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Interleukin-1 inhibits glucose-induced Ca^{2+} uptake by islets of Langerhans

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Recombinant interleukin-1 (rIL-1) is known to inhibit glucose-induced insulin secretion by islets of Langerhans, a novel target tissue of cytokine. We have investigated whether rIL-1 pretreatment affects biochemical mechanisms known to be involved in the regulation of Ca^{2+} homeostasis during glucose-induced insulin secretion. Glucose-induced Ca^{2+} uptake by intact islets through the plasma membrane was dramatically inhibited (96%) by rIL-1 (2 nM). rIL-1, however, did not affect Ca^{2+} uptake by, or Ins 1,4,5-P_3-induced Ca^{2+} efflux from, the endoplasmic reticulum in digitonin-permeabilized islets, although glucose-induced accumulation of inositol trisphosphates was inhibited (38%). These results suggest that perturbation of intracellular Ca^{2+} homeostasis in islets is involved in inhibition of insulin secretion by rIL-1.

Interleukin-1; Ca^{2+} uptake; Glucose; Inositol phosphate; (Pancreatic islet)

1. INTRODUCTION

The endocrine pancreas has recently been recognized to be a target tissue for interleukin-1 (IL-1) [1–5]. Recombinant IL-1 (rIL-1) exerts potent effects on glucose-induced insulin secretion by the β-cells of islets, the direction of which is determined by the concentration of rIL-1 and the duration of exposure [5]. Incubation of islets for several days with rIL-1 is believed to result in β-cell death [6]. Nerup and co-workers [6] have therefore proposed that rIL-1-mediated β-cell destruction may be an important pathogenic event in the development of insulin-dependent diabetes mellitus. The biochemical mechanisms underlying the cytotoxic effects of rIL-1 are unknown. Moreover, it is difficult to assess the events involved in β-cell destruction once cytotoxicity has progressed to an irreversible stage. We have therefore begun to examine the biochemical actions of rIL-1 on β-cells at a time when suppression of insulin secretion is observed but before cytotoxicity has developed. An incubation period of 18 h was selected because at that time inhibition of glucose-induced insulin secretion is reversible upon removal of rIL-1, and the rIL-1-treated islets display no signs of cytotoxicity [3].

This experimental design has been employed to investigate the effects of rIL-1 on the biochemical mechanisms controlling Ca^{2+} homeostasis in islets [7–9].

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2. MATERIALS AND METHODS

2.1. *Islet isolation and culture*

Islets of Langerhans were isolated from 8–15 rats per experiment by collagenase digestion, followed by separation on a discontinuous Ficoll gradient [10]. Isolated islets were cultured under an atmosphere of 95% air/5% CO_2 for 18 h at 37°C in 2.5 ml complete tissue culture medium (CMRL-1066 containing

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5 mM D-glucose, 1% L-glutamine, 10% heat-inactivated fetal bovine serum, 0.5% penicillin and 0.5% streptomycin) supplemented with 2 nM rIL-1 (a gift from Dr P.T. Lomedico, Hoffman LaRoche, Inc., Nutley, NJ) or the vehicle [3]. Islets were then washed extensively in fresh complete tissue culture medium prior to use. Protein content of rIL-1-treated islets was identical to control islets [0.87 ± 0.04 (n = 34) μg protein/islet for control, 0.94 ± 0.05 (n = 35) μg protein/islet for rIL-1-treated islets].

2.2. *Ca^{2+} uptake and Ca^{2+} efflux by intact islets*

Ca^{2+} uptake by intact islets was assessed by measuring the $^{45}Ca^{2+}$ content of islets in a static incubation design (30 islets/tube) using a dual-isotope technique with [^{3}H]sucrose as the extracellular marker as described [11,12]. rIL-1-pretreated (2 nM for 18 h) and control islets were preincubated for 30 min at 37°C in 0.1 ml Hepes/Krebs buffer (25 mM Hepes, 115 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, pH 7.4) supplemented with 3 mM glucose and then incubated for 2, 5, and 60 min in 0.1 ml fresh Hepes/Krebs buffer containing $^{45}Ca^{2+}$ (2.5 mM, 12 mCi/mmol final specific activity) and [^{3}H]sucrose (5 mM, 6 mCi/mmol final specific activity) and 3 or 28 mM glucose. Ca^{2+} efflux experiments were performed on rIL-1-pretreated and control islets labeled overnight with $^{45}Ca^{2+}$ to isotopic equilibrium [13]. Radiolabeled islets were placed in a perifusion apparatus (450 islets/chamber) and perifused (1 ml/min) at 37°C with a modified Hepes/Krebs buffer supplemented with 3 or 28 mM glucose and which contained no added Ca^{2+} (final Ca^{2+} concentration = 0.003 mM) as in [12,13].

2.3. *Inositol phosphate accumulation*

Isolated islets were labeled to isotopic equilibrium with [^{3}H]inositol. In brief, islets were incubated for 135 min at 37°C with 400 μCi [^{3}H]inositol in 0.1 ml Hepes/Krebs buffer supplemented with 28 mM glucose [10,14]. Radiolabeled islets were then divided into two groups, each group being incubated for another 18 h at 37°C in 2.5 ml complete tissue culture medium supplemented with 50 μCi [^{3}H]inositol and 2 nM rIL-1 or vehicle. Radiolabeled islets were washed 3 times in Hepes/Krebs buffer to remove unincorporated [^{3}H]inositol, placed in silanized vials (400 islets/vial), preincubated for 30 min in Hepes/Krebs buffer with 3 mM glucose, and then incubated for another 30 min at 37°C in fresh Hepes/Krebs buffer with 3 or 28 mM glucose. Inositol phosphates were extracted and separated by anion-exchange HPLC as in [10,14].

2.4. *Ca^{2+} fluxes in the endoplasmic reticulum of digitonin-permeabilized islets*

rIL-1 pretreated (2 nM for 18 h) and control islets were permeabilized with digitonin as reported in [12]. Permeabilized islets (30/tube) were then incubated for 30 min in 100 μl of Pipes buffer mimicking intracellular conditions (100 mM Pipes, 100 mM KCl, 7 mM $MgCl_2$, ± 5 mM ATP, 2.25 μg/ml ruthenium red, pH 7.0). The free Ca^{2+} concentration of this buffer was set at 0.2 μM by including EGTA at a concentration of 0.11 mM. The $^{45}Ca^{2+}$ steady-state (30 min) content, the efflux of $^{45}Ca^{2+}$ (10 min) induced by Ins 1,4,5-P_3 (10 μM), and the efflux of $^{45}Ca^{2+}$ induced by A23187 (2 μM) from the endoplasmic reticulum of digitonin-permeabilized islets were measured as in [12].

3. RESULTS

3.1. *Effect of rIL-1 on glucose-induced Ca^{2+} uptake and Ca^{2+} efflux by intact islets*

Glucose-induced Ca^{2+} uptake by intact islets from the extracellular space was dramatically decreased in islets incubated for 18 h with 2 nM rIL-1. As shown in table 1, Ca^{2+} uptake in rIL-1-pretreated islets was inhibited by 69, 70 and 96% at 2, 5 and 60 min incubation ($p < 0.01$, $p < 0.05$, $p < 0.001$, respectively) following incubation with 28 mM glucose. rIL-1 pretreatment (2 nM for 18 h) had no effect on $^{45}Ca^{2+}$ accumulation of islets incubated with a basal concentration of glucose (3 mM).

In order to assess whether rIL-1 was exerting any effects on Ca^{2+} efflux from islets, a perifusion experimental design was employed. In the absence of extracellular Ca^{2+}, glucose is known to reduce Ca^{2+} efflux from perifused control islets loaded to isotopic equilibrium with $^{45}Ca^{2+}$ [8]. It was observed that rIL-1 pretreatment did not affect inhibition of Ca^{2+} efflux by glucose in such a perifusion system in comparison with control islets indicating that the efflux of Ca^{2+} from intact islets was not affected by pretreatment with rIL-1 (not shown).

Table 1

Effect of rIL-1 pretreatment (18 h) on glucose-induced Ca^{2+} uptake by intact islets

Incubation time (min)	Glucose-induced Ca^{2+} uptake (pmol/islet)	
	Control	2 nM rIL-1
2	1.18 ± 0.20	0.37 ± 0.19[b]
5	1.16 ± 0.24	0.34 ± 0.27[a]
60	3.36 ± 0.70	0.14 ± 0.64[c]

[a] $p < 0.05$ vs control
[b] $p < 0.01$ vs control
[c] $p < 0.001$ vs control

Glucose-induced Ca^{2+} uptake is calculated as the difference between the $^{45}Ca^{2+}$ content obtained at 28 mM glucose minus that at 3 mM glucose for each experimental condition as described in section 2. Control Ca^{2+} content at 3 mM glucose was 0.8 ± 0.2, 1.2 ± 0.3 and 7.7 ± 0.6 pmol/islet at 2, 5 and 60 min, respectively. rIL-1 pretreatment had no effect on the Ca^{2+} content at 3 mM glucose (0.8 ± 0.3, 1.2 ± 0.3 and 7.3 ± 0.7 pmol/islet at 2, 5 and 60 min, respectively). Results are shown as means ± SE of 24–38 observations per condition from 4–5 experiments

3.2. *Effect of rIL-1 on glucose-induced accumulation of inositol phosphates*

Islets were labeled to isotopic equilibrium (overnight incubation) with [^{3}H]inositol in the presence or absence of 2 nM rIL-1. [^{3}H]Inositol-prelabeled islets were then incubated for 30 min with 3 or 28 mM glucose under conditions identical to those reported for insulin secretion studies [3]. In control islets, 28 mM glucose resulted in a significant increase (vs 3 mM glucose) in the accumulation of Ins P_1 (24.9 ± 10.1%, $p < 0.05$), Ins P_2 (45.0 ± 11.9%, $p < 0.001$), Ins 1,3,4-P_3 (241.7 ± 51.1%, $p < 0.001$), Ins 1,4,5-P_3 (64.5 ± 19.8%, $p < 0.02$), and Ins 1,3,4,5-P_4 (49.7 ± 15.2%, $p < 0.005$) (table 2). In rIL-1-pretreated islets, basal (3 mM glucose) accumulation of inositol phosphates was not significantly different from that of control islets. With rIL-1-pretreated islets however, accumulation of inositol phosphates induced by 28 mM glucose was inhibited by 22% for Ins P_2 ($p < 0.02$), 38% for Ins 1,3,4-P_3 ($p < 0.01$), 20% for Ins 1,4,5-P_3 and 34% for Ins 1,3,4,5-P_4 ($p < 0.005$) (table 2). rIL-1 pretreatment had no effect on the inositol phospholipid content of islets (not shown).

Table 2

Effect of rIL-1 (2 mM) pretreatment (18 h) on glucose-induced accumulation of inositol phosphates

Incubation conditions	[^{3}H]Inositol phosphate accumulation (cpm)		
	Ins 1,4,5-P_3	Ins 1,3,4,5-P_4	Ins 1,3,4-P_3
3 mM glucose	129 ± 19	201 ± 17	68 ± 4
28 mM glucose	212 ± 26	301 ± 31	232 ± 35
3 mM glucose + 2 nM rIL-1 pretreatment	143 ± 22	161 ± 14	65 ± 6
28 mM glucose + 2 nM rIL-1 pretreatment	169 ± 19	199 ± 21	144 ± 21
% inhibition of 28 mM glucose-induced accumulation due to rIL-1	20 ± 9	34 ± 7[b]	38 ± 9[a]

[a] $p < 0.01$ vs control
[b] $p < 0.05$ vs control

Isolated islets were prelabeled with [^{3}H]inositol and incubated for 18 h in the presence or absence of 2 nM rIL-1 as described in section 2. Radiolabeled islets (300–350/tube) were preincubated for 30 min with 3 mM glucose and then incubated for another 30 min with 3 or 28 mM glucose. Inositol phosphates were extracted and analyzed by anion-exchange HPLC [10,14]. rIL-1 did not affect the incorporation of [^{3}H]inositol into phospholipids and inositol phosphates (802554 ± 52764 cpm [^{3}H]inositol incorporated in controls vs 819635 ± 77113 cpm for rIL-1-treated islets). Results are shown as means ± SE of 5 separate experiments, each involving duplicate determinations

3.3. *Effect of rIL-1 on Ca^{2+} fluxes in the endoplasmic reticulum of permeabilized islets*

Islets were pretreated for 18 h with 2 nM rIL-1 or with vehicle (control) and then permeabilized with digitonin to measure Ca^{2+} handling by the endoplasmic reticulum in situ. rIL-1 had no effect on the ATP-dependent Ca^{2+} steady-state content of the endoplasmic reticulum (0.38 ± 0.02 pmol/islet in control vs 0.39 ± 0.02 pmol/islet in rIL-1-pretreated islets; n = 22–24). Ins 1,4,5-P_3 (10 μM) mobilized $^{45}Ca^{2+}$ equally well from the endoplasmic reticulum of control (0.17 ± 0.03 pmol Ca^{2+}/islet) and from rIL-1-pretreated (0.21 ± 0.03 pmol Ca^{2+}/islet) islets. Similarly, the Ca^{2+} ionophore A23187 mobilized the same amount of Ca^{2+} from the endoplasmic reticulum of control islets (0.19 ± 0.02 pmol/islet) and from rIL-1-pretreated islets (0.18 ± 0.03 pmol/islet). These observations indicate that rIL-1 pretreatment for 18 h does not influence the ability of the endoplasmic reticulum to accumulate Ca^{2+} in the presence of ATP or to release Ca^{2+} in response to Ins 1,4,5-P_3 or the Ca^{2+} ionophore A23187.

4. DISCUSSION

Glucose-induced Ca^{2+} uptake by the β-cells was profoundly inhibited following rIL-1 pretreatment for 18 h. Basal Ca^{2+} uptake (in the presence of 3 mM glucose) was not affected. The lack of effect under basal conditions suggests that the integrity of the β-cell plasma membrane remained intact. Glucose stimulation of the normal β-cell results in plasma membrane depolarization, which is believed to be mediated by ATP-sensitive, voltage-independent K^+ channels [15]. Plasma membrane depolarization opens voltage-dependent Ca^{2+} channels which leads to an influx of extracellular Ca^{2+} [8]. It is currently believed that glucose-induced Ca^{2+} uptake, as measured in this study, represents the influx of Ca^{2+} through the voltage-dependent Ca^{2+} channels [8]. Such uptake is inhibited by Ca^{2+} channel blockers, and voltage-

dependent inward Ca^{2+} currents have been directly recorded in islet cells (review [8]). Inhibition of Ca^{2+} uptake by rIL-1 may be due to a decrease in ATP concentrations which would prevent membrane depolarization mediated by ATP-induced closure of the K^+ channels. rIL-1 has been shown to inhibit glucose oxidation in islets [4]. We are currently assessing ATP levels in rIL-1-treated islets.

The effect of rIL-1 on Ca^{2+} entry through the plasma membrane is apparently selective because rIL-1 did not influence Ca^{2+} handling by the endoplasmic reticulum. The participation of the endoplasmic reticulum in the control of islet cytosolic Ca^{2+} concentration is thought to be essential in glucose-induced insulin secretion [9]. rIL-1 influenced neither Ca^{2+} uptake by the endoplasmic reticulum nor Ca^{2+} efflux induced by the second messenger Ins 1,4,5-P_3. These observations indicate that the endoplasmic reticulum itself functions normally after 18 h of exposure to rIL-1 even though glucose-stimulated inositol trisphosphate accumulation is perturbed.

In conclusion, we have shown that rIL-1 inhibits glucose-stimulated voltage-dependent entry of Ca^{2+} which suggests that perturbation of intracellular Ca^{2+} homeostasis may be involved in rIL-1 inhibition of insulin secretion.

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