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Inhibition of fetal rat pancreatic β-cell replication by interleukin-1β in vitro is not mediated through pertussis toxin-sensitive G-proteins, a decrease in cyclic AMP, or protease activation

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It has been proposed that the cytokine interleukin-1β (IL-1β), secreted by islet-infiltrating macrophages, may be involved in the pathogenesis of insulin-dependent diabetes mellitus by participation in β-cell destruction. Addition of IL-1β to isolated pancreatic islets in vitro results in cytotoxic effects on β-cell function, but there is little information on the intracellular events that convey the actions of the cytokine. In the present study, fetal rat pancreatic islets containing a high fraction of β-cells were exposed in culture to IL-1β. It was found that IL-1β markedly decreased β-cell DNA synthesis, insulin secretion and cyclic AMP content. In order to explore whether the decrease in cAMP resulted from IL-1β interaction with GTP-binding proteins coupled to adenylyl cyclase, islets were treated for 24 h with pertussis toxin prior to addition of cytokine. While this treatment restored the decrease in cAMP, the reduced DNA synthesis and insulin secretion persisted. Pertussis toxin treatment without the addition of IL-1β resulted in increases in cAMP, DNA synthesis and insulin secretion. Addition of the stimulatory cAMP analog Sp-cAMPS also increased DNA synthesis and insulin secretion, but failed to affect the decrease in these functions evoked by IL-1β. The protease inhibitor *Nα-p*-tosyl-L-lysine chloromethyl ketone, recently shown to protect completely against IL-1β-induced suppression of insulin production and secretion, was found to markedly reduce DNA synthesis without affecting insulin secretion. When the protease inhibitor was combined with IL-1β, the suppressed secretion was counteracted while DNA synthesis inhibition was not. It is concluded that cAMP stimulates DNA synthesis and insulin secretion in β-cells, but that the inhibitory effect of IL-1β on these functions cannot be ascribed to the decrease in cAMP evoked by the cytokine. However, the repressive effect of the cytokine on insulin secretion, but not DNA synthesis, may be prevented by protease inhibition.

Cytokine; Pancreatic islet; DNA synthesis; Insulin secretion; Cyclic AMP; Protease

1. INTRODUCTION

Recent findings have shown that certain cytokines are able to exert both inhibitory and cytotoxic actions on pancreatic islet cells in vitro [1–8]. Cytokines are also known to be secreted by leukocytes infiltrating the islets early in the development of insulin-dependent diabetes mellitus [8]. These combined findings gave rise to the important notion that cytokines may be involved in the pathogenesis of this disease [5–8]. The most extensively studied cytokine in this context, IL-1β, inhibits insulin secretion at least in part by selectively interfering with mitochondrial substrate oxidation, leading to a reduced ATP-production [9]. One way by which the striking loss of β-cells, occurring during the development of insulin-dependent diabetes mellitus, can be compensated for, is via de novo formation of β-cells by replication. In the present study we have investigated the impact of IL-1β on DNA synthesis by cultured fetal pancreatic islets containing a high fraction of β-cells. In addition, the long-term influence of the cytokine also on regulation of islet insulin secretion was evaluated. Attempts were also made to elucidate whether IL-1β affects the β-cell by interaction with different parts of the cAMP signalling system or through protease activation.

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2. MATERIALS AND METHODS

2.1. *Materials*

Pertussis toxin and TLCK were from Sigma, St. Louis, MO. The Sp-diastereomer of cyclic adenosine-3′,5′-monophosphothioate (Sp-cAMPS) was delivered by Biolog Life Science Institute, Bremen, Germany. [*methyl*-^{3}H]Thymidine (5 Ci/mmol) and the cAMP assay kit (Cat. no. RPA.509) were purchased from Amersham International, UK. Recombinant human IL-1β was kindly provided by Dr Klaus Bendtzen, Laboratory of Medical Immunology, Rigshospitalet, University Hospital, Copenhagen, Denmark. The biological activity of the IL-1β was 5 U/pg, as determined by comparison with an interim international standard IL-1β preparation (NIBSC, London, UK) in the mouse thymocyte co-stimulatory assay and the EL4 murine T-cell line [10]. The endotoxin content after adding the interleukin was <1 pg/ml, as measured in the *Limulus* amoebocyte assay. We have previously found that neither 1 pg/ml nor 10 pg/ml of endotoxin affected the growth or viability of insulin-producing cells [11].

2.2. *Methods*

Pregnant Sprague–Dawley rats belonging to a local stock were killed by cervical dislocation on day 21 of gestation and the fetuses rapidly removed. Fetal rat islets were prepared from pancreatic glands as previously described [12–14]. At the end of a 5-day culture period

in medium RPMI 1640, groups of 50 islets were transferred to fresh media containing 1% fetal calf serum, with or without IL-1β or other test substances and cultured free-floating for 24 h. When pertussis toxin was included, islets were exposed to the toxin 24 h prior to IL-1β addition in order to ensure that the effects of the toxin were expressed.

The methods for analyzing islet cell DNA synthesis [13,14], DNA content [15,16] and insulin secretion [17] have been published previously.

For cAMP measurements, cultured islets in groups of 50 were quickly washed in PBS and then transferred to tubes containing 150 μl 6% trichloroacetic acid. These were immediately sealed, plunged into liquid nitrogen and stored at −80°C, pending analysis. The samples were thawed by sonication on ice and centrifuged at 2000 × g (4°C) for 15 min. The pellet was re-sonicated in 200 μl of redistilled water and used for measurements of DNA and [^{3}H]thymidine incorporation as described above. The supernatant was washed 4 times with 5 vols of water-saturated diethyl ether. The aqueous extract was freeze-dried and the content of cAMP measured by RIA (using [^{125}I]cAMP) exactly as described by the manufacturer of the assay kit. In order to increase the sensitivity of the method, samples were acetylated.

3. RESULTS

Exposure of fetal rat islets to 25 U/ml of IL-1β for 24 h resulted in a marked suppression of DNA synthesis, as assessed by measurements of [^{3}H]thymidine incorporation into DNA (Table I). IL-1β also significantly inhibited secretion of insulin from these islets over the 24-h exposure period and lowered their content of cAMP by some 50% (Table I). Addition of pertussis toxin (50 ng/ml) in the absence of cytokine resulted in an increased cAMP content and a marked stimulation of DNA synthesis and insulin secretion (Table I). A 24-h pertussis toxin pretreatment period was, however, not able to reverse the inhibition of DNA synthesis and insulin secretion evoked by IL-1β, despite a normalization of the cAMP content of these islets. Addition of the membrane-permeant stimulatory cAMP analog Sp-cAMPS (50 μM) also substantially enhanced DNA synthesis and insulin secretion rates (Table I), again effects that completely vanished in the presence of IL-1β (Table I). There were, however, no corresponding changes in islet DNA content in any of the groups (not shown), averaging 15 ± 2 ng/islet, a finding which is not surprising when considering that less than 10% of the fetal islet cells traverse through the cell cycle [18].

Treatment of islets for 24 h with the serine protease inhibitor TLCK (100 μM) inhibited islet cell DNA synthesis by approximately 75%, while leaving insulin secretion unaffected (Table II). TLCK also did not influence the islet contents of insulin or DNA (not shown). When the protease inhibitor was combined with IL-1β, the inhibited [^{3}H]thymidine incorporation rate persisted, while there was a complete protection against the reduction of insulin secretion evoked by the cytokine (Table II).

4. DISCUSSION

The present study shows that the cytokine IL-1β exerts inhibitory actions not only on insulin secretion, but also on the proliferation, of fetal rat pancreatic islet cells in vitro. This cytokine is known to be produced by macrophages, which invade the pancreatic islets early in the course of insulin-dependent diabetes mellitus [5,7,8]. In addition, elevated serum levels of IL-1 in diabetic patients were recently noted [19]. It is therefore quite possible that this cytokine plays a key role in the development of insulin-dependent diabetes. There is little information on the influence of cytokines on β-cell replication, in spite of the fact that de novo formation of β-cells by replication may be an important way to compensate for the loss of β-cells occurring in insulin-dependent diabetes. Nevertheless, it has been shown that IL-1β decreases DNA synthesis in adult islet cells of rats [20] and mice [21]. However, since adult islets contain only 70–80% β-cells, it cannot be excluded that these results reflect effects on non-β-cells.

In other cell types, IL-1β has been shown to activate intracellular proteases [22]. Recent evidence also in β-cells suggests that a primary event in IL-1β action might

Table I

Long-term effects of IL-1β, pertussis toxin and Sp-cAMPS on islet DNA synthesis, insulin secretion and cAMP content

Islet culture			DNA synthesis (cpm/μg DNA)	Insulin secretion (ng/μg DNA per ml × 24 h)	cAMP content (fmol/μg DNA)
IL-1β	PTX	Sp-cAMPS			
−	−	−	1510 ± 176	211 ± 29	107 ± 12
−	+	−	3055 ± 293*	446 ± 59*	161 ± 21*
−	−	+	2891 ± 320*	399 ± 44*	N.D.
+	−	−	485 ± 37*	92 ± 20*	48 ± 10*
+	+	−	511 ± 57*,†	113 ± 16*,†	97 ± 8#†
+	−	+	458 ± 72*,§	106 ± 13*,§	N.D.

Groups of 50 fetal rat islets were cultured free-floating for 24 h in medium RPMI 1640 containing 1% fetal calf serum and supplemented with (+) or without (−) IL-1β (25 U/ml), pertussis toxin (PTX) or Sp-cAMPS (50 μM). When PTX was included, islets were treated with the toxin (50 ng/ml) 24 h prior to and during the entire IL-1β exposure period. Values are means ± SEM for 4–8 experiments. *(vs. control islets), #(vs. IL-1β-treated islets), §(vs Sp-CAMPS-treated islets) and †(vs. PTX-treated islets) denote 95% multicomparison significance level using one-way factorial ANOVA.

be protease activation, since a complete protection against IL-1β inhibition of insulin production and secretion in adult rat islets can be afforded by certain protease inhibitors [23]. The presently observed protective effect of the serine protease inhibitor $N\alpha$-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) on the impairment of long-term insulin secretion evoked by IL-1β fits nicely with these data. The tosyl-lysine group of TLCK forms a complex with enzymes recognizing basic and hydrophobic amino acid residues, whereas the chloromethyl ketone group accounts for irreversible inactivation of the enzyme by alkylating a catalytically active histidine [24]. Importantly, IL-1β itself is not affected by TLCK [23]. The unexpected finding of a potent growth inhibitory action of this protease inhibitor, makes it clear that the drug certainly cannot be used to counteract the repressive influence of IL-1β on β-cell DNA synthesis. The mechanism by which TLCK inhibits DNA synthesis might be through its inhibition of tyrosine kinases [25], enzymes that are believed to be instrumental in the transduction of mitogenic signals by many growth factors.

The presently reported reduction in the content of cyclic AMP in IL-1β-treated islets might be suspected to contribute to the inhibitory actions of the cytokine on insulin secretion and DNA synthesis, since both processes reportedly are stimulated by cyclic AMP [26,27]. One way by which a decrease in cyclic AMP can be brought about is through interference with GTP-binding proteins that connect a cell surface receptor to adenylyl cyclase [28]. Certain of these inhibitory GTP-binding proteins are sensitive to the toxin of *Bordetella pertussis*, which alleviates the adenylyl cyclase from an inhibitory constraint [28]. However, our present findings of a lack of protection by pertussis toxin pretreatment despite a prevention of the decrease in cAMP suggest that IL-1β does not convey its inhibitory actions primarily by interfering with this pathway. Moreover, addition of the cAMP analog Sp-cAMPS was found to increase β-cell DNA synthesis and insulin secretion, but failed to affect the inhibitory action of IL-1β on these parameters. These findings indicate that the decrease in cAMP elicited by IL-1β treatment is not responsible for the inhibition of DNA synthesis or insulin secretion evoked by the cytokine. Sp-cAMPS is a stimulatory analog of the natural signal molecule cAMP in which one of the two exocyclic oxygen atoms in the cyclic phosphate moiety is replaced by sulfur by axial modification. It mimics all biological effects of natural cAMP, is extremely resistant to cyclic nucleotide phosphodiesterases and is an agonist of cAMP-dependent protein kinases I and II [29–32]. The use of the membrane-permeant Sp-cAMPS makes it possible to avoid undesired side-effects of other cAMP analogs; e.g. stimulation of adenosine receptors by 8-bromo cAMP or 'butyrate effects' by dibutyryl cAMP. We have found that butyrate itself inhibits islet DNA synthesis and completely blocks the mitogenicity of the adenylyl cyclase activator forskolin (Å.S., unpublished). Thus, the true role of cAMP in β-cell replication may be severely obscured by using dibutyryl cAMP, a problem that can be avoided by using Sp- cAMPS.

Table II

Long-term effects of IL-1β and TLCK on islet DNA synthesis and insulin secretion

Islet culture	DNA synthesis (cpm/μg DNA)	Insulin secretion (ng/μg DNA per ml × 24 h)
Control	1093 ± 139	123 ± 16
IL-1β (25 U/ml)	401 ± 51*	61 ± 8*
TLCK (100 μM)	271 ± 33*	139 ± 19
IL-1β + TLCK	243 ± 21*#	133 ± 16#

Groups of 50 fetal rat islets were cultured free-floating for 24 h in medium RPMI 1640 containing 1% fetal calf serum and supplemented as indicated in the Table. During the final 5 h of culture, 1 μCi/ml [^{3}H]thymidine was present in the culture medium and DNA synthesis rates were determined by measuring the radioactivity incorporated into trichloroacetic acid-precipitable material. The secretion of insulin into the culture medium during the 24 h of culture was determined radioimmunologically. Values are expressed as means ± SEM for 6 experiments. *(vs. controls) and #(vs. IL-1β-treated islets) denote 95% multicomparison significance level using one-way factorial ANOVA.

It is concluded that long-term IL-1β exposure inhibits both the proliferation and insulin secretion by fetal β-cells. Our results furthermore indicate that cAMP stimulates DNA synthesis and insulin secretion in β-cells, but that the inhibitory effect of IL-1β on these functions cannot be ascribed to the decrease in cAMP evoked by the cytokine. However, the repressive effect of IL-1β on insulin secretion, but not DNA synthesis, may be counteracted by protease inhibition. These direct effects of IL-1β on the insulin-secreting cells may prove valuable for the understanding of the putative role of cytokines in the development of insulin-dependent diabetes mellitus.

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