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Effect of human lymphoblastoid interferon on insulin synthesis and secretion in isolated human pancreatic islets

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Summary. Human islets of Langerhans were isolated from the pancreas removed from a 13-year-old female transplant donor. The islets were incubated in a culture medium for 24 h in the presence of human lymphoblastoid interferon (1000 units/ml). Insulin secretion, proinsulin biosynthesis, total protein biosynthesis and total insulin content were assessed at various concentrations of glucose in the presence of interferon. In interferon-treated islets glucose-stimulated insulin secretion was unaltered from that of control islets; however, glucose-stimulated proinsulin biosynthesis was specifically inhibited by interferon (48%, $p < 0.025$). Total protein biosynthesis and total insulin content were not significantly affected by interferon.

Key words: Interferon, insulin biosynthesis, insulin secretion, human pancreatic islets.

Interferon is able to protect most types of mammalian cells from a viral infection by modifying the protein synthesizing system of the host cell [1, 2]. At low concentrations, (< 50 units/ml), interferon preferentially inhibits viral replication, leaving host cell protein synthesis untouched [1]. However, at higher concentrations (> 150 units/ml), interferon also may reduce host cell protein synthesis [3], and inhibit the synthesis of certain inducible proteins [4, 5].

A number of viruses are known to induce diabetes in experimental animals by direct effects on the islets of Langerhans [6]. Viruses also can induce the production of interferon in some cells [1]. While interferons under some circumstances can protect against subsequent inoculation of such diabetogenic viruses [7, 8], it is not known whether high local concentrations of interferon may have other effects on β-cell function. With this in mind, we have investigated the effect of a high concentration of interferon on human pancreatic islet β-cell function.

Materials and methods

Human islet isolation

Part of the tail of the pancreas from a renal transplant donor was removed surgically in a minimum ischaemic period and placed in ice-cold sterile RPMI 1640 tissue culture medium containing glucose (5 mmol/l), 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (0.1 mg/ml), for carriage back to the laboratory. Removal of the pancreas was conducted under the guidelines of The London Hospital Ethical Committee. The patient was a 13-year-old female with a brain tumour. There was no previous history of diabetes or other metabolic disorder. The pancreas was washed in sterile bicarbonate buffered medium gassed with $O_2 + CO_2$ (95%:5%) immediately before use [9] and then cut into pieces of approximately 1 g weight. These pieces were distended by injection with bicarbonate buffered medium (pH 7.4), containing collagenase (1 mg/ml) (Type I, Sigma (London), Poole, Dorset, UK), chopped into small pieces (2–3 mm^3), centrifuged (2,500 rev/min for 20 s) and the collagenase solution decanted. The pancreas pieces were then suspended in fresh bicarbonate buffer (10 ml) [9], containing collagenase (3 mg/ml) and shaken for 30 min at 37 °C, using a mechanical flask shaker (Griffin, London, UK). From the resultant digest, islets were harvested as described by Howell and Taylor [10], using a drawn out Pasteur pipette under aseptic conditions and viewing under a dissection microscope. From approximately 5 g of pancreas 543 islets were obtained.

Islet treatment and assay of islet β-cell function

Harvested islets were placed in groups of 100/ml in RPMI 1640 tissue culture medium containing glucose (2 mmol/l), 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (0.1 mg/ml). The islets were incubated for 24 h either with or without addition of human lymphoblastoid interferon (1000 units/ml, Burroughs Wellcome, Beckenham, Kent, UK), in a CO_2 incubator (LEEC, Leicester, UK) at 37 °C. After washing in bicarbonate buffer [9], islets were placed in groups of 10–15 in microcentrifuge tubes. Each group was incubated in bicarbonate buffer (150 µl) with glucose (2 or 20 mmol/l) and L-phenyl-[2,3-^{3}H]-alanine (80 µCi/ml) (Amersham International, Amersham, Bucks, UK) for 90 min at 37 °C in a shaking water bath. After centrifugation (2,500 rev/min for 20 s), the supernatant was removed for radioimmunoassay of insulin secreted [11]. (Human insulin stan-

Table 1. Effects of human leucocyte interferon on various parameters of isolated human islet function at basal and raised glucose concentrations

	Control islets		Interferon-treated islets	
	Glucose (2 mmol/l)	Glucose (20 mmol/l)	Glucose (2 mmol/l)	Glucose (20 mmol/l)
Total protein biosynthesis (10^3 cpm ^{3}H-phenylalanine incorporation · μg DNA^{-1} · h^{-1})	14.41 ± 2.38 (5)	25.24 ± 4.81 (5)[a]	11.41 ± 1.54 (4)	18.60 ± 2.16 (6)[a]
Proinsulin biosynthesis (10^3 cpm ^{3}H-phenylalanine incorporation · μg DNA^{-1} · h^{-1})	0.47 ± 0.14 (5)	4.26 ± 0.87 (5)[b]	0.56 ± 0.09 (5)	2.23 ± 0.18 (6)[b,c]
Insulin secretion (pg · μg DNA^{-1} · min^{-1})	65.32 ± 7.90 (5)	234.79 ± 38.24 (5)[b]	86.03 ± 10.09 (5)	253.56 ± 25.17 (6)[b]
Total insulin content (ng/μg DNA)	114.05 ± 26.47 (5)	97.34 ± 21.57 (5)	104.28 ± 19.13 (5)	87.49 ± 17.37 (6)

Results are given as mean ± SEM with the number of observations in parentheses. [a] $p < 0.05$ or [b] $p < 0.005$ statistically different from equivalent 2 mmol/l glucose-treated islets [c] $p < 0.025$ statistically different from equivalent control islets.

dard and binding reagent were from Wellcome Research Reagents, Beckenham, Kent, UK). The islets were then washed in phosphate buffered saline (PBS, 1 ml), containing phenylalanine (10 mmol/l) and resuspended in 100 μml PBS and sonicated (25 watts for 15 s, Branson – B15P, Dawe Instruments, London, UK). The resulting sonicate was assayed for islet total insulin [11] and DNA content [12]. Incorporation of labelled phenylalanine into total protein and immuno-extractable proinsulin was assessed by the method of Berne [13]. In these experiments, no attempt was made to distinguish between proinsulin and insulin.

Statistical analysis

Results are expressed as mean ± SEM, with the number of observations in parentheses. Significant differences between results were assessed by Student's t-test. Values with $p < 0.05$ were regarded as significant.

Results

The rates of proinsulin biosynthesis and insulin secretion at glucose (20 mmol/l) were specifically increased in control isolated human islets when compared with the rate at glucose (2 mmol/l) (Table 1). The rate of total protein biosynthesis was similarly raised, but to a much lesser extent. Total insulin content of control islets was not significantly affected by altering glucose concentrations.

In islets treated with interferon (1000 units/ml), insulin secretion and total insulin content were unaltered, giving results similar to those obtained in control islets incubated with glucose (2 and 20 mmol/l). By contrast, interferon specifically and significantly inhibited 20 mmol/l glucose-induced proinsulin biosynthesis ($p < 0.025$). There was a slight reduction in total protein biosynthesis with glucose (2 and 20 mmol/l) in interferon-treated islets; however, this was not statistically significant.

The DNA content of the islets (0.137 ± 0.007 μg/islet) did not vary significantly between groups of islets and was comparable to that of other human islet studies.

Discussion

That high glucose concentration induces an increase in insulin secretion and proinsulin biosynthesis in isolated human islets has been reported elsewhere [14–16]. Total protein biosynthesis (to a lesser extent) is also significantly raised by high glucose concentrations, and this may be a reflection of increased glucose utilisation [14], as well as increased proinsulin biosynthesis.

In this study, a high level of human lymphoblastoid interferon (1000 units/ml) inhibits glucose-induced proinsulin biosynthesis in isolated human pancreatic islets. Similar observations for interferon inhibition of the synthesis of inducible proteins have been made in other systems [4, 5, 17]. At this high interferon concentration, total protein biosynthesis may also be reduced [3].

Interferon is thought to inhibit protein biosynthesis by inducing at least two enzyme systems via specific receptors on the host cell [1, 2]. One of the enzymes induced by interferon, 2′-5′oligoadenylate synthetase, synthesises certain oligoadenylates from ATP. These oligoadenylates activate latent endoribonucleases which cleave free single-stranded RNA (including free cytoplasmic mRNA), resulting in a decrease in new protein synthesis in the host cell. The other interferon-induced enzyme is a protein kinase, which phosphorylates an initiation factor involved in the initiation of protein synthesis, known as eIF2. Once phosphorylated, eIF2 activity is inhibited, hence the initiation of new protein biosynthesis in the cell is reduced. We suggest that interferon is inducing these enzyme systems in the isolated human islet β cells, which in turn inhibits glucose-induced proinsulin biosynthesis.

It is uncertain whether the effect of high concentrations of interferon are important in virus-induced diabetes, in which under some circumstances proinsulin biosynthesis may be decreased [18]. With regard to this, a high local concentration of interferon in the vicinity of the islet β cells might temporarily diminish proinsulin biosynthesis.

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