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Interleukin-1β inhibition of insulin release in rat pancreatic islets: possible involvement of G-proteins in the signal transduction pathway

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Summary In vitro exposure of rat pancreatic beta cells to interleukin-1β (IL-1β) inhibits glucose-stimulated insulin release (2140 ± 239 and 323 ± 80 pg · islet^{-1} · h^{-1} at glucose levels of 16.7 mmol/l in control and IL-1β-exposed islets, respectively, $n = 7$, $p < 0.001$). Cholera toxin (2 μg/ml) or pertussis toxin (0.5 μg/ml) potentiated, as expected, glucose-induced insulin release in control islets, but, in addition, when added together with IL-1β, were able to prevent the IL-1β mediated inhibition of glucose-stimulated insulin secretion (2087 ± 301 and 1662 ± 173 pg · islet^{-1} · h^{-1}, respectively, $p < 0.05$ vs islets exposed to IL-1β alone). To investigate the mechanism by which the toxins prevent the IL-1β effect, we then measured nitrite levels, glucose oxidation and Ca^{2+} uptake. Nitrite levels in the culture medium were 4.2 ± 1.4 and 24.0 ± 5 pmol · islet^{-1} · 24 h^{-1} in control islets and in IL-1β-exposed islets, respectively ($n = 6$, $p = 0.05$). In islets exposed to IL-1β and cholera or pertussis toxins, nitrite levels were 9.1 ± 3 and 12.4 ± 6 pmol · islet^{-1} · 24 h^{-1}, respectively ($n = 6$, NS vs control islets). Glucose oxidation at 16.7 mmol/l glucose was 31.1 ± 2.9 pmol · islet^{-1} · 120 min^{-1} in control islets and 16.8 ± 2.7 pmol · islet^{-1} · 120 min^{-1} in IL-1β-treated islets ($p < 0.05$). The addition of cholera or pertussis toxins simultaneously to IL-1β prevented the inhibition of glucose oxidation at 16.7 mmol/l glucose (32.9 ± 3.8 and 31.7 ± 3.3 pmol · islet^{-1} · 120 min^{-1} in the presence of cholera or pertussis toxins, respectively). Glucose-stimulated $^{45}Ca^{2+}$ uptake was also significantly inhibited in IL-1β-treated islets when compared to control islets (7.1 ± 0.9 and 16.8 ± 3.2 pmol · islet^{-1} · 20 min^{-1}, respectively, $p < 0.05$). This inhibition was prevented by the presence of cholera or pertussis toxins (14.0 ± 3.8 and 11.2 ± 2.7 pmol · islet^{-1} · 20 min^{-1}, respectively). In conclusion, our data show that cholera and, to a lesser extent, pertussis toxins are able to partially prevent the IL-1β-induced increase in nitrite levels and block the inhibitory effects of IL-1β on different steps leading to glucose-induced insulin secretion. These findings support the possibility that in pancreatic beta cells, G-proteins may be involved or interfere with the cytokine signal transduction. [Diabetologia (1995) 38: 779–784]

Key words Interleukin-1β, pertussis toxin, cholera toxin, pancreatic islets, insulin secretion, G-proteins, nitric oxide.

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Abbreviations: IL-1β, Interleukin 1β; CT, cholera toxin; PT, pertussis toxin; NO, nitric oxide; FCS, fetal calf serum; KRHB, Krebs-Ringer Hepes buffer.

Interleukin-1β (IL-1β) is believed to play a role in the process leading to beta-cell dysfunction [1–3]. Several in vitro studies have shown that chronic exposure of rat pancreatic islets and purified beta cells to IL-1β inhibits glucose-induced insulin release [4–10]. The exact biochemical mechanism whereby IL-1β affects insulin secretion, however, is still under debate. Recent data indicate that IL-1β activates the inducible form of nitric oxide synthase (iNOS), with the subsequent accumulation of nitric oxide (NO) [11–14].

The increased NO levels are likely to play a major role in rat islets and purified beta cells in mediating the IL-1β-induced inhibition of insulin secretion and glucose oxidation, since these effects are prevented by inhibitors of NO synthase [15]. Moreover, a complete recovery of insulin secretion is observed 8 h after inhibition of NO synthase [16].

IL-1β, as well as several hormones and secretagogues, exerts its biological effects on pancreatic islets by binding to a specific IL-receptor [17–20]. IL-1β receptors have been identified in normal rat pancreatic beta cells [21], and in the insulinoma cells RIN-m5F and HIT [22, 23]. In a variety of cell types, including fibroblasts and T- and B-lymphocytes, the biological effects of IL-1β are pertussis toxin sensitive, suggesting the involvement of a Gi-like protein in IL-1β signalling [24–26]. G-proteins seem to play an important role as modulators of insulin secretion [27]. They link the hormone binding surface receptor to its effector system such as adenylate cyclase, phospholipases and ion channels located in the intracellular surface of the plasma membrane [28, 29]. Little is known about the intracellular signalling mechanism for the IL-1β/IL-1β receptor complex in pancreatic beta cells and the involvement of G-proteins in IL-1β effects on these cells has never been demonstrated.

In the present study we investigated whether G-proteins might be involved in the inhibitory effects of IL-1β on beta-cell function. For this purpose, rat pancreatic islets were pre-exposed to IL-1β alone or in combination with cholera (CT) or pertussis (PT) toxins, two agents commonly used for studying the G-protein function. In these cells nitrite levels (as an indicator of NO production) and glucose-induced insulin secretion were measured, as well as two important steps in the cascade of intracellular events leading to glucose-induced insulin release: glucose oxidation and Ca^{2+} uptake.

Materials and methods

Materials. Crude collagenase was obtained from Boehringer Mannheim (Mannheim, Germany). Culture medium CMRL-1066, heat inactivated fetal calf serum (FCS), glutamine and gentamycin were obtained from Gibco (Glasgow, UK). Human recombinant interleukin-1β (IL-1β) (specific activity 5×10^8 U/mg), $^{45}CaCl_2$ (25 mCi/mg) and D-[U-^{14}C]glucose were from Amersham (Amersham, Bucks., UK). Cholera and pertussis toxins, and Antimycin A were purchased from Sigma (London, UK). Silicone oil (density 1.040) was from Merck (Darmstadt, Germany). All other chemicals were of analytical grade.

Islet preparation and culture conditions. Pancreatic islets were isolated by the collagenase method from 200- to 250-g fed male Wistar rats injected i.p. with 0.2 ml of a 0.2 % pilocarpine solution 2 h before killing by decapitation. With this technique, 300–400 islets were isolated from each pancreas [30]. The whole procedure was completed within 120 min. Purified islets were cultured in CMRL-1066 medium (5.5 mmol/l glucose) containing 10 % FCS, 2 mmol/l L-glutamine and gentamycin at 37 °C in a 95 % air/5 % CO_2 atmosphere with or without IL-1β ± CT or PT for 24 h. After this period, either insulin secretion, nitrite levels, glucose oxidation or $^{45}Ca^{2+}$ uptake were studied.

Insulin secretion. To study insulin secretion, triplicate groups of five purified islets were incubated in Krebs-Ringer Hepes buffer (KRHB, containing mmol/l 115 NaCl, 5.4 KCl, 2.38 $CaCl_2$, 0.8 $MgSO_4$, 1 Na_2HPO_4, 10 Hepes, 0.5 % bovine serum albumin, pH 7.35) containing either 2.8 (basal) or 16.7 mmol/l glucose. Insulin in the medium was measured by radioimmunoassay after 1 h incubation at 37 °C. Results are expressed as insulin released in the medium ($pg \cdot islet^{-1} \cdot h^{-1}$).

Nitrite determination. Nitrite, the stable end product of NO, was determined in culture medium as described by Welsh et al. [13]. Briefly, 150 islets were incubated in 0.6 ml of culture medium for 24 h. Triplicate samples of 90 µl were then removed from the medium and added to 10 µl of 0.5 % N-(1-naphtyl)ethylendiamine dihydrochloride (NED), 5 % sulphanilamide in a 25 % H_3PO_4 solution, prepared less than 12 h before use. The reaction was carried out at 60 °C for 2 min, and the absorbance at 546 nm was measured in a Packard spectrophotometer (Packard, Groningen, The Netherlands) against a standard curve. The detection limit was 50 pmol, and the variability of the assay was less than 5 %.

Glucose oxidation. Glucose oxidation was determined by measuring the formation of $^{14}CO_2$ from [U-^{14}C]glucose [31]. After 24 h preincubation with or without 50 U/ml IL-1β ± CT or PT, groups of 15 islets were incubated in 100 µl of Krebs-Ringer bicarbonate buffer [(mmol/l) 118 NaCl, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$] supplemented with 10 mmol/l Hepes (pH 7.4) containing 3 µCi D-[U-^{14}C]glucose (specific activity, 270 mCi/mmol) plus non- radioactive glucose to a final concentration of either 1.5 or 16.7 mmol/l. The vials, suspended in standard 20-ml glass scintillation flasks, were gassed with air: CO_2 (95:5) and capped air tight. The flasks were then shaken continuously at 37 °C for 120 min. The metabolism was stopped by injecting 100 µl of 0.05 mmol/l Antimycin A (dissolved in 70 % ethanol) into the centre vial. This was immediately followed by an injection of 250 µl hyamine hydroxide (New England Nuclear, Boston, Mass., USA) into the outer flasks. $^{14}CO_2$ was liberated from the incubation medium by a subsequent injection into the centre vial of 100 µl of 0.4 mmol/l Na_2HPO_4 solution adjusted to pH 6.0. After 1 h at room temperature (to allow the liberated $^{14}CO_2$ to be trapped by the hyamine hydroxide) 10 ml of a scintillation fluid was added to each flask and the radioactivity determined in a liquid scintillation counter.

Calcium uptake. $^{45}Ca^{2+}$ uptake was measured according to the method described by Henquin and Lambert [32]. After a 24-h pre-incubation with or without 50 U/ml IL-1β ± CT and/or PT, groups of 15 islets were transferred into 50 µl Krebs-bicarbonate buffer modified by replacing phosphate and sulphate with equimolar amounts of chloride [33] and layered on silicone oil. The uptake period was started by adding 50 µl of medium containing $^{45}Ca^{2+}$ (2.5 mmol/l) and glucose (final concentration 2.8 or 16.7 mmol/l). The reaction was stopped by centrifuging the islets for 2 min in a microfuge (Beckman Instruments, Palo Alto, Calif., USA) through the layer of silicone oil. The bottoms of the 400-µl tubes (Beckman) were then cut and the radioactivity of the pellet counted by liquid

Table 1. Effect of IL-1β with or without either CT (2 μg/ml) or PT (0.5 μg/ml) on insulin secretion (pg · islet^{-1} · h^{-1}) induced by different stimuli

	Culture conditions					
	Control	IL-1β	CT	CT + IL-1β	PT	PT + IL-1β
Glucose 2.8 mmol/l	117 ± 32	126 ± 39	198 ± 43	176 ± 28	157 ± 28	145 ± 38
Glucose 16.7 mmol/l	2140 ± 239	323 ± 80[b]	2975 ± 271[a]	2087 ± 301	3090 ± 225[a]	1662 ± 173

Data represent mean ± SEM of seven separate experiments.
[a] $p < 0.05$; [b] $p < 0.001$ vs control islets

scintillation. Tubes without islets were run as blanks. The uptake of [U-^{14}C]-sucrose was measured to correct for label in the extracellular space.

Statistical analysis

Statistical significance was assessed by Student's *t*-test for unpaired comparison.

Results

Insulin release. In control rat islets insulin release in the presence of a non-stimulatory glucose concentration (2.8 mmol/l) was 117 ± 32 pg · islet^{-1} · h^{-1}, (mean ± SEM, $n = 7$), and increased significantly in response to 16.7 mmol/l glucose (2140 ± 239, $n = 7$). In islets pre-exposed for 24 h to 50 U/ml IL-1β, basal insulin was not significantly affected (Table 1) but glucose-stimulated insulin release was markedly reduced (323 ± 80, $p < 0.001$ vs control islets, $n = 7$).

When increasing concentrations of either CT (dose range 0.1–5 μg/ml) or PT (0.1–2 μg/ml) were added simultaneously to 50 U/ml IL-1β at the beginning of the 24-h culture period, the inhibition of glucose-stimulated insulin release was progressively prevented (Fig. 1). The 50% of maximal effect was reached at a concentration of approximately 0.5 μg/ml for CT and 0.2 μg/ml for PT. The maximal effect was observed at 2 μg/ml CT and 0.5 μg/ml PT (glucose-stimulated insulin release 2087 ± 301, and 1662 ± 173 pg · islet^{-1} · h^{-1}, respectively). In islets treated for 24 h with 2 μg/ml CT or 0.5 μg/ml PT alone basal insulin release was unchanged, but glucose-stimulated insulin secretion was potentiated (Table 1).

To examine the possibility that the CT and PT effect on IL-1β inhibition of insulin release could be reversed by agents that increase cAMP levels without affecting G-proteins, we incubated groups of islets in the presence or the absence of IL-1β and forskolin (an agent known to increase cAMP levels by directly activating the enzyme adenyl cyclase). Unfortunately, in islets incubated for 24 h in the presence of forskolin (5 μmol/l) glucose-stimulated insulin release was reduced (690 pg · islet^{-1} · h^{-1}, $n = 4$, $p < 0.01$ vs control islets), thus making the interpretation of the results difficult. In islets exposed to IL-1β (50 U/ml) and forskolin (5 μmol/l) glucose-stimulated insulin release was 489 pg · islet^{-1} · h^{-1} ($n = 4$).

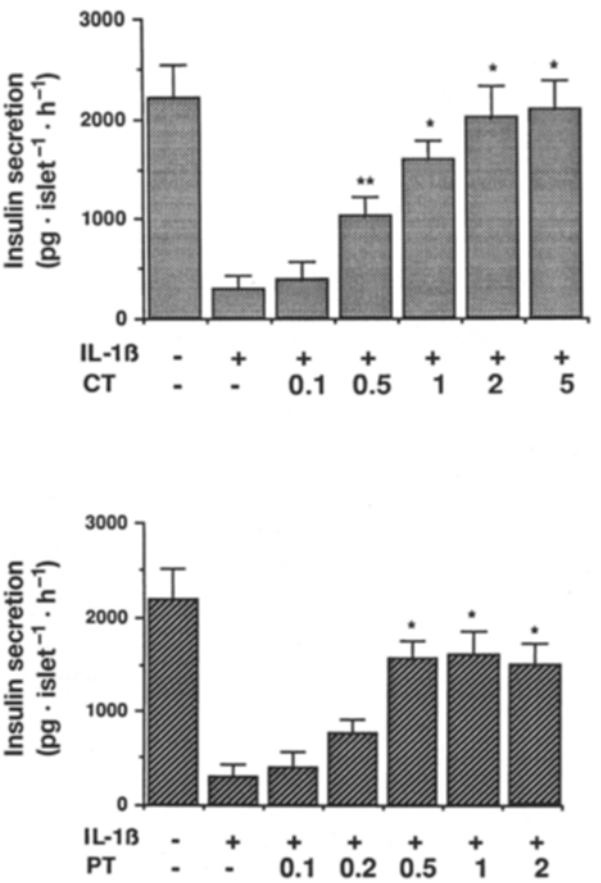

Fig. 1. Glucose-stimulated insulin release in rat pancreatic islets cultured for 24 h in the absence or presence of 50 U/ml IL-1β plus increasing concentrations of CT (upper panel) or PT (lower panel). At the end of the 24 h pre-incubation period, islets were washed three times and incubated for 1 h at 37 °C in KRHB containing 16.7 mmol/l glucose. Results (mean ± SEM of four separate experiments) are expressed as insulin released in the medium (pg · islet^{-1} · h^{-1}). **$p < 0.05$, *$p < 0.005$ vs islets treated with IL-1β alone

Nitrite levels. Nitrite levels in the culture medium were 4.2 ± 1.4 and 24.0 ± 5 pmol · islet^{-1} · 24 h^{-1} in control islets and in IL-1β-exposed islets, respectively ($n = 6$, $p < 0.05$). In islets exposed to IL-1β and CT or

Table 2. Effect of IL-1β with or without CT and/or PT on glucose oxidation (pmol · islet^{-1} · 120 min^{-1})

	Culture conditions					
	Control	IL-1β	CT	IL-1β + CT	PT	IL-1β + PT
Secretagogues						
Glucose 1.5 mmol/l	7.3 ± 0.8	9.0 ± 1.2	10.9 ± 1.4	11.3 ± 2.1	8.8 ± 1.0	8.1 ± 1.1
Glucose 16.7 mmol/l	31.1 ± 2.9	16.8 ± 2.7[a]	33.4 ± 4.0	32.9 ± 3.8	32.5 ± 3.6	31.7 ± 3.3

[a] $p < 0.005$ in respect to control islets

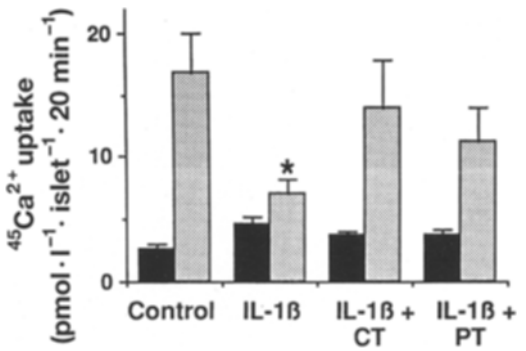

Fig. 2. Basal (■) and glucose-stimulated (▦) Ca^{2+} uptake in rat pancreatic islets cultured for 24 h in the absence (control) or in the presence of 50 U/ml IL-1β, with or without CT (2 μg/ml) or PT (0.5 μg/ml). At the end of the 24-h pre-incubation period, islets were washed three times and incubated for 20 min at 37 °C in buffer containing either 2.8 or 16.7 mmol/l glucose. Ca^{2+} uptake is expressed as pmol · l^{-1} · islet^{-1} · 20 min^{-1}. Data represent mean ± SEM of six separate experiments. *$p < 0.005$ vs control islets

PT, nitrite levels were 9.1 ± 3 and 12.4 ± 6 pmol · islet^{-1} · 24 h^{-1}, respectively ($n = 6$, NS vs control islets). In islets exposed to CT or PT alone, in the absence of IL-1β, nitrite levels were not different from control islets (4.6 ± 2.0 and 4.8 ± 1.9, respectively).

Glucose oxidation. In control islets cultured in the absence of IL-1β, glucose oxidation increased from 7.3 ± 0.8 pmol · islet^{-1} · 120 min^{-1} at 1.5 mmol/l glucose to 31.1 ± 2.9 pmol · islet^{-1} · 120 min^{-1} at 16.7 mmol/l of glucose. In islets cultured for 24 h with 50 U/ml IL-1β, glucose oxidation at 1.5 mmol/l glucose was similar to the control value, but the response to 16.7 mmol/l glucose was significantly impaired (Table 2).

When islets were cultured in the presence of both IL-1β and either CT or PT, the basal oxidation rate (at glucose 1.5 mmol/l) was slightly higher than that observed in control islets, although the difference was not significant (Table 2). Moreover, in these islets glucose oxidation in the presence of 16.7 mmol/l glucose was similar to control islets (Table 2). Neither toxin had a significant effect on glucose oxidation in the absence of IL-1ß (Table 2).

Ca^{2+} uptake. In control islets, $^{45}Ca^{2+}$ uptake was 2.6 ± 0.4 pmol · islet^{-1} · 20 min^{-1} (mean ± SEM, $n = 6$) under basal conditions (i.e. in the presence of 2.8 mmol/l glucose) and increased to 16.8 ± 3.2 pmol · islet^{-1} · 20 min^{-1} in the presence of 16.7 mmol/l glucose. In pancreatic islets pre-exposed to 50 U/ml IL-1β, calcium uptake under basal conditions was higher than in control islets (4.4 ± 0.5 pmol · islet^{-1} · 20 min^{-1}, $p < 0.05$), but only slightly increased after 16.7 mmol/l glucose stimulation (7.1 ± 0.9 pmol · islet^{-1} · 20 min^{-1}, $p < 0.005$ vs control islets) (Fig. 2). When 2 μg CT or 0.5 μg PT were added simultaneously to IL-1β, both basal and glucose-induced calcium uptake were similar to control islets (3.7 ± 0.3 and 3.7 ± 0.4 pmol · islet^{-1} · 20 min^{-1}, basal values, and 14.0 ± 3.8 and 11.2 ± 2.7 pmol · islet^{-1} · 20 min^{-1}, glucose-stimulated values in the presence of CT or PT, respectively, Fig. 2). Neither CT nor PT alone modified basal or glucose-stimulated $^{45}Ca^{2+}$ uptake (data not shown).

Discussion

The present study confirms that IL-1β is able to increase NO production, as measured by the nitrite levels, and to inhibit glucose oxidation, glucose-stimulated Ca^{2+} uptake and the glucose-induced insulin secretion in isolated rat pancreatic islets. Our data also demonstrate that the presence of CT or PT together with IL-1β prevents all the inhibitory effects of the cytokine, therefore suggesting that they are mediated by CT and PT substrates. Since both toxins prevent, at least partially, the IL-1β-induced increase in nitrite levels, they probably interfere with IL-1β signal transduction, and are not mainly acting by stimulating insulin secretion.

The free radical NO has been proposed to play a major role in mediating the IL-1β-induced inhibition of insulin secretion in rat islets [11–14]. NO is formed by a cytokine-inducible NO synthase, and inhibits key enzymes involved in glucose metabolism and energy production [13]. As a consequence, signals arising from it and coupling glucose metabolism and insulin secretion are impaired. In the cascade of intracellular events leading to insulin secretion, glucose metabolism is an early step that (by increasing the ATP content) leads to membrane depolarisation

and subsequent Ca^{2+} influx, the suggested trigger for insulin release. The impaired Ca^{2+} uptake and insulin release observed after islet exposure to IL-1β may, therefore, be secondary to the cytokine inhibitory effect of NO on glucose metabolism. In our experiments, CT and PT, by partially preventing the increase in NO levels, may hinder the inhibition of glucose oxidation and restore the signalling pathway that leads to glucose-induced Ca^{2+} uptake and, eventually, insulin release. This possibility is supported by the observation that in IL-1β-exposed islets both Ca^{2+} uptake and insulin release are normal in response to glyburide [34]. Therefore, since in IL-1β-exposed islets Ca^{2+} uptake and insulin release in response to a stimulation different from glucose are normal, it is likely that the IL-1β inhibitory effect at the level of glucose metabolism is the primary dysfunction caused by the cytokine in pancreatic beta cells.

Our study is unable to precisely identify the effector system involved in the transmission signal of the IL-1β/IL-1β receptor complex. The G-proteins seem to play an important role as modulators of insulin secretion [27, 35, 36]. They specifically mediate the inhibitory effects of several hormones such as somatostatin, galanine and epinephrine. These effects are prevented by beta-cell treatment with PT [37–40]. CT and PT have been reported to specifically and irreversibly activate different G-proteins (Gi_{1-3}, $Go_{1,2}$) [35, 41]. The observations that both CT and PT are able to prevent the IL-1β-induced effects and that CT is more effective than PT suggest that multiple G-proteins are involved in the signal transmission of the IL-1β receptor, and that the activation of CT and PT substrate pathway may overcome the block. However, it has been previously reported that the G-protein bound to the IL-1β receptor may be a common substrate for both CT and PT [25], and that CT can ribosylate PT-sensitive substrates [41]. As an alternate possibility G-proteins may not be directly involved in IL-1β signal transmission, but the CT and PT effects may be mediated by an increase in cAMP levels. We tried to examine this possibility by incubating islets with forskolin (an agent known to increase cAMP levels by directly activating the enzyme adenyl cyclase), in the presence or absence of IL-1β. These results, however, are difficult to interpret because in forskolin-exposed islets glucose-induced insulin release was decreased. Similar results have been previously obtained [42], and may be due to islet desensitization [43].

Previous studies were unable to demonstrate an effect of PT on IL-1β inhibition of insulin secretion in fetal [44], adult rat islets [45] or RIN cells [46]. However, the experimental design was quite different. In particular, in the study by Sjöholm [44] the fetal islets were treated for 24 h with PT prior to the addition of IL-1β; in the study by Eizirik et al. [45], islets were exposed for 1 h to IL-1β and studied 12 h later. Our study shows that the contemporary presence of CT and PT together with IL-1β during the 24-h culture period is necessary to prevent the inhibitory effects of the cytokine. The different results in RIN cells [46] may be due to the large difference in glucose metabolism between normal and tumoural cells. This difference may be of critical significance, mitochondrial glucose metabolism being among the proposed targets of IL-1β action in pancreatic beta cells.

In conclusion, our data confirm that IL-1β has an inhibitory effect on glucose-induced insulin release in pancreatic beta cells and show that this effect may be prevented by the presence of CT or PT, suggesting the involvement of a G-protein-dependent pathway. The effector system involved, however, remains unknown.

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