Inhibition of insulin secretion from rat islets of Langerhans by interleukin-6

An effect distinct from that of interleukin-1

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Glucose-induced insulin secretion from islets cultured in the presence of interleukin-6 (IL-6) for 12–24 h was inhibited to a similar extent as when islets were treated with interleukin-1 β (IL-1 β). However, unlike IL-1 β , IL-6 did not potentiate insulin secretion during an acute (30 min) exposure of islets to the cytokine, nor did it inhibit DNA synthesis during a 24 h culture period. A 12 h pretreatment of islets with tumour necrosis factor- α (TNF- α) combined with IL-1 β potentiated the inhibitory effect of IL-1 β on secretion, such that 20 mM-glucose-induced insulin secretion was abolished. No synergistic inhibition of secretion was observed with TNF- α and IL-6. However, IL-1 β and IL-6 were found to inhibit insulin secretion in an additive manner. These results suggest that IL-6 inhibits insulin secretion in a manner distinct from that of IL-1 β , and that IL-6 is unlikely to mediate the inhibitory effects of IL-1 β or TNF- α on rat islets of Langerhans.

INTRODUCTION

It has been suggested that cytokines released from activated macrophages during the process of insulinitis may contribute to the destruction of the islet β -cell in insulin-dependent diabetes mellitus (IDDM) (Nerup *et al.*, 1987). The report of raised serum levels of interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) in recently diagnosed IDDM patients (Hussain *et al.*, 1990) further supports an involvement of these cytokines in the development of this autoimmune disease. Acute exposure (< 1 h) of islets to IL-1 β potentiates glucose-induced insulin secretion, whereas a more prolonged exposure results in the inhibition of insulin secretion and DNA synthesis, decreased glucose oxidation, and ultimately cell death (for review see McDaniel *et al.*, 1988). Another macrophage-derived product, TNF- α , has been shown to synergize with IL-1 β to potentiate the inhibition of insulin secretion (Mandrup-Poulsen *et al.*, 1987) and give rise to islet cell lysis (Pukel *et al.*, 1988). Specific plasma membrane receptors for IL-1 β have been demonstrated on numerous cell types (see Dower & Urdal, 1987), and more recently on an insulin-secreting β -cell line (Hammonds *et al.*, 1990). Thus IL-1 β may act directly upon the islet β -cell. Individually, IL-1 β and TNF- α induce the secretion of the cytokine interleukin-6 (IL-6) from a variety of cell types including macrophages, fibroblasts and endothelial cells (Wong & Clark, 1988), and, moreover, IL-1 β and TNF- α stimulate fibroblast IL-6 production in a synergistic manner (Elias & Lentz, 1990). Pancreatic islet β -cells may also produce IL-6 (Campbell *et al.*, 1989). Thus IL-6 has been considered as a potential mediator of the effects of IL-1 β . In this study we investigate the effects of IL-6 on insulin secretion and DNA synthesis in cultured rat islets of Langerhans to determine whether IL-6 is a likely mediator of the effects of IL-1 β . We also examine the interactions of IL-6, IL-1 β and TNF- α on cultured rat islets of Langerhans to assess the potential contribution of these cytokines to the development of impaired islet β -cell function in IDDM.

MATERIALS AND METHODS

Cytokines

Recombinant human cytokines were from NIBSC: IL-1 β (code 86/526B), IL-6 (unglycosylated) (code 87/736) and TNF- α (code 87/630).

Islet isolation and tissue culture

Islets of Langerhans were isolated from groups of four female Sprague–Dawley rats (180–200 g) by a collagenase digestion technique (Howell & Taylor, 1968). Islets were separated from pancreatic acinar tissue by centrifugation (800 g, 5 min) in 23% (w/v) Dextran T70 (Pharmacia) in a bicarbonate-buffered medium (Gey & Gey, 1936), and collected using a finely drawn out Pasteur pipette. Islets were precultured for a period of 48 h in RPMI-1640 medium supplemented with penicillin (60 000 units/litre), streptomycin (100 mg/l) and 5% foetal calf serum (Gibco) prior to all studies.

Insulin secretion studies

To determine the acute effects of cytokine treatment, islets were removed from tissue culture and preincubated in medium (Gey & Gey, 1936) containing 2 mM-glucose for 1 h at 37 °C. Groups of five islets were then incubated for 30 min in medium containing 8 mM-glucose in the presence or the absence of cytokines (10 pM–1 nM). Insulin secreted into the medium was measured by radioimmunoassay (Green *et al.*, 1983).

To determine the effect of prolonged cytokine treatment, islets were cultured for a further 6, 12 or 24 h in the presence or the absence of cytokines. Islets were rinsed and preincubated for 1 h in medium containing 2 mM-glucose. Groups of three islets were then incubated for 30 min in medium containing 2 mM- or 20 mM-glucose. Insulin secretion and total islet insulin contents (extracted in ethanol/water/conc. HCl; 50:15:1, by vol., for 24 h at 4 °C) were determined as above.

Islet DNA content and DNA synthesis

Groups of 30 islets, cultured for 24 h in the presence of 0.5 nM cytokines in medium supplemented with 2 μ Ci of [3 H]-thymidine/ml (Amersham) were washed extensively and sonicated in 500 μ l of distilled water. Portions of 100 μ l were mixed with 500 μ l of 5% trichloroacetic acid, and [3 H]thymidine incorporation was determined as previously described (Swenne, 1990). Total islet DNA was determined in 50 μ l samples of the islet sonicate (Green & Taylor, 1972).

Statistics

Results were analysed using an unpaired Student's *t* test.

RESULTS

Insulin secretion studies

IL-1 β (0.1–1 nM) potentiated 8 mM-glucose-induced insulin secretion from islets exposed to the cytokine during a 30 min incubation, but IL-6 over the same concentration range did not affect insulin secretion (Table 1). However, the treatment of islets for 24 h with IL-6 resulted in a dose-dependent inhibition of insulin secretion in response to a subsequent 20 mM-glucose challenge (Table 2). IL-6 gave a similar degree of inhibition of insulin secretion as that produced by IL-1 β at a concentration of 1 nM, and both IL-1 β and IL-6 significantly raised basal insulin

Table 1. Effects of short-term IL treatment on insulin secretion

Insulin secretion was measured in groups of five rat islets of Langerhans incubated for 30 min in medium containing 8 mM-glucose in the presence or the absence of IL-1 β or IL-6 at the concentrations shown. IL-1 β potentiated 8 mM-glucose-induced insulin secretion (**P* < 0.05; ***P* < 0.01). The results are means $\pm$ S.E.M. of two separate islet preparations, *n* = 10.

Treatment	Insulin secretion (fmol/30 min per islet)
Control	57.4 $\pm$ 6.8
IL-6 (10 pM)	57.2 $\pm$ 6.8
IL-6 (0.1 nM)	53.7 $\pm$ 3.4
IL-6 (1.0 nM)	60.2 $\pm$ 6.9
IL-1 β (10 pM)	77.9 $\pm$ 10.0
IL-1 β (0.1 nM)	85.3 $\pm$ 9.4*
IL-1 β (1.0 nM)	89.6 $\pm$ 6.2**

Table 2. Effects of long-term IL treatment on insulin secretion

Insulin secretion was measured from islets pretreated for 24 h in tissue culture in the presence or the absence of IL-6 or IL-1 β at the concentrations shown. After tissue culture, islets were preincubated for 1 h in medium containing 2 mM-glucose. Groups of these islets were then incubated for 30 min in medium containing 2 mM- or 20 mM-glucose, and insulin secretion was determined by radioimmunoassay. The results are means $\pm$ S.E.M. of two separate experiments (numbers of measurements in parentheses); **P* < 0.05, ***P* < 0.01 and ****P* < 0.008 versus controls.

Treatment (24 h)	Insulin secretion (fmol/30 min per islet)	
	2 mM-Glucose	20 mM-Glucose
Control	9.8 $\pm$ 2.3 (14)	94.9 $\pm$ 11.0 (16)
IL-6 (0.1 pM)	11.8 $\pm$ 2.2 (12)	76.5 $\pm$ 8.3 (13)
IL-6 (10 pM)	12.2 $\pm$ 2.1 (12)	65.3 $\pm$ 5.6 (13)*
IL-6 (1 nM)	23.1 $\pm$ 2.6 (15)***	44.2 $\pm$ 7.5 (14)**
IL-1 β (1 nM)	24.8 $\pm$ 3.3 (13)***	46.4 $\pm$ 4.8 (11)**

release in the presence of 2 mM-glucose (Table 2). An inhibitory effect of IL-1 β (0.5 nM), but not of IL-6 (0.5 nM), on 20 mM-glucose-induced insulin secretion was observed as early as after 6 h of exposure to the cytokine (35.1 $\pm$ 4.2 and 88.6 $\pm$ 11.3 versus 71.2 $\pm$ 12.4 fmol/30 min per islet respectively; *P* < 0.005, *n* = 19–24 from two separate experiments). When the exposure period was increased to 12 h, IL-6 (0.5 nM) was found to inhibit insulin secretion to the same degree as IL-1 β (0.5 nM), and TNF- α (0.5 nM) pretreatment was without effect (Table 3). Insulin secretion induced by 20 mM-glucose was abolished after a 12 h exposure to the combinations of IL-1 β plus IL-6 (0.5 nM), or IL-1 β plus TNF- α (0.5 nM), so that insulin secretion was not significantly different from basal release in the presence of 2 mM-glucose (Table 3). However, when TNF- α was combined with IL-6, the inhibitory effect on insulin secretion was no greater than that observed with IL-6 alone. Basal insulin release in the presence of 2 mM-glucose and total islet insulin contents were not affected by the 12 h cytokine treatments described in Table 3 (results not shown).

Islet DNA and insulin contents and DNA synthesis

Total islet DNA and insulin contents were not affected by a 24 h exposure to IL-1 β or IL-6 (0.5 nM) (Table 4). During 24 h of exposure to 0.5 nM cytokines, IL-6 did not affect the incorporation of [3 H]thymidine into DNA, whereas IL-1 β significantly lowered incorporation by 44%. In contrast with the additive effects on the inhibition of insulin secretion, the combination of

Table 3. Effects of cytokines on glucose-stimulated insulin secretion

Insulin secretion induced by 20 mM-glucose during a 30 min incubation in groups of three islets pretreated for 12 h with 0.5 nM cytokines (alone or in combination) was measured as shown (means $\pm$ S.E.M. of two separate experiments; numbers of measurements in parentheses). Basal insulin release from islets incubated in the presence of 2 mM-glucose was 12.9 $\pm$ 1.4 fmol/30 min per islet. **P* < 0.001 versus control; †*P* < 0.0001 for combined cytokine treatments versus the effect of either cytokine alone.

Treatment (12 h)	Insulin secretion (fmol/30 min per islet)
Control	77.6 $\pm$ 8.1 (18)
IL-1 β	44.8 $\pm$ 5.7 (19)*
IL-6	35.3 $\pm$ 4.4 (17)*
TNF- α	83.1 $\pm$ 8.1 (19)
IL-1 β + IL-6	16.2 $\pm$ 3.6 (18)*†
TNF- α + IL-1 β	14.4 $\pm$ 2.6 (18)*†
TNF- α + IL-6	43.6 $\pm$ 4.7 (20)*

Table 4. Effects of ILs on islet DNA and insulin contents and DNA synthesis

[3 H]Thymidine incorporation was measured in rat islets of Langerhans cultured for 24 h with IL-1 β and/or IL-6 (alone and in combination at 0.5 nM). The results are means $\pm$ S.E.M. of two separate experiments (*n* = 15); **P* < 0.0001 versus control.

Treatment (24 h)	DNA content (ng/islet)	[3 H]Thymidine incorporation (c.p.m./ng of DNA)	Insulin content (pmol/islet)
Control	22.9 $\pm$ 2.0	11.0 $\pm$ 0.6	2.5 $\pm$ 0.2
IL-1 β	21.5 $\pm$ 1.6	6.1 $\pm$ 0.3*	2.4 $\pm$ 0.2
IL-6	19.2 $\pm$ 1.4	10.9 $\pm$ 0.3	2.1 $\pm$ 0.2
IL-1 β + IL-6	21.3 $\pm$ 0.8	6.9 $\pm$ 0.3*	2.8 $\pm$ 0.3

IL-1 β plus IL-6 did not affect the inhibition of DNA synthesis observed with IL-1 β alone (Table 4).

DISCUSSION

Human recombinant IL-6 inhibited in a dose-dependent manner glucose-induced insulin secretion from cultured rat islets of Langerhans pretreated with the cytokine *in vitro*. However, acute exposure of islets to IL-6 did not give the potentiation of glucose-induced insulin secretion associated with IL-1 β (Spinass *et al.*, 1986). The similar degree of inhibition obtained with IL-1 β and IL-6 (1 nM), and the raised basal insulin release following a 24 h exposure to these cytokines (Table 2), are consistent with the hypothesis that IL-6 is a mediator of the long-term inhibitory, and possibly cytotoxic, effects of IL-1 β on islet β -cell function. However, at the time points studied here, no cell death was indicated, as islet DNA and insulin contents were not affected. A cytotoxic effect of IL-6 on islets has previously been suggested (Buschard *et al.*, 1989; Sandler *et al.*, 1990). Islets exposed to IL-6 for 48 h showed raised levels of insulin in the culture medium but had a decreased secretory response to a subsequent glucose challenge, with a small but significant decrease in islet DNA content (Sandler *et al.*, 1990). Ultrastructural abnormalities similar to those observed in IL-1 β -treated islets have been described in islets exposed to IL-6 (Buschard *et al.*, 1989). We found that IL-6, unlike IL-1 β , did not lower the incorporation of [³H]thymidine into DNA, nor did it alter the effect of IL-1 β on DNA synthesis when these cytokines were used in combination, suggesting that IL-6 is not a mediator for the cytotoxic effect of IL-1 β on islet DNA synthesis. Further differences in the actions of IL-1 β and IL-6 on insulin secretion were also observed. Inhibition of secretion after pretreatment with IL-1 β (6 h) was more rapid than with IL-6 (12 h); moreover, the combination of IL-6 plus IL-1 β (12 h) was found to inhibit insulin secretion in an additive manner, in contrast with the report that IL-6 preserves the secretory response from islets co-cultured with an inhibitory dose of IL-1 β (Buschard *et al.*, 1989). Thus our results support the existence of distinct modes of action of IL-6 and IL-1 β on the islet β -cell, which is in agreement with the findings that a major effect of IL-1 β on the islet β -cell is to decrease glucose oxidation and oxygen uptake (Sandler *et al.*, 1987), whereas IL-6 increases both of these parameters (Sandler *et al.*, 1990). We further differentiated between the actions of IL-6 and IL-1 β by pre-treating islets with these cytokines in combination with TNF- α .

Although a synergistic inhibitory effect of IL-1 β and TNF- α on insulin secretion was clearly demonstrated, synergism between IL-6 and TNF- α was not observed. In conclusion, prolonged exposure of rat islets of Langerhans to IL-6 results in the inhibition of glucose-induced insulin secretion, the effect of which is more pronounced in the presence of IL-1 β . However, from the differences in the actions of IL-6 and IL-1 β described here, IL-6 is unlikely to mediate the actions of IL-1 β on islet function. Thus IL-6, along with IL-1 β and TNF- α , when released in the vicinity of the islet β -cell as a consequence of an autoimmune process, may contribute to the impaired β -cell function characteristic of the early stages of IDDM.

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