al. 1988c).

**Islet glucose metabolism and light microscopy**

For D-[6-14C] glucose (55 mCi/mmol, Amersham International, Amersham, UK) oxidation, triplicate groups of 10 islets were transferred to glass vials (Keen et al. 1963) containing 100 µl of a bicarbonate buffer (Krebs & Henseleit 1932) supplemented with 10 mmol/l HEPES (hereafter designated KRBH buffer) and non-radioactive glucose to a final concentration of 16.7 mmol/l glucose. Islet glucose oxidation was subsequently measured as described in detail elsewhere (Andersson & Sandler 1983). The utilization of glucose by the isolated islets was determined as the formation of 3H2O from D-[5-3H] glucose (21.1 Ci/mmol, Amersham, Amersham, UK) (Ashcroft et al. 1972). Triplicate groups of 10 islets each were incubated in 15 µl of KRBH buffer and non-radioactive glucose to a final concentration of 16.7 mmol/l glucose, and the glucose utilization measured as previously described (Borg et al. 1979). In the present series of experiments, the recovery of 3H from known amounts of 3H2O was 61.7%. The values obtained for glucose utilization were not corrected for the recovery.

For morphological studies, groups of 25–30 islets were washed in Hanks' solution, fixed in Bouin's solution, embedded, cut and stained in hematoxylin-cosin, as described in detail elsewhere (Sandler et al. 1987).

**Statistical analyses**

Data are given as means ± SEM and groups of data were compared using the Wilcoxon rank sum test, two-tailed (Colton 1974).

**Results**

In a first series of experiments, the possibility of a potentiating effect of rTNF (25 µg/l) on the rIL-1 β induced inhibition of glucose oxidation was tested in the presence of three rIL-1 concentrations: 10, 30 and 150 pmol/l (Fig. 1). The oxidation of D-[6-14C] glucose in the control group was 283 ± 32 pmol/90 min × 10 islets (N = 7), and was not affected by rTNF alone (276 ± 44 pmol/90 min × 10 islets, N = 6). The lower concentration of rIL-1 tested (10 pmol/l) did not affect the oxidation of glucose. However, at both 30 and 150 pmol/l, rIL-1 induced a clear decrease in glucose oxidation, further potentiated by the presence of rTNF. At 150 and 300 pmol/l rIL-1 (not shown), the islets became irregularly shaped and ragged, and tissue debris was noticeable in the culture medium, when

![Figure 1](image-url)
viewed in the stereomicroscope. At those rIL-1 concentrations, light microscopical examination (not shown) of the rIL-1 exposed islets, independently of the presence of rTNF, were very similar to previous observations (Sandler et al. 1987), revealing varying numbers of pyknotic nuclei in the central part of the islets. Control islets very seldom showed pyknotic figures.

In order to further characterize the potentiating role of rTNF on rIL-1β mediated impairment in B-cell glucose metabolism, we used the lowest effective rIL-1 concentration, 30 pmol/l. At that dosage, the islet morphological changes described above were not present, but there was a 50% decrease in glucose oxidation, in comparison with the control group (*P < 0.01) (Table 1). rTNF alone again did not affect glucose oxidation. However, when 25 µg/l rTNF were added to 30 pmol/l rIL-1, there was a further 30% decrease in glucose oxidation, in comparison with islets incubated in the presence of rIL-1 only (*P < 0.01). Glucose utilization was not affected by the presence of either rIL-1 alone or rIL-1 plus rTNF.

### Discussion

The objectives of this study were to extend our previous suggestions of a rIL-1 induced blockage of glucose metabolism at the mitochondrial level (Sandler et al. 1988), and to relate this observation to the recently described potentiating effect of rTNF on rIL-1 induced B-cell impairment (Mandrup-Poulsen et al. 1987; Pukel et al. 1988). For this purpose, the glucose metabolism was studied with the parallel use of two differently labelled D-glucose molecules (Sener & Malaisse 1987). The production of 3H2O from D-[5-3H] glucose (glucose utilization, mainly a cytosolic phenomenon) occurs during the generation of triose phosphates, and also when 2-phosphoglycerate is converted into phosphoenolpyruvate. The production of 14CO2 from D-[6-14C] glucose (glucose oxidation, a mitochondrial event) occurs when acetyl-CoA is subsequently oxidized in the tricarboxylic acid cycle. rIL-1 or an association of rIL-1 and rTNF did not induce any decrease in glucose utilization. However, there was a significant decrease in glucose oxidation in the presence of 30 pmol/l rIL-1, further potentiated by the addition of 25 µg/l rTNF. rTNF alone had no effect on glucose oxidation, similarly to previous findings suggesting that this cytokine does not impair islet function (Sandler et al. 1987). The observed potentiating effect of rTNF on rIL-1 induced impairment in islet oxidative metabolism of glucose is in good agreement with the previously proposed potentiating role of rTNF on rIL-1 action on the B-cells, in terms of inhibition of insulin release and cell lysis (Mandrup-Poulsen et al. 1987; Pukel et al. 1988). Furthermore, these data suggest that this potentiation, at least in terms of impairment of glucose metabolism, can occur at the mitochondrial level. It is conceivable that in the long range the observed impairment in mitochondrial function can contribute to cell death.

The present findings of a normal glucose utilization, together with a defective glucose oxidation after rIL-1 or rIL-1 plus rTNF exposure, have also been observed immediately after exposure to al-
loxan (Borg et al. 1979) and in islets maintained in culture for 7 days after exposure to streptozotocin (Eizirik et al. 1988a), a treatment that induces a long-lasting impairment in glucose-stimulated insulin release (Eizirik et al. 1988b). These observations contrast with the previous suggestions that glucokinase could be the main ‘glucose sensor’ in B-cells (Meglaskos & Matschinsky 1984), and that a specific inactivation of glucokinase could explain alloxan-induced diabetes in experimental animals (Lenzen & Panten 1988) and some cases of human diabetes (Meglaskos & Matschinsky 1984). On the contrary, in the light of the present and previous findings (Borg et al. 1979; Malaisse & Sener 1985; Sener & Malaisse 1987; Eizirik et al. 1988a), it seems likely that regulatory sites distal to glucose phosphorylation may have a more important role in the stimulus-secretion coupling in glucose-induced insulin release. Furthermore, an impaired mitochondrial handling of glucose can be a common feature of B-cells after exposure to different assaults.

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References


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