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Suppression of bank vole pancreatic islet function by proinflammatory cytokines

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Key words: proinflammatory cytokines, interleukin -1 β , tumor necrosis factor - α , interferon - γ , pancreatic islets, bank vole.

Summary

We recently characterized β -cell function of pancreatic islets from normal and glucose intolerant/diabetic bank voles. These animals had features of both human type 1 and type 2 diabetes. Presently, we studied how pancreatic islets isolated from normal i.e. glucose tolerant bank voles are affected by proinflammatory cytokines *in vitro*.

IL-1 β alone or in combination with TNF- α + IFN- γ markedly reduced insulin gene expression and the (pro)insulin biosynthesis rate, which was accompanied by a profound depletion of the islet insulin content. The decrease of islet insulin content was not prevented by the preferential inducible NO synthase inhibitor aminoguanidine.

Our findings suggest that the reduction in islet insulin content is not attributed to enhanced exocytosis or related to altered glucose metabolism, but rather are due to a decline in insulin production. The suppressive effects of islet functions elicited by cytokines seem to be mediated by an NO-independent mechanism. In relation to previous studies on cytokine effects on islets from various species, the bank vole islets show a pattern which more resembles human islets than rat or murine islets.

Abstract

Bank voles kept in captivity may develop diabetes. We recently characterized β -cell function of pancreatic islets from normal and glucose intolerant/diabetic bank voles. These animals had features of both human type 1 and type 2 diabetes. Cytokines may impair β -cell function in both types of diabetes. Presently, we studied how pancreatic islets isolated from normal i.e. glucose tolerant bank voles are affected by proinflammatory cytokines *in vitro*.

Islets were exposed to hIL-1 β (25 U/ml) alone or in combination with hTNF- α (1000 U/ml) + mIFN- γ (1000 U/ml) for 48 hours, whereupon islet functions were assessed.

Cytokines markedly reduced insulin gene expression and the (pro)insulin biosynthesis rate, which was accompanied by a profound depletion of the islet insulin content. The cytokines did not affect the culture medium insulin accumulation and the glucose oxidation rate, but caused a modest increase in medium nitrite, an indicator of nitric oxide (NO) generation. Cytokine-induced decrease of islet insulin content was not prevented by the preferential inducible NO synthase inhibitor aminoguanidine.

These findings suggest that the reduction in islet insulin content is not attributed to enhanced exocytosis or related to altered glucose metabolism, but rather are due to a decline in insulin production. The suppressive effects of islet functions elicited by cytokines seem to be mediated by an NO-independent mechanism. In relation to previous studies on cytokine effects on islets from various species, the bank vole islets show a pattern which more resembles human islets than rat or murine islets.

Introduction

Bank voles kept in captivity develop polydipsia and polyuria and subsequently diabetes to a significant extent (Blixt et al., 2007; Schoenecker et al., 2000). We have recently characterized β -cell function of pancreatic islets isolated from normal and glucose intolerant/diabetic bank voles and found that this animal model may have features of both human type 1 and type 2 diabetes (Blixt et al., 2007). At least in the wild state a novel picornavirus (Ljungan virus) have been found in the pancreas of the bank voles (Niklasson et al., 1999). Its possible role in the development of diabetes in the bank voles remain to be proven. It has been shown that the CD1 mouse offspring develop diabetes when the mother is inoculated with the Ljungan virus during gestation (Niklasson et al., 2006).

Cytokines play a crucial role in modulation of the immune cells during immune reactions, but they are also believed to be important for the impairment of the β -cell function during the autoimmune destruction in type 1 diabetes (Rabinovitch et al., 1998; Sandler et al., 1991). It has also been suggested that inflammatory mediators like cytokines may be involved in the development of type 2 diabetes both at the β -cell level and in peripheral tissues (Donath et al., 2003; Kolb et al., 2005; Larsen et al., 2007; Sjöholm et al., 2006). Proinflammatory cytokines like IL-1 β and TNF- α , secreted from activated macrophages and T-cells, are proposed to be responsible for the activation of inducible nitric oxide synthase (iNOS) (Mandrup-Poulsen, 1996). Activated iNOS produce nitrogen oxide (NO) free radicals that may perturb islet function and even be toxic to the β -cell and ultimately lead to cell death (Eizirik et al., 1992; Southern et al., 1990; Welsh et al., 1991). In rodent islets iNOS is activated when exposed to IL-1 β alone or when added in combination with TNF- α or IFN- γ or both. However, in human islets a combination of IL-1 β + IFN- γ or IL-1 β + TNF- α + IFN- γ are needed to activate iNOS (Eizirik et al., 1994).

Presently, we have studied how pancreatic islets isolated from normal i.e. glucose tolerant bank voles are affected by prolonged exposure to proinflammatory cytokines *in vitro*. We have chosen to use IL-1 β alone and in combination with TNF- α and IFN- γ to ascertain iNOS activation if possible. Indeed, it has been shown that pancreatic islets of various species may exhibit differences in response to these cytokines. Herein we report that bank vole islets are affected by cytokine exposure, but in contrast to rat and mouse islets treated in a similar way the functional changes seem to be NO independent.

Materials and methods

Animals

Bank voles (*Myodes glareolus*) were housed at Astrid Fagreu Laboratory, Karolinska Institute, Sweden with free access to water and standard laboratory chew. The experimental procedures were approved by the animals' ethical committee in Stockholm (N248/03) and in accordance with international guidelines (NIH publications no.85-23, revised 1985).

Male bank voles 15-20 weeks of age were tested with a glucose tolerance test (GTT) where 2 g glucose/kg body weight was injected IP. Blood glucose measurement was performed with an automated glucose meter (Accu-Chek Aviva; Roche Diagnostics, Bromma, Sweden) immediately prior to injection (0 minutes), at 60 and 120 minutes after glucose injection. Animals with 120 min blood glucose < 11.1 mM were classified as glucose tolerant. The pancreas in such animals was resected and placed in Hanks' balanced salt solution (HBSS; SBL Vaccine, Stockholm, Sweden) supplemented with 50 U/ml benzylpenicillin and 0.05 mg/ml streptomycin. The animals were anaesthetized with Isoflurane (Abbott Scandinavia, Solna, Sweden) prior to testing and killing.

Islet isolation, culture and cytokine exposure

Pancreatic islets were isolated by collagenase digestion as described elsewhere (Blixt et al., 2007). Islets from individual bank voles were maintained in medium RPMI 1640 with 11.1 mM glucose supplemented with 10 % fetal calf serum, 2 mM glutamine and 50 U/ml benzylpenicillin and 0.05 mg/ml streptomycin. Islets were precultured at 37 °C, in humidified air + 5 % CO₂ for 5 days whereupon the pre-culture islets were transferred to new culture dishes containing culture medium as above, with or without addition of IL-1 β (25 U/ml corresponding to 144.5 pM; human; PeproTech EC Ltd, London, UK) alone or in combination with TNF- α (1000 U/ml corresponding to 143.7 pM; human; PeproTech) + IFN- γ (1000 U/ml corresponding to 160.3 pM; murine; PeproTech) and cultured for 48 hours before tested. In separate experiments we also added aminoguanidine, which is a preferential inhibitor of iNOS (Holstad et al., 1997), to islet cultures with or without the cytokine combination during the 48 hours culture period.

Medium insulin accumulation, glucose-stimulated insulin release and islet insulin content

Culture medium was collected for determination of medium insulin accumulation. Islets in triplicate of 10 were incubated at 1.7 mM glucose in KRBH supplemented with 2 mg/ml BSA at 37 °C (O₂/CO₂; 95/5 %) for 1 hour. Subsequently the buffer was replaced with KRBH supplemented 16.7 mM glucose and incubated in an additional hour. After the incubations the islets were pooled in groups of 30 and dispersed through sonication in H₂O. The islet insulin was extracted at 4 °C in 70 % ethanol supplemented with 0.13 mM HCl. The insulin concentrations were measured with rat insulin ELISA and high range rat insulin ELISA kit (Merckodia AB, Uppsala, Sweden).

Islet total protein and (pro)insulin biosynthesis and glucose oxidation rates

Groups of 10 islets in duplicate were incubated in KRBH containing either 1.7 mM or 16.7 mM glucose and ³H-labeled leucine (protein and (pro)insulin biosynthesis), or KRBH supplemented 16.7 mM glucose and D-[U-¹⁴C]glucose (glucose oxidation rate) and further analysis was performed as described previously (Blixt et al., 2007).

Medium nitrite

Culture medium samples were mixed with 0.05 % N-(1-naphtyl)-ethylenediamine dihydrochloride and 0.5 % sulfanilamide dissolved in phosphoric acid and incubated at 60 °C in 2 minutes. Nitrite levels were determined through absorbance measurement at 546 nm in a spectrophotometer. Culture media collected from wells without islets was used as measurement of the original nitrite level in the media.

RNA isolation, cDNA synthesis and Real Time PCR

50 islets from each group were used to extract total RNA with RNeasy Micro kit (Qiagen, Hilden, Germany) complemented with RNase-free DNase (Qiagen) and eluted in 14 µl of RNase free water. The RNA amount and purity was tested using the Nanodrop ND-1000 system (NanoDrop Technologies, Delaware, USA).

Synthesis of cDNA was performed with Reverse Transcription System (Promega, Madison, USA) using 1:2 volume of total RNA and 0.025 µg/µl Oligo(dT)15 primer. The reactions were incubated at 42 °C in 60 minutes followed by 99 °C in 5 minutes.

The LightCycler Instrument (Roche) combined with sequence independent detection with SYBR Green I was used to amplify and analyze generated cDNA. The sequence of the primers used were: insulin (mouse) forward 5'-

CCATCAGCAAGCAGGTTAT-3', reverse 5'-GGGTGTGTAGAAGAAGCCA-3', PDX-1 (mouse) forward 5'-GGTGCCAGAGTTCAGCGCTA -3' reverse 5'-TTGTTTTCTCCTCGGGTTCCGC-3' and β -actin (mouse) forward 5'-CCACCGATCCACACAGAGTACTTG-3' reverse 5'-GCTCTGGCTCCTAGCACC-3' (TIB MOLBIOL Syntheselabor GmbH, Berlin, Germany) PCR amplification of 0.1 μ g of cDNA sample were performed with 0.2 μ M of each insulin or PDX-1 primer and 0.5 μ M of each β -actin primer in SYBR Green JumpStart ready mixture (FastStart Taq DNA polymerase, dNTP mix and SYBR Green I dye; Roche) and 3.5 mmol/l $MgCl_2$. After an initial 95 °C in 30 seconds the following was cycled 40 times; 94 °C in 5 seconds, 47 °C in 10 seconds and 72 °C in 15 seconds. Cycle threshold (Ct) values were obtained for individual samples with the second derivative maximum method (Gibson et al., 1996; Heid et al., 1996). The relative expression was calculated from the formula $2^{-(\Delta Ct)}$.

Viability

Islet viability was determined using Hoechst 33342 and propidium iodide. In each test 10 islets were incubated with 5 μ g/ml bisbenzimidazole and 20 μ g/ml propidium iodide for 20 minutes. The islets were washed in PBS and the emitted fluorescence was observed at 461 nm and 615 nm respectively after excitation with UV light. The nucleus morphology was assessed and cells were counted by a blinded observer using a fluorescence microscope.

Statistical analysis

A mean was calculated from each duplicate or triplicate groups of islets and then considered as one separate observation. Each observation represents an individual bank vole islet donor. Values are expressed as means $\pm$ S.E.M., and groups of data were compared using Student's t-test, ANOVA with a Bonferroni correction or Mann-Whitney rank sum test. Tests are considered statistically significant when $P \leq 0.05$. Statistical analysis was performed using SigmaStat® (SPSS Inc., Chicago, IL, USA).

Results

Islets isolated from glucose tolerant bank voles and exposed to IL-1 β or a combination of IL-1 β + TNF- α + IFN- γ showed similar medium insulin accumulation as compared to non-cytokine exposed control islets (Table 1). Islet insulin secretion was affected by the proinflammatory cytokines. After culture it was seen that the basal insulin release at low glucose (1.7 mM) was elevated from islets cultured with the cytokine combination, but not to a significant extent after addition of IL-1 β alone (Table 1). At high glucose (16.7 mM) there was a trend towards a decline in insulin release after cytokine addition. However, the relative stimulation of insulin secretion at high glucose vs. low glucose was lowered following addition of IL-1 β alone or of the cytokine mixture. The islet insulin content was markedly decreased after culture in the presence of either IL-1 β or the combination of IL-1 β + TNF- α + IFN- γ (Table 1). Also the islet oxidative metabolism was assessed by determination of glucose oxidation rates at 16.7 mM glucose (Table. 1). Thus, it was found that neither IL-1 β nor the combination of cytokines affected the glucose oxidation rates.

Next, we measured the islet capacity to synthesize proteins and (pro)insulin at a low and high glucose concentration (Table 2). Due to scarcity of islets, incubations at 1.7 mM glucose after culture with the cytokine combination were not performed. IL-1 β did not affect the (pro)insulin or total protein biosynthesis rates at low glucose. The (pro)insulin biosynthesis rate at high glucose was impaired after culture with both IL-1 β alone and the cytokine combination. The total protein biosynthesis was also reduced after addition of the cytokine mixture. The (pro)insulin biosynthesis rates were more suppressed compared to protein biosynthesis rates, which resulted in a substantial decline in the relative contribution of labeled (pro)insulin to the total pool of labeled proteins, although for the cytokine combination this was barely not statistically not significant ($P = 0.06$).

In subsequent experiments we assessed transcription level of the insulin and PDX-1 (Table 3). Insulin gene transcription was reduced by approximately 50 % after cytokine addition. PDX-1 was also expressed in bank vole islets and also this transcript tended to be reduced, but this difference did not attain statistical significance.

We also determined the amount of non-viable islet cells by staining with Hoechst 33342 and propidium iodide after culture with the cytokine combination. There was not an increase in death of bank vole islet, but rather on the contrary there was

tendency ($P = 0.06$) towards a decrease in the number of non-viable islet cells after exposure to IL-1 β + TNF- α + IFN- γ compared to control islets (Figure 1).

As an indicator of NO formation nitrite accumulation in the culture medium was measured. There was a modest increase in nitrite after addition of IL-1 β alone, but no significant increase after adding the cytokine combination (Table 1). In order to examine if the NO production may be attributed to iNOS activity, aminoguanidine (2 mM) a preferential iNOS inhibitor (Holstad et al., 1997) was used (Figure 2A). Addition of aminoguanidine reduced the medium nitrite level both from non-cytokine and cytokine exposed islets. However, the reduction in islet insulin content induced by the cytokine combination was not counteracted by aminoguanidine (Figure 2B), suggesting that the decline in islet insulin content was not NO-dependent.

Finally, we also performed some experiments with addition of IL-1 β (25 U/ml) alone for only 24 h. This resulted in a decrease in the islet insulin content (ng/10 islets; Control: 292 ± 70 and IL-1 β : 90.5 ± 14.6 ; $P < 0.01$ ($n = 6$)), but no significant effects on medium insulin accumulation and glucose-stimulated insulin release was seen (data not shown).

Discussion

The aim of the present study was to investigate if proinflammatory cytokines affect the function of pancreatic islets isolated from bank voles *in vitro*. This was considered of interest since the bank voles develop diabetes in captivity and the animals show signs of both type 1 and type 2 diabetes, and cytokines may have a role in both types of diabetes (Donath et al., 2003; Kolb et al., 2005; Larsen et al., 2007; Mandrup-Poulsen, 1996; Rabinovitch et al., 1998). We used human and mouse preparations of cytokines in our experiments because bank vole specific cytokines are not available. Despite this the cytokines elicited herein various effects when islet functions were examined.

In the present study we found that the cytokines markedly reduced the insulin gene expression and the (pro)insulin biosynthesis rate and this was accompanied by a profound depletion of the islet insulin content. The cytokines also induced a slight inhibition of glucose-stimulated insulin secretion, but they did not affect the culture medium insulin accumulation and the glucose oxidation rate. This finding suggest that the reduction in islet insulin content was not attributed to an enhanced exocytosis and not related to an altered glucose metabolism, but rather was due to a decline in the formation of insulin.

This pattern is partly similar and partly it differs to what have been seen in experiments using islet preparations from other species. In rat pancreatic islets IL-1 β induced strong suppressive effects on all functions described above and it is likely that impaired glucose metabolism is a key factor (Sandler et al., 1987; Sandler et al., 1989; Welsh et al., 1991). Concerning mouse pancreatic islets exposed to IL-1 β the insulin content and (pro)insulin biosynthesis rates were depressed, medium insulin accumulation and glucose oxidation rates essentially unaffected, but glucose-stimulated insulin release reduced by about 50% (Eizirik et al., 1990). Furthermore, human islets cultured with IL-1 β alone for a prolonged period show a stimulated β -cell function (Eizirik et al., 1993), and when a combination of cytokines was added a strong increase in medium insulin accumulation, a decrease in islet insulin content, a decline in relative glucose-induced insulin release (16.7/1.7), but no change in glucose oxidation rate was seen (Eizirik et al., 1994). When analyzing the absence and presence of functional alterations observed after prolonged treatment of bank vole islets to IL-1 β or the cytokine combination, the islets of this species more resemble isolated human islets than isolated rat or murine islets.

Especially NO, via induction of the iNOS, has been found to be an important mediator of cytokine actions in β -cell preparations; for a review see (Eizirik et al., 1996). When iNOS is induced this normally leads to very marked increase in NO formation above basal NO generation formed from constitutive NOS (cNOS) enzymes. NO is mostly assessed by nitrite medium accumulation in cell culture experiments. Herein we found only a modest increase in medium nitrite after culture of bank vole islets with IL-1 β and no significant increase when IL-1 β + TNF- α + IFN- γ were added. Currently, addition of aminoguanidine reduced nitrite generation from both control and cytokine exposed islets, but this effect of aminoguanidine is probably due to inhibition of cNOS activity since aminoguanidine is not a completely selective blocker of iNOS (Holstad et al., 1997). However, this effect was not accompanied by a protection against cytokine mediated decrease in islet insulin content (Figure 2B). This suggests that it is unlikely that iNOS and excessive NO production would have contributed to the inhibitory actions we observed in the present study. Thus, NO-independent suppressive actions elicited by the cytokines must be considered in this context. Indeed, such mechanisms have been suggested to occur based on experiments with human islets (Eizirik et al., 1994), rat-derived β -cell preparations (Dunger et al., 1996; Suarez-Pinzon et al., 1994) and pancreatic islets from mice with an inactivated iNOS gene (Andersson et al., 2001). In experiments performed with the latter type of islets it has been shown that mRNA expression for the transcription factor PDX-1 was reduced by cytokines (Andersson

et al., 2005). Presently, we observed a reduction in insulin gene expression by 50% after exposure to IL-1 β + TNF- α + IFN- γ and a tendency ($P = 0.087$) that PDX-1 mRNA was reduced.

An unresolved issue is that it can not be excluded that the colony of bank voles examined in our study is infected by the Ljungan virus. If so and whether this would have influenced our findings when cytokines were added to the isolated pancreatic islets is unclear. In this context it is of interest that it was recently reported that BB rat model for type 1 diabetes is infected with the Ljungan virus (Niklasson et al., 2007).

In the present study we assessed if the combinations of cytokines elicited increased cell death, but we did not observe such an effect. This lack of effect could be due to that the period of cytokine treatment was too short and/or the concentrations of cytokines were insufficient, a species (bank vole) specific cytokine action might be required, and of course it can not be excluded that the proinflammatory cytokine combination does not induce cytotoxicity to the bank vole islet cells. If anything it could be that the particular cytokine treatment protocol may reduce bank vole islet cell death *in vitro*. The mechanism underlying such a putative effect is unclear but one speculation is that IFN- γ may interfere with cell death signals occurring in the bank vole islet cells *in vitro*.

Another question is if the islet function of islets isolated from bank voles that will develop diabetes, is similar to the islet function observed in the present study after cytokine addition *in vitro*. In our previous study on islets isolated from glucose intolerant/diabetic bank voles and examined acutely after isolation, we observed that this subpopulation of islets exhibited an enhanced function perhaps due to an enhanced functional demand on the remaining islets (Blixt et al., 2007). In contrast, in the present study in which islets were isolated from glucose tolerant bank voles and exposed to proinflammatory cytokines showed a suppression of islet functions. However, from these combined observations it can nevertheless not be established whether or not direct actions of proinflammatory cytokines on islet function *in vivo* play a role during development of diabetes in bank voles.

In conclusion, pancreatic islets isolated from glucose tolerant bank voles are affected by prolonged exposure to proinflammatory cytokines. This effect seems to be essentially NO-independent, and a key cellular event appears to be a decreased level of insulin mRNA leading to a decline in insulin translation and a depletion of insulin stores in the β -cells.

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Legends to the figures

Figure 1.

Percent non-viable islet cells in isolated bank vole islets cultured in 48 hours with or without IL-1 β (25 U/ml) + TNF- α (1000 U/ml) + IFN- γ (1000 U/ml). Islets were stained with Hoechst 33342 and propidium iodide, and cells were counted by a blinded observer using a fluorescence microscope. The number of non-viable cells are expressed as percent of total islet cell count and presented as means $\pm$ S.E.M. for n observations. Data are compared using ANOVA with a Bonferroni correction where x denotes $P = 0.06$ vs. control islets.

Figure 2

Islets in groups of 70 were cultured for 48 hours with or without IL-1 β (25 U/ml) + TNF- α (1000 U/ml) + IFN- γ (1000 U/ml) and in the presence and absence of aminoguanidine (2 mM). Medium nitrite concentration (**A**) and islet insulin content (**B**) was subsequently determined. Values are means $\pm$ S.E.M. for n observations. Data are compared using ANOVA with a Bonferroni correction where *, ** and *** denote $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ vs. control islets (no addition) x and xx denote $P \leq 0.05$ and $P \leq 0.01$ vs. cytokine exposed islets.

Table 1. Effects of proinflammatory cytokines on insulin secretion, glucose metabolism and nitrite production of pancreatic islets isolated from bank voles.

	Control	IL-1 β	IL-1 β + TNF- α + IFN- γ
Medium insulin accumulation (ng/10 islets x 24 h)	481 $\pm$ 57.7 (20)	548 $\pm$ 90.1 (10)	404 $\pm$ 34.6 (14)
Islet insulin content (ng/10 islets)	297 $\pm$ 27.0 (8)	81.1 $\pm$ 13.5 (6) ***	91.9 $\pm$ 6.8 (8) ***
Islet insulin release (ng/10 islets x 1 h)			
1.7 mM glucose	18.0 $\pm$ 1.4 (8)	24.1 $\pm$ 2.6 (6)	25.8 $\pm$ 1.7 (8) *
16.7 mM glucose	57.8 $\pm$ 5.1 (8)	38.8 $\pm$ 6.0 (6)	46.6 $\pm$ 3.6 (8)
16.7/1.7 (fold increase)	3.6 $\pm$ 0.5 (8)	1.7 $\pm$ 0.3 (6) **	2.5 $\pm$ 0.2 (8) **
Islet glucose oxidation rate (pmol/10 islets x 60 min)	492 $\pm$ 80.6 (13)	387 $\pm$ 56.5 (9)	395 $\pm$ 64.5 (8)
Medium nitrite concentration (nM/10 islets)	52.8 $\pm$ 6.7 (19)	93.6 $\pm$ 16.7 (10) *	72.6 $\pm$ 7.2 (15)

Islets from glucose tolerant bank voles were isolated and precultured for 5 days in medium RPMI 1640 + 10% fetal calf serum, then the islets were further cultured in fresh medium for 48 hours without or with addition of 25 U/ml IL-1 β or in combination with 1000 U/ml TNF- α + 1000 U/ml IFN- γ as indicated. Values are means $\pm$ S.E.M. for the number observations given in parenthesis. *, ** and *** denote $P < 0.05$, $P < 0.01$ and $P < 0.001$ vs. control islets, using Student's unpaired t-test.

Table 3. Islet insulin and PDX-1 gene expression after 48 hours exposure to IL-1 β + TNF- α + IFN- γ .

mRNA ($2^{-(\Delta Ct)}$)	Control	IL-1 β + TNF- α + IFN- γ
Insulin	42.58 $\pm$ 8.51 (8)	22.46 $\pm$ 2.68 (9) *
PDX-1	0.0038 $\pm$ 0.0016 (6)	0.0016 $\pm$ 0.0003 (9) x

The relative islet insulin and PDX-1 gene expression compared to beta actin gene expression calculated from the difference in the Ct values. The islets used were isolated from glucose tolerant bank voles and precultured in 5 days in RPMI 1640 + 10% fetal calf serum, then the islets were further cultured in fresh medium for 48 hours with 25 U/ml IL-1 β , 1000 U/ml TNF- α + 1000 U/ml IFN- γ as indicated. Values are means $\pm$ S.E.M. for the number of observation stated in parenthesis. Data were compared using Mann-Whitney rank sum test where * and x denotes $P \leq 0.05$ and $P = 0.087$ vs. control islets incubated at the same glucose concentrations.

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Table 2. Islet (pro)insulin biosynthesis and total protein biosynthesis rates after 48 hours exposure to IL-1 β alone or in combination with TNF- α + INF- γ .

	1.7 mM glucose		16.7 mM glucose		
	Control	IL-1 β	Control	IL-1 β	IL-1 β + TNF- α + IFN- γ
(Pro)insulin (dpm x 10 ³ / 10 islets x 2 h)	15 $\pm$ 2.4 (4)	14 $\pm$ 2.5 (4)	42 $\pm$ 3.1 (7)	20 $\pm$ 4.0 (4) **	12 $\pm$ 3.6 (3) ***
Total protein (dpm x 10 ³ / 10 islets x 2 h)	74 $\pm$ 6.7 (4)	97 $\pm$ 13 (4)	118 $\pm$ 10 (7)	105 $\pm$ 6.3 (4)	56 $\pm$ 9.1 (3) **
(Pro)insulin / total protein x 100 (%)	20 $\pm$ 2.3 (4)	15 $\pm$ 2.0 (4)	36 $\pm$ 2.5 (7)	20 $\pm$ 5.0 (4) *	21 $\pm$ 4.5 (3) x

Islet total biosynthesis and (pro)insulin synthesis rate at low (1.7 mM) glucose and high (16.7 mM) glucose conditions. Islets isolated from glucose tolerant bank voles and precultured in 5 days in RPMI 1640 + 10% fetal calf serum, then the islets were further cultured in fresh medium for 48 hours with or without addition of 25 U/ml IL-1 β alone or in combination with 1000 U/ml TNF- α + 1000 U/ml IFN- γ as indicated. Values are means $\pm$ S.E.M. for the number of observation stated in parenthesis. Data are compared using ANOVA with a Bonferroni correction where *, **, *** and x denote $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ and $P = 0.06$ vs. control islets incubated at the same glucose concentrations.