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# Interleukin-1β inhibits glucokinase activity in clonal HIT-T15 β-cells

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Interleukin-1β (IL-1β) has been implicated in the pathogenesis of insulin-dependent diabetes mellitus. In the present study we have investigated the effects of IL-1β on glucose metabolism in clonal HIT-T15 β cells. In the short-term (1 h), 25 U/ml IL-1β significantly increased the rates of insulin release and glucose utilisation, but not glucose oxidation. In contrast, after 48 h, IL-1β inhibited insulin release and glucose utilisation and oxidation. By assaying enzymes (hexokinase, glucokinase, pyruvate dehydrogenase, glucose 6-phosphatase) and nucleotides (ATP, ADP) associated with the regulation of glycolysis and glucose oxidation, we conclude that the inhibitory effects of IL-1β may be due to impaired glucokinase activity.

HIT-T15; Insulin-dependent diabetes mellitus; Interleukin-1β; Glucokinase; Pyruvate dehydrogenase

## 1. INTRODUCTION

The cytokine IL-1β has been proposed by Nerup et al. to be the main effector of β-cell damage during insulitis [1,2], the inflammatory process which precedes the onset of insulin-dependent diabetes mellitus.

Previous studies have shown that short-term (1 h) exposure of isolated pancreatic islets to IL-1β stimulates, whereas [3,4] longer term (48 h) incubations inhibit insulin release and biosynthesis [5,6]. Further exposure to the cytokine eventually causes β-cell destruction and death [5,6]. In vivo, the effects of IL-1β are similar to in vitro findings: lower doses of IL-1β reduce, whereas higher doses promote, the onset of diabetes in the BB rat [7]. In addition, whilst acute administration of IL-1β is hypoglycaemic in mice [8,9], chronic administration inhibits glucose-stimulated insulin release from the perfused pancreas of treated rats [10].

Several groups have investigated the effects of IL-1β on β-cell glucose metabolism. Acute exposure to IL-1β increases [11] and chronic exposure decreases [6,12] the rate of glucose oxidation in pancreatic islets. This suggests that IL-1β may act through the inhibition of β-cell mitochondrial function.

The pancreatic islet comprises a mixed population of cell types, and the possibility that IL-1β may have different effects on the metabolism of each cell type cannot be excluded. We have therefore investigated the effects of IL-1β on glucose metabolism in clonal HIT-T15 cells. The inhibitory effects of long-term exposure to IL-1β on glucose oxidation are confirmed. In addition, we show for the first time, that long-term exposure to IL-1β decreased both glucokinase activity and glycolytic flux in HIT-T15 cells. This suggests that the primary effect of IL-1β on glucose metabolism in HIT cells may be at the level of glucose phosphorylation.

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## 2. EXPERIMENTAL

### 2.1. *Materials*

RPMI 1640, antibiotics and foetal calf serum were from Gibco Laboratories, Grand Island, NY. Human recombinant IL-1β ($2.5–5 \times 10^7$ LAF units/mg) was either from Collaborative Research Inc., Bedford, MA. or from the Glaxo Institute of Molecular Biology, Geneva, Switzerland. Radiochemicals were from Amersham International, Arlington Heights, IL. All other chemicals and biochemicals were of analytical grade and were purchased from Sigma Chem. Co., St. Louis, MO, Fisher Scientific, Pittsburgh, PA or Boehringer Mannheim Biochemicals, Indianapolis, IN.

### 2.2. *Cell culture and insulin secretion*

HIT-T15 cells were cultured in RPMI 1640 medium as we have previously described [14]. On the day of the experiment, culture medium was aspirated and replaced with medium supplemented with additions as shown. Insulin release was measured in media aliquots by Enzyme-Linked Immunosorbent Assay (ELISA) as we have previously described [13].

### 2.3. *Glucose utilisation and oxidation*

Rates of glucose utilisation and oxidation were determined in HIT-T15 cells ($\sim 3 \times 10^6$ cells/assay) by the rates of $[^3H]$ $H_2O$ formation over 1 h from 10 mM D-[5-$^3$H]glucose (423–456 dpm/nmol) [15] or 10 mM D-[6-$^3$H]glucose (404–446 dpm/nmol) [16] respectively.

### 2.4. *Enzyme activities*

Hexokinase activity was assayed in HIT-T15 cell homogenates ($\sim 1 \times 10^6$ cells/assay) by the rate of D-[U-$^{14}$C]glucose-6-phosphate formation from 1 mM D-[U-$^{14}$C]glucose (1891–1920 dpm/nmol) [17]. Soluble glucokinase activity was assayed in the same experiment in 100 000 *g* supernatants prepared from HIT-T15 cell homogenates ($\sim 7.5 \times 10^6$ cells/assay), by the rate of D-[U-$^{14}$C]glucose-6-phosphate formation from 100 mM D-[U-$^{14}$C]glucose (159–175 dpm/nmol) [26]. D-[1-$^{14}$C]glucose-6-phosphate formed was

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separated from D-[1-$^{14}$C]glucose by ion-exchange chromatography on Dowex-2 formate resin [18].

Glucose-6-phosphatase (G6Pase) activity was assayed in HIT-T15 cell homogenates ($\sim 2.5 \times 10^6$ cells/assay) as described in [19], except that glucose-6-phosphate was measured radiochemically by the rate of D-[1-$^{14}$C]glucose formation from 5 mM D-[1-$^{14}$C]glucose-6-phosphate (439–470 dpm/nmol) [20].

Pyruvate dehydrogenase (PDH) complex activity (active and total forms) was assayed by $^{14}CO_2$ formation from 8 mM [1-$^{14}$C] pyruvate (7000–8000 dpm/nmol) in HIT-T15 cell homogenates ($\sim 2.5 \times 10^5$ cells/assay) prepared and assayed as described in [21]. Total PDH complex activity (dephosphorylated form) was determined by incubating HIT-T15 cell homogenates in the presence of a broad specificity protein phosphatase [21].

One unit of enzyme activity is defined as that activity catalysing the formation of 1 μmol of product per minute at 30°C.

### 2.5. *Adenine nucleotides*

Adenine nucleotides were determined in neutralised perchloric acid extracts [22] of HIT-T15 cells by modified reverse-phase HPLC analysis, using tetrabutylammonium hydrogen sulphate (THBS) as a pairing agent on a Waters Nova-Pak® C18 column [23]. Nucleotides were detected spectrophotometrically at 254 nm. Peak identification was confirmed against ATP and ADP standards.

## 3. RESULTS AND DISCUSSION

We have recently demonstrated [13] that both the short-term stimulatory and the longer term inhibitory effects of IL-1β on HIT-T15 insulin release were maximal at a concentration of 25 U/ml ($\sim$1 pM). This concentration of IL-1β was used in all experiments described in the present study.

Fig. 1 shows the effects of 1 and 48 h exposure to IL-1β on HIT-T15 insulin release, glucose utilisation and glucose oxidation. Overall, in short-term incubations (1 h), IL-1β induced a 57.1 ± 6.4% potentiation of insulin release. In contrast, longer-term (48 h) exposure to IL-1β resulted in a 60% inhibition of insulin release. These findings are in general agreement with our previous study with HIT-T15 cells [13] and also with the effects of IL-1β on isolated pancreatic islets [1,2].

In general, the effects of IL-1β on HIT-T15 insulin secretion were accompanied by similar changes in the rates of glucose utilisation, but not glucose oxidation. Thus, short-term exposure to IL-1β significantly ($P < 0.01$) increased glucose utilisation, but was without effect on glucose oxidation. In contrast, the rates of both glucose utilisation and oxidation were inhibited following long-term (48 h) exposure to IL-1β. It is pertinent to note that the ratio of glucose utilisation:oxidation, which was 6.20 ± 0.54 (3) in control incubations (n = 3), was not affected by acute or chronic exposure to IL-1β (not shown). This might suggest that any effect of IL-1β on glucose oxidation is likely to be indirect, via changes in the rate of glucose utilisation, rather than through direct inhibition of enzymes of the tricarboxylic acid cycle.

The demonstration that 48 h exposure of HIT-T15 cells to IL-1β resulted in inhibition of glucose oxidation

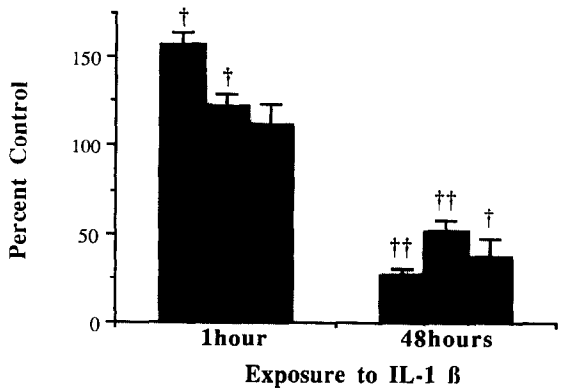


Fig. 1. Effects of IL-1β on insulin secretion, glucose utilisation and oxidation in HIT-T15 cells. HIT-T15 cells were cultured in the presence or absence of IL-1β (25 U/ml) for the times shown. Rates of glucose utilisation and oxidation were measured by incubating the cells in the presence of D-[5-$^3$H]glucose and D-[6-$^3$H]glucose respectively. Insulin release was determined by ELISA. Results are expressed as percent of control and are for 3 and 12 experiments for glucose utilisation/oxidation and insulin release respectively. Absolute values for control insulin secretion at 1 and 48 hours were 561 ± 80 and 13026 ± 1836 μU/ml/$10^6$ cells respectively. Absolute values for control glucose utilisation and oxidation were 31.6 ± 0.9 and 5.3 ± 0.5 nmol/hr/$10^6$ cells respectively. Statistics: **$p < 0.01$; ***$p < 0.001$ for differences from control (Student's t-test).

is in agreement with studies using isolated pancreatic islets [6,11,24]. However, no significant effects of IL-1β on glucose utilisation have been reported in islets [11,24]. We have confirmed and extended our observations on HIT-T15 glucose utilisation by demonstrating precise effects of IL-1β on the activities of individual hexose phosphorylating enzymes (Table I). In the short-term, exposure of HIT-T15 cells to IL-1β (1 h) decreased hexokinase activity by 42% ($P < 0.05$) but was without effect on glucokinase. In contrast, longer term (48 h) exposure to IL-1β decreased glucokinase activity by 32% ($P < 0.05$), but caused no further inhibition of hexokinase activity above that seen at 1 h exposure. These findings contrast with those of Eizirik et al. [25], who reported that 48 h exposure of pancreatic islets to IL-1β (48 h) was without effect on glucokinase and hexokinase activities. A possible explanation for this discrepancy is that in liver, glucokinase exists in two immunologically distinct, soluble and particulate isoforms which may be separated by high speed centrifugation [17]. In the present study, glucokinase activity was assayed in a 100 000 × *g* supernatant. Here, the soluble form of glucokinase alone will be measured [17]. However, Eizirik et al. [25] measured glucokinase activity in islet homogenates; conditions which would presumably detect both forms. Therefore, it is possible that the assay conditions used in [25] would not be as sensitive to changes in glucokinase activity as those described here.

A recent study [26] has shown that islet G6Pase activity is higher than previously reported [27,28]. This suggests that, in addition to hexose phosphorylation,

Table I

Effects of IL-1$\beta$ on the activity of glycolytic enzymes in HIT-T15 cells

| Enzyme activities ($\mu$U/$10^6$ cells) | Control | IL-1$\beta$ (1 h) | IL-1$\beta$ (48 h) |
|---|---|---|---|
| Glucokinase | 113 ± 18 | 92 ± 10 | 69 ± 7* |
| Hexokinase | 744 ± 101 | 464 ± 22* | 341 ± 46* |
| Glucose-6-phosphatase | 112 ± 52 | 156 ± 11 | 148 ± 2 |

Glycolytic enzyme activities were assayed in HIT-T15 cells (~$30 \times 10^6$ cells) as described in the Experimental section and were calculated by least squares regression analysis. Results are means ± SE, for 3 experiments. Statistics: *$P < 0.05$ for difference from control (Student's $t$-test).

Table II

Effect of IL-1$\beta$ on the activity of PDH-complex and adenine nucleotides in HIT-T15 cells

| | Control | IL-1$\beta$ (1 h) | IL-1$\beta$ (48 h) |
|---|---|---|---|
| PDH active form (% total active) | 41.1 ± 5.0 | 38.6 ± 4.6 | 69.3 ± 7.3* |
| PDH total complex ($\mu$U/$10^6$ cells) | 292 ± 44 | 179 ± 45 | 335 ± 12 |
| ATP/ADP | 11.5 ± 2.9 | 14.0 ± 1.9 | 12.6 ± 3.4 |

PDH complex activity was assayed in HIT-T15 cell homogenates (~$60 \times 10^6$ cells) and is expressed as the percentage of total complex in the active form. Adenine nucleotides were determined as described in section 2. Retention times for ATP and ADP were 7.87 ± 0.21 (16) and 18.27 ± 0.22 (16) min respectively. Intracellular concentrations of ATP and ADP in controls were 3.18 ± 0.70 (4) and 0.26 ± 0.04 (4) mM respectively (calculated assuming a HIT-T15 intracellular water space of 0.81 pl/cell [31]). Results are means ± SE, for 3 experiments. Statistics: *$P < 0.05$ for differences from control (Student's $t$-test).

significant re-conversion of glucose-6-phosphate back to glucose, i.e. substrate cycling, occurs in the $\beta$-cell and may offer an additional site for controlling glycolysis. In HIT-T15 cells, G6Pase activity was not significantly affected by exposure to IL-1$\beta$ (Table I). This suggests that inhibitory effects on glucokinase activity, and concomitant inhibition of flux through the glycolytic pathway, may be larger than predicted by maximal enzyme activities alone.

The long-term inhibitory effect of IL-1$\beta$ on HIT-T15 glucose oxidation was further studied at the level of pyruvate dehydrogenase (PDH), since a decrease in PDH complex activity is one conceivable site of cytokine-mediated inhibition. The PDH complex is a mitochondrial multienzyme complex catalysing the decarboxylation of pyruvate to acetylCoA. The complex is regulated by reversible phosphorylation (phosphorylation is inactivating), catalysed by an intrinsic kinase and by a phosphatase, and by end product inhibition [29].

The effects of IL-1$\beta$ on both total complex and the activity state of PDH complex in HIT-T15 cells are shown in Table II. Total PDH complex activity (phosphorylated and dephosphorylated forms) was unaffected by exposure to IL-1$\beta$. In contrast, whilst short-term exposure of HIT-T15 cells to IL-1$\beta$ was without effect, 48 h exposure resulted in a 70% increase in the activity state of PDH complex. In view of the decrease in glucose oxidation reported in this, and other studies [6,11,24], the increase in the activity state of PDH complex in response to long-term exposure to IL-1$\beta$ appears paradoxical. However, Eizirik et al. [25] have shown that the rate of [1-$^{14}$C] pyruvate decarboxylation in islets exposed to IL-1$\beta$ (48 h) increased 2-fold from control whilst, at the same time, the rate of D-[6-$^{14}$C]glucose oxidation was decreased by approximately 50% from control. These results, together with the present findings might suggest that the primary effect of IL-1$\beta$ on glucose metabolism may be proximal to the PDH reaction; thus whilst IL-1$\beta$ may increase the activity state of PDH complex, this may not result in increased glucose oxidation.

One likely consequence of a decreased rate ot glucose oxidation in response to IL-1$\beta$ (48 h) would be a decrease in the intracellular ATP/ADP ratio. This would result in inhibition of PDH kinase activity and would therefore increase the activity state of PDH complex [29]. However, there was no significant effect of IL-1$\beta$ on either the ATP/ADP ratio (Table II) or the total adenine nucleotide content (results not shown) of HIT-T15 cells following either 1 h or 48 h exposure to IL-1$\beta$. In addition, there was no effect of IL-1$\beta$ on the NADH/NAD ratio; as indexed by the lactate:pyruvate ratio (results not shown). The precise mechanism by which IL-1$\beta$ increases the activity state of PDH complex remains unclear, although modulation of PDH phosphatase and/or PDH kinase activities is presumably involved.

In HIT-T15 cells, glucose metabolism is normally limited by the rate of entry of glucose into the cell [30,31]. We propose that in HIT-T15 cells exposed to IL-1$\beta$, hexose phosphorylation becomes rate-limiting for glycolysis. This could explain the decrease in glucose utilisation in HIT-T15 cells exposed to IL-1$\beta$ (48 h). Further, the contribution of hexokinase to hexose phosphorylation in the intact $\beta$-cell under physiological concentrations of glucose is low and thus glucokinase activity is likely to be the major determinant of the rate of hexose phosphorylation [32]. This suggests that the primary effect of IL-1$\beta$ on glucose utilisation may result from a decrease in glucokinase activity.

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