Effect of interferon and double-stranded RNA on B-cell function in mouse islets of Langerhans

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1. The direct effects of α - and β -interferons on isolated mouse pancreatic islets were investigated *in vitro* and found to be similar. 2. After 7 h incubation with interferon concentrations above 350 units/ml, glucose-stimulated (pro)insulin biosynthesis was significantly inhibited, with only a slight inhibition of total protein biosynthesis. Inhibition could be abolished in the additional presence of an anti-interferon antibody. Interferon did not affect insulin release, total insulin content, or glucose oxidation of the islets. 3. The stimulation of (pro)insulin biosynthesis by adenosine, D-glyceraldehyde, mannose, N-acetylglucosamine and leucine was also inhibited by interferon, with no effect on insulin release. 4. At concentrations of dsRNA (double-stranded RNA) said to induce interferon (1–100 μ g/ml), glucose-stimulated (pro)insulin biosynthesis was inhibited without significantly affecting insulin release. The dsRNA may itself inhibit stimulated (pro)insulin biosynthesis or may function indirectly by the induction of interferon.

Interferons are a group of proteins that can be produced in most types of vertebrate cells in response to a virus infection, and are generally thought to be effective by halting virus replication in host cells (Lockhart, 1973). Interferons are able to achieve this, through specific host-cell-surface receptors (Aguet, 1980), and then it is thought by inducing various enzyme systems that inhibit the initiation of protein synthesis in the host cell (Yabrov, 1980; Kerr *et al.*, 1981; Lengyel, 1982; Lebleu & Content, 1982). Generally, at low interferon concentrations, virus replication is preferentially inhibited; however, on raising the interferon concentration, total host-cell protein synthesis may also be decreased (Falcoff *et al.*, 1975). The induction of specific host-cell proteins may also be inhibited by raised concentrations of interferon (Beck *et al.*, 1974; Illinger *et al.*, 1976; Rossi *et al.*, 1977).

A number of viruses are known to induce diabetes in experimental animals by direct effects on pancreatic islets (Craighead & McLane, 1968; Coleman *et al.*, 1973; Notkins *et al.*, 1981; Notkins & Yoon, 1983; Müntefering, 1984). To date, such viruses have not been shown to induce interferon production directly in islets. Nevertheless, insulin

synthesis in mice has been shown to be greatly diminished *in vivo* and in islets in tissue culture by a diabetogenic strain of encephalomyocarditis virus (Petersen *et al.*, 1975; Hellqvist *et al.*, 1981), or by Cocksackie B4 virus (T. M. Szopa & K. W. Taylor, unpublished work). Some of these inhibitory effects may reflect locally high concentrations of interferon apart from more direct effects of a virus.

For these reasons, we have studied both the direct effects of a synthetic dsRNA, which is an interferon inducer (Field *et al.*, 1967), and those of interferon itself, on the function of isolated mouse islets. A preliminary report on some aspects of this work has been published (Rhodes & Taylor, 1984).

Experimental

Animals

Male DBA/2 mice (8–12 weeks old; Bantin and Kingman, Hull, Humberside, U.K.) were allowed free access to water and standard pellet food. They were killed by cervical dislocation.

Materials

Unless otherwise stated, all biochemical reagents were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K., or from BDH Chemicals, Poole, Dorset, U.K., and were of the highest grade or purity available. Poly(I)•poly(C)

Abbreviation used: dsRNA, double-stranded RNA.

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was from P-L Biochemicals, Northampton, U.K. All radiochemicals were supplied by Amersham International, Amersham, Bucks., U.K. Human insulin standard and insulin-binding reagent were from Wellcome Research Reagents, Beckenham, Kent, U.K.

Two sources of mouse β -interferon were used. One was given by Professor L. Collier, London Hospital Medical College, London E1, U.K.; the other was purchased from Enzo Biochemicals, New York, NY, U.S.A. The α -interferon was given by Dr. I. M. Kerr, Imperial Cancer Research Fund Laboratory, London W.C.1, U.K. All interferons were tested for antiviral activity and were all found to be active against viral replication between 1 and 10 units/ml. The rabbit antiserum to mouse β -interferon was purchased from Calbiochem-Behring, Bishop's Stortford, Herts, U.K.; it is specific for β -interferons and had no cross-reactivity with α - or γ -interferons, as stated by the manufacturer.

Isolation of islets

Islets were isolated under aseptic conditions by collagenase digestion of the pancreas (Howell & Taylor, 1968). Throughout this procedure bicarbonate-buffered medium was used (Gey & Gey, 1936), equilibrated with O₂/CO₂ (19:1) and containing bovine serum albumin (1 mg/ml) and glucose (2 mM). This basal medium was also used in washing islets and in some assays of islet function, where the glucose was replaced by other secretagogues.

Treatment of islets with interferon and dsRNA

For islets incubated with interferon or dsRNA for more than 2 h, batches of 100 islets were placed in 1 ml of RPMI 1640 tissue-culture medium containing glucose (2 mM), penicillin (100 units/ml), streptomycin (0.1 mg/ml), 10% (v/v) heat-inactivated foetal-calf serum, and different concentrations of α - or β -interferon or dsRNA. No interferon or dsRNA was added for control islets. The islets were then incubated for various time periods up to 48 h in a CO₂ incubator (L.E.E.C., Leicester, U.K.) at 37°C under 5% CO₂ in air. For islets incubated for less than 2 h, conditions were used as described below for the assay of islet B-cell function.

Determination of islet B-cell function

After the pretreatment, batches of 15–20 islets per observation were placed in Microfuge tubes (Beckman; 1.5 ml) and washed twice with 2 × 1 ml of bicarbonate-buffered medium containing 2 mM-glucose. The islets were then resuspended in 0.15 ml of the bicarbonate-buffered medium containing 2 mM- or 20 mM-glucose or an alternative

secretagogue in the presence of 50 μ Ci of L-[4,5-³H]leucine/ml. When leucine stimulation of islets was investigated, 80 μ Ci of L-[2,3-³H]phenylalanine/ml was used as an alternative labelled amino acid to monitor incorporation into protein, because of excessive amounts of unlabelled leucine present. The islets were then incubated for 2 h at 37°C in a shaking water bath. Interferon- or dsRNA-treated islets were incubated in the presence of interferon or dsRNA at concentrations equal to those used in the previous treatment incubation. At the end of this second incubation, the islets were centrifuged down at 1500g for 30 s, and 0.1 ml of the supernatant was taken for assay of insulin released, by radioimmunoassay (Hales & Randle, 1963) using human insulin standard and ¹²⁵I-insulin. The L-[4,5-³H]leucine present did not interfere with ¹²⁵I-insulin in this assay, and the curves for human and rodent insulin were similar over the range of insulin standards used.

The islets were then washed twice with 2 × 1 ml of 0.4 mM-phosphate-buffered saline (pH 7.4) containing 10 mM-leucine and then suspended in 0.1 ml of phosphate-buffered saline. The islets were then disrupted by sonication at 25 W for 15 s with a Branson Sonifier (Branson Sonic Power, Danbury, CT, U.S.A.). L-[4,5-³H]Leucine incorporation into 10% (w/v)-trichloroacetic acid-precipitable protein (total protein) and into immunoprecipitable (pro)insulin was assessed by the method of Berne (1975). No attempt was made to distinguish between proinsulin and insulin in this assay. Total insulin content of the islets was assayed from the sonicated material by radioimmunoassay (Hales & Randle, 1963), as was total DNA content, which was assayed by the method of Vytásek (1982). Results were expressed in terms of islet DNA.

Presentation of results

All results are presented as means $\pm$ S.E.M., with the numbers of observations per group in parentheses. Statistical significance of differences between experimental groups was determined by Student's *t* test.

Results

Effects of interferon on isolated mouse islets

The basal rate of insulin release and (pro)insulin biosynthesis at 2 mM-glucose can be specifically increased by a stimulatory 20 mM-glucose concentration ($P < 0.01$), as shown in control islets (Table 1). The rate of total protein biosynthesis was also raised ($P < 0.01$), but to a lesser extent. Total insulin content of the islets remains unaltered.

However, when the islets have been exposed to 1000 units of α - or β -interferon/ml for 24 h, 20 mM-glucose-stimulated (pro)insulin biosynthesis was

Table 1. *Effects of α - and β -interferons on isolated mouse islets*

Islets were incubated for a total of 24 h with 1000 units of α - or β -interferon/ml and islet function was assayed at 2 mM- and 20 mM-glucose in the presence of 50 μ Ci of L-[4,5- 3 H]leucine/ml (see the Experimental section). Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. Results that significantly differ from the corresponding untreated controls with $P < 0.01$ are denoted by *, and those that differ from the equivalent observation at 2 mM-glucose with $P < 0.01$ are denoted by †.

Treatment	Glucose (mM)	$10^{-3} \times$ Total protein synthesis (c.p.m./h per μ g of DNA)	$10^{-2} \times$ (Pro)insulin synthesis (c.p.m./h per μ g of DNA)	Insulin secretion (pg/min per μ g of DNA)	Total insulin content (ng/ μ g of DNA)
Control	2	25.9 $\pm$ 3.8 (10)	3.4 $\pm$ 0.5 (10)	48 $\pm$ 7 (10)	228 $\pm$ 31 (10)
	20	45.7 $\pm$ 4.7 (10)†	22.9 $\pm$ 2.7 (10)†	226 $\pm$ 15 (10)†	241 $\pm$ 20 (10)
α -Interferon	2	18.5 $\pm$ 3.1 (5)	3.7 $\pm$ 0.6 (5)	49 $\pm$ 12 (5)	276 $\pm$ 35 (5)
	20	34.6 $\pm$ 6.0 (5)†	11.3 $\pm$ 2.4 (5)*†	216 $\pm$ 19 (5)†	268 $\pm$ 40 (5)
β -Interferon	2	22.5 $\pm$ 4.2 (5)	4.4 $\pm$ 1.1 (5)	32 $\pm$ 5 (5)	274 $\pm$ 43 (5)
	20	36.1 $\pm$ 6.0 (5)†	13.2 $\pm$ 2.4 (5)*†	214 $\pm$ 16 (5)†	244 $\pm$ 27 (5)

significantly inhibited in comparison with the controls ($P < 0.01$). There was no difference between the response in control and interferon-treated islets incubated at 2 mM-glucose. The rate of total protein biosynthesis at 2 mM- and 20 mM-glucose was lower in α - and β -interferon-treated islets, though this was not statistically significant. Insulin release at 2 mM- or 20 mM-glucose and total insulin content were unaltered by α - or β -interferon.

When the concentration of β -interferon exposed to islets for 24 h was varied (Fig. 1), 20 mM-glucose-stimulated (pro)insulin biosynthesis was significantly inhibited ($P < 0.01$) by β -interferon concentrations above 350 units/ml. The rate of (pro)insulin biosynthesis at 2 mM-glucose was not significantly altered. Insulin release from the islets at 2 mM- or 20 mM-glucose was unaltered, at any concentration of β -interferon, in comparison with the control values.

The rate of 20 mM-glucose-stimulated (pro)insulin biosynthesis was significantly inhibited ($P < 0.02$) after 7 h in β -interferon-treated islets, with no significant effect on the rate at 2 mM-glucose in comparison with control islets (Fig. 2). This effect persisted up to 50 h exposure time to interferon. The rate of insulin released at 2 mM- or 20 mM-glucose in interferon-treated islets was no different from the controls, up to 50 h exposure to interferon.

In islets pre-treated with α -interferon for 24 h, glucose-stimulated (pro)insulin biosynthesis was significantly inhibited ($P < 0.005$) above 10 mM-glucose, resulting in a 'flattened' dose-response curve (Fig. 3). In contrast, for insulin release, a sigmoidal glucose-dose-response curve was observed in control islets, which was very similar to that for α -interferon-treated islets.

Fig. 1. *Effect of varying the β -interferon concentration on the rate of (pro)insulin biosynthesis in isolated mouse islets*

Concentrations of β -interferon between 0 and 1000 units/ml were incubated with islets for a total of 24 h at 2 mM-glucose, and then the islets were assayed at 2 mM- and 20 mM-glucose for a further 2 h period (see the Experimental section). Results (●, 20 mM-glucose; ○, 2 mM-glucose) represent the means for at least five individual determinations, with S.E.M. values shown by bars. Values that differ from that at zero β -interferon with $P < 0.005$ are denoted by *.

Fig. 2. Effect of varying the incubation time of isolated mouse islets with 500 units of β -interferon/ml. β -Interferon (500 units/ml) was incubated with islets for times between 1 and 48 h at 2 mM-glucose and then the islets were assayed at 2 mM- and 20 mM-glucose for a further 2 h period (see the Experimental section). Results (Δ , 20 mM-glucose, control; $\circ$, 2 mM-glucose, control; $\blacktriangle$, 20 mM-glucose + interferon; $\bullet$, 2 mM-glucose + interferon) represent the means for at least five individual determinations, with S.E.M. shown. Values that differ from the equivalent control with $P < 0.02$ are denoted by *.

The inhibition of 20 mM-glucose-stimulated (pro)insulin biosynthesis by β -interferon could be abolished when an excessive amount of rabbit antiserum for mouse β -interferon was also present (Table 2). An equivalent volume of normal rabbit serum incubated with islets had no significant effect when compared with the controls. The decrease of total protein biosynthesis by β -interferon also reverted to control values in the added presence of the anti- β -interferon antibody. Again, no effect on insulin released or total insulin content of treated islets was observed.

Stimulation of (pro)insulin biosynthesis in islets by D-glyceraldehyde (1.5 mM), adenosine (10 mM), mannose (20 mM) and N-acetylglucosamine

Fig. 3. Effect of α -interferon on isolated mouse islets, assayed at various glucose concentrations. α -Interferon (1000 units/ml) was incubated with islets for a total of 24 h at 2 mM-glucose and then the islets were assayed over a range of glucose concentrations (0–20 mM) for a further 2 h period (see the Experimental section). Results ($\bullet$, control islets; $\circ$, interferon-treated islets) represent the means for at least five individual determinations with S.E.M. shown. Values that differ from the equivalent control with $P < 0.005$ are denoted by *.

(20 mM) was significantly inhibited ($P < 0.05$) in the presence of α -interferon (1000 units/ml), similar to observations made for 20 mM-glucose (Table 3). There was no significant effect of α -interferon on total protein biosynthesis, insulin release or total insulin content with these secretagogues. Similar results for 20 mM-leucine stimulation of islets were observed where (pro)insulin biosynthesis was significantly inhibited ($P < 0.01$) in the presence of α -interferon (Table 4).

Effects of dsRNA [poly(I)·poly(C)] on isolated mouse islets

In islets exposed to concentrations of poly(I)·poly(C) above 1.0 μ g/ml for 24 h, 20 mM-glucose stimulation of (pro)insulin biosynthesis is significantly inhibited ($P < 0.01$) in comparison with control islets (Table 5). The rate of total protein biosynthesis is decreased at both 2 mM- and 20 mM-glucose at poly(I)·poly(C) concentrations above 1.0 μ g/ml, but is only significantly so in 20 mM-glucose-stimulated islets in the presence of 100 μ g

Table 2. *Effect of the action of β -interferon and dsRNA on isolated mouse islets in the presence of rabbit antiserum for mouse β -interferon*

Islets were incubated for a total of 24 h with 1000 units of β -interferon/ml or 10 μ g of poly(I)·poly(C)/ml, with or without enough antibody specific for β -interferon present to neutralize 2500 units of β -interferon/ml. Islets were assayed at 2 mM- and 20 mM-glucose in the presence of 50 μ Ci of L-[4,5- 3 H]leucine/ml (see the Experimental section). An equivalent volume of normal rabbit serum was also similarly incubated with islets as an additional control. Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. Results which significantly differ from the equivalent no-treatment control with $P < 0.01$ are denoted by *, and those that differ from the equivalent observation at 2 mM-glucose with $P < 0.01$ are denoted by †.

Treatment (24 h incubation)	Glucose (mM)	$10^{-3} \times$ Total protein synthesis (c.p.m./h per μ g of DNA)	$10^{-2} \times$ (Pro)insulin synthesis (c.p.m./h per μ g of DNA)	Insulin secretion (pg/min per μ g of DNA)	Total insulin content (ng/ μ g of DNA)
Control	2	24.4 $\pm$ 3.6 (11)	3.4 $\pm$ 0.8 (9)	59 $\pm$ 9 (11)	258 $\pm$ 40 (11)
	20	54.9 $\pm$ 3.2 (11)†	19.2 $\pm$ 1.7 (10)†	254 $\pm$ 23 (11)†	313 $\pm$ 34 (11)
Normal rabbit serum (15 μ l/ml)	2	26.5 $\pm$ 3.3 (11)	4.1 $\pm$ 0.9 (10)	69 $\pm$ 9 (11)	290 $\pm$ 37 (11)
	20	51.5 $\pm$ 3.8 (11)†	18.7 $\pm$ 1.9 (9)†	286 $\pm$ 27 (11)†	277 $\pm$ 45 (11)
Poly(I)·poly(C) (10 μ g/ml)	2	24.8 $\pm$ 5.9 (5)	3.2 $\pm$ 0.7 (4)	55 $\pm$ 11 (5)	313 $\pm$ 48 (5)
	20	45.0 $\pm$ 3.3 (5)†	9.2 $\pm$ 1.4 (5)*†	250 $\pm$ 30 (5)†	262 $\pm$ 33 (5)
Poly(I)·poly(C) (10 μ g/ml) + β -interferon antibody	2	21.1 $\pm$ 3.1 (5)	2.9 $\pm$ 0.5 (9)	68 $\pm$ 12 (5)	279 $\pm$ 37 (5)
	20	46.3 $\pm$ 5.0 (5)†	9.9 $\pm$ 1.5 (5)*†	258 $\pm$ 39 (5)†	282 $\pm$ 41 (5)
β -Interferon (1000 units/ml)	2	21.3 $\pm$ 4.6 (6)	3.5 $\pm$ 0.5 (5)	58 $\pm$ 9 (6)	302 $\pm$ 44 (6)
	20	49.9 $\pm$ 4.1 (6)†	10.3 $\pm$ 2.1 (6)*†	266 $\pm$ 31 (6)†	327 $\pm$ 50 (6)
β -Interferon (1000 units/ml) + β -interferon antibody	2	27.3 $\pm$ 2.4 (5)	3.8 $\pm$ 0.9 (5)	53 $\pm$ 7 (5)	296 $\pm$ 43 (5)
	20	59.9 $\pm$ 6.6 (6)†	18.3 $\pm$ 2.4 (6)†	264 $\pm$ 34 (6)†	329 $\pm$ 51 (6)

Table 3. *Effect of α -interferon on the stimulation of (pro)insulin biosynthesis and insulin secretion in mouse islets by various secretagogues*

Islets were incubated for a total of 24 h with 1000 units of α -interferon/ml. Islet function was assayed in the presence of 50 μ Ci of L-[4,5- 3 H]leucine/ml (see the Experimental section) at concentrations of secretagogues said to stimulate the rate of (pro)insulin biosynthesis, in comparison with the basal rate at 2 mM-glucose. Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. Results that significantly differ from the equivalent control with $P < 0.05$ are denoted by *, or $P < 0.01$ denoted by **, and those that differ from the equivalent observation at 2 mM-glucose with $P < 0.01$ are denoted by †.

Secretagogue	Treatment	$10^{-3} \times$ Total protein synthesis (c.p.m./h per μ g of DNA)	$10^{-2} \times$ (Pro)insulin synthesis (c.p.m./h per μ g of DNA)	Insulin secretion (pg/min per μ g of DNA)	Total insulin content (ng/ μ g of DNA)
2 mM-Glucose	Control	24.1 $\pm$ 2.0 (15)	2.9 $\pm$ 0.5 (14)	46 $\pm$ 5 (15)	245 $\pm$ 29 (15)
	+ interferon	22.0 $\pm$ 2.3 (15)	2.8 $\pm$ 0.3 (15)	52 $\pm$ 13 (15)	276 $\pm$ 35 (15)
20 mM-Glucose	Control	52.7 $\pm$ 3.6 (11)†	21.0 $\pm$ 1.8 (10)†	275 $\pm$ 19 (11)†	283 $\pm$ 39 (10)
	+ interferon	41.0 $\pm$ 5.0 (11)†	13.4 $\pm$ 1.6 (10)**†	275 $\pm$ 30 (10)†	256 $\pm$ 42 (10)
1.5 mM-D-Glyceraldehyde	Control	16.8 $\pm$ 1.9 (5)†	8.9 $\pm$ 1.1 (5)†	87 $\pm$ 8 (5)†	249 $\pm$ 59 (5)
	+ interferon	13.1 $\pm$ 1.3 (5)†	6.6 $\pm$ 0.6 (5)*†	103 $\pm$ 7 (5)†	296 $\pm$ 6 (5)
10 mM-Adenosine	Control	21.4 $\pm$ 2.0 (10)	11.2 $\pm$ 1.5 (9)†	205 $\pm$ 48 (10)†	257 $\pm$ 25 (10)
	+ interferon	21.3 $\pm$ 1.3 (10)	7.6 $\pm$ 1.2 (10)*†	238 $\pm$ 28 (10)†	227 $\pm$ 29 (10)
20 mM-Mannose	Control	45.4 $\pm$ 3.4 (5)†	14.8 $\pm$ 2.0 (5)†	241 $\pm$ 38 (5)†	232 $\pm$ 36 (5)
	+ interferon	41.3 $\pm$ 6.0 (5)†	8.7 $\pm$ 1.0 (5)**†	230 $\pm$ 38 (5)†	231 $\pm$ 46 (5)
20 mM-N-Acetylglucosamine	Control	38.2 $\pm$ 5.2 (10)†	12.9 $\pm$ 2.0 (10)†	130 $\pm$ 18 (10)†	282 $\pm$ 40 (10)
	+ interferon	29.2 $\pm$ 4.8 (10)	6.1 $\pm$ 0.9 (10)**†	140 $\pm$ 14 (10)†	253 $\pm$ 31 (10)

Table 4. *Effect of α -interferon on 20mM-leucine stimulation of isolated mouse islets*

Islets were incubated for a total of 24 h with 1000 units of α -interferon/ml. Islet function was assayed in the presence of 80 μ Ci of L-[2,3- 3 H]phenylalanine/ml at 2mM- or 20mM-glucose or 20mM-leucine (see the Experimental section). Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. Results that significantly differ from the equivalent control with $P < 0.01$ are denoted by *, and those that differ from the equivalent observation at 2mM-glucose with $P < 0.01$ are denoted by †.

Secretagogue	Treatment	$10^{-3} \times$ Total protein synthesis (c.p.m./h per μ g of DNA)	$10^{-2} \times$ (Pro)insulin synthesis (c.p.m./h per μ g of DNA)	Insulin secretion (pg/min per μ g of DNA)	Total insulin content (ng/ μ g of DNA)
2mM-Glucose	Control	25.8 $\pm$ 3.6 (10)	5.4 $\pm$ 1.1 (10)	39 $\pm$ 10 (10)	270 $\pm$ 48 (10)
	+ interferon	25.0 $\pm$ 4.1 (10)	3.5 $\pm$ 0.7 (10)	36 $\pm$ 7 (10)	304 $\pm$ 40 (10)
20mM-Glucose	Control	50.2 $\pm$ 6.6 (10)†	24.0 $\pm$ 2.9 (9)†	235 $\pm$ 33 (10)†	257 $\pm$ 39 (10)
	+ interferon	43.7 $\pm$ 5.2 (10)†	16.4 $\pm$ 1.3 (10)*†	225 $\pm$ 43 (10)†	306 $\pm$ 53 (10)
20mM-Leucine	Control	16.4 $\pm$ 2.0 (10)	18.4 $\pm$ 2.0 (10)†	188 $\pm$ 27 (10)†	312 $\pm$ 46 (10)
	+ interferon	14.3 $\pm$ 1.6 (10)	9.0 $\pm$ 0.9 (9)*†	177 $\pm$ 26 (10)†	298 $\pm$ 37 (10)

Table 5. *Effect of dsRNA [poly(I)·poly(C)] on isolated mouse islets*

Islets were incubated for a total of 24 h with concentrations of poly(I)·poly(C) of 0.1–100 μ g/ml. Islet function was assayed at 2mM- and 20mM-glucose in the presence of 20 μ Ci of L-[4,5- 3 H]leucine/ml (see the Experimental section). Mean values ($\pm$ S.E.M.) are shown, together with the numbers of individual determinations in parentheses. Results that significantly differ from the equivalent no-treatment control with $P < 0.01$ are denoted by *, and those that differ from the equivalent observation at 2mM-glucose with $P < 0.01$ are denoted by †.

Treatment (24 h incubation)	Glucose (mM)	$10^{-3} \times$ Total protein synthesis (c.p.m./h per μ g of DNA)	$10^{-2} \times$ (Pro)insulin synthesis (c.p.m./h per μ g of DNA)	Insulin secretion (pg/min per μ g of DNA)
No treatment	2	7.7 $\pm$ 0.8 (15)	3.5 $\pm$ 0.7 (15)	83 $\pm$ 13 (15)
	20	18.5 $\pm$ 1.8 (16)†	14.5 $\pm$ 1.3 (14)†	266 $\pm$ 16 (16)†
Poly(I)·poly(C) (0.1 μ g/ml)	2	9.7 $\pm$ 1.1 (5)	2.5 $\pm$ 0.7 (5)	84 $\pm$ 19 (5)
	20	18.6 $\pm$ 2.5 (6)†	13.1 $\pm$ 1.5 (6)†	231 $\pm$ 15 (6)†
Poly(I)·poly(C) (1.0 μ g/ml)	2	6.3 $\pm$ 1.1 (5)	2.5 $\pm$ 0.8 (4)	67 $\pm$ 13 (5)
	20	16.5 $\pm$ 1.1 (5)†	8.9 $\pm$ 2.2 (4)*†	320 $\pm$ 18 (5)†
Poly(I)·poly(C) (10.0 μ g/ml)	2	6.3 $\pm$ 0.6 (5)	4.1 $\pm$ 0.8 (4)	95 $\pm$ 14 (5)
	20	17.7 $\pm$ 1.3 (4)†	6.8 $\pm$ 1.4 (5)*	248 $\pm$ 27 (5)†
Poly(I)·poly(C) (100.0 μ g/ml)	2	5.8 $\pm$ 0.4 (5)	2.5 $\pm$ 0.6 (5)	134 $\pm$ 34 (5)
	20	9.8 $\pm$ 0.8 (6)*†	5.6 $\pm$ 1.2 (5)*	305 $\pm$ 39 (6)†

of poly(I)·poly(C)/ml. The dsRNA did not significantly alter the rate of insulin release at 2mM- or 20mM-glucose, though the release rate at 2mM-glucose in the presence of 100 μ g of poly(I)·poly(C)/ml was unusually high. These observations may indicate B-cell damage mediated by dsRNA toxicity (Lindsay *et al.*, 1969; Absher & Stinebring, 1969).

Rabbit antiserum to mouse β -interferon had no effect on the inhibition of (pro)insulin biosynthesis by 10 μ g of poly(I)·poly(C)/ml (Table 2). This is unlike the effect of β -interferon, where a similar inhibition is abolished by the antibody. This set of data also shows that poly(I)·poly(C) has no significant effect on total insulin content of the islets.

Discussion

Interferon action in islet (pro)insulin biosynthesis

Glucose stimulation of (pro)insulin biosynthesis in comparison with other islet cell protein has been described previously (Taylor *et al.*, 1964; Howell & Taylor, 1966; Permutt & Kipnis, 1970; Morris & Korner, 1970; Pipeleers *et al.*, 1973).

In the present work, α - and β -interferons have been shown specifically to inhibit glucose-stimulated (pro)insulin biosynthesis (Table 1). As α - and β -interferons may bind to a common cell-surface receptor without exciting co-operative effects (Aguet & Blanchard, 1981) and have similar biological activities (Yabrov, 1980), it is not surprising that they exert parallel effects on isolated islets.

Such an inhibition of an inducible host-cell protein by high concentrations of interferon has been observed in other mammalian systems (Beck *et al.*, 1974; Illinger *et al.*, 1976; Rossi *et al.*, 1977). The concentration of interferon necessary to observe this effect is high in comparison with that required for antiviral activity. However, this study suggests that it is a specific effect of interferon, since its effects on isolated islets could be abolished in the presence of anti-interferon antiserum (Table 2). It has been shown that cell-bound interferon can be displaced in the presence of lectins or antibodies (Chany, 1981).

The antiviral effect of interferon involves the inhibition of new protein synthesis in the cell via interferon-induced enzyme systems (Yabrov, 1980; Kerr *et al.*, 1981; Lengyel, 1982; Lebleu & Content, 1982). In pancreatic islets treated with interferon, it is possible that these interferon-induced enzyme systems are active and may thus inhibit glucose-stimulated (pro)insulin biosynthesis. The short-term stimulation of (pro)insulin biosynthesis by glucose appears to involve translational control, as no net new synthesis of proinsulin mRNA is required (Itoh & Okamoto, 1980). In this instance it has been suggested that there is an increased flux of cytoplasmic free proinsulin mRNA to membrane-bound polyribosomes (the site of proinsulin biosynthesis), at stimulatory concentrations of glucose (Okamoto, 1981). In interferon-treated islets, free mRNA may be degraded and the formation of initiation complexes inhibited, so that the glucose-stimulated translational control of (pro)insulin biosynthesis is lost. The slight decrease in the rate of total protein synthesis observed in interferon-treated islets could be similarly explained.

Stimulation of (pro)insulin biosynthesis by 1.5 mM-D-glyceraldehyde (Jain *et al.*, 1975), 10 mM-adenosine (Andersson, 1978), 20 mM-mannose (Parry & Taylor, 1966; Ashcroft *et al.*, 1978), 20 mM-N-acetylglucosamine (Ashcroft *et al.*, 1976) and 20 mM-leucine (Andersson, 1976) was significantly inhibited in interferon-treated islets in a similar fashion to that of glucose stimulation (Tables 3 and 4), even though the mechanisms for this stimulation of (pro)insulin biosynthesis by these compounds may differ. It is tempting to suppose, however, that the regulation of insulin biosynthesis, even by this diverse group of substances, involves common effects at the translational level.

Action of dsRNA on pancreatic islet cells

Synthetic dsRNA [poly(I)·poly(C)] is able to induce interferon in animal cells, *in vivo* and *in vitro* (Field *et al.*, 1967). Above 1 µg of poly(I)·poly(C)/ml, glucose-stimulated (pro)insulin bio-

synthesis is significantly inhibited (Table 5). This is similar to effects observed for interferon-treated islets. At this concentration of poly(I)·poly(C) significant amounts of interferon are thought to be induced, and it may be that this induced interferon is the primary cause of inhibiting induced (pro)insulin biosynthesis in poly(I)·poly(C)-treated islets. However, this inhibition could not be abolished in the presence of anti-β-interferon antibody in poly(I)·poly(C)-treated islets as it was in β-interferon-treated islets (Table 2). This suggests that either γ- or α-interferon is induced from islets or that poly(I)·poly(C) is acting as a protein-synthesis inhibitor in its own right.

Physiological implications of interferon and dsRNA treatment on pancreatic islets

Interferon inhibition of (pro)insulin biosynthesis may only be of slight physiological significance, for the following reasons. (a) High concentrations of interferon are required for this inhibition, in comparison with much smaller concentrations which produce an antiviral effect. (b) Blood glucose concentrations *in vivo* in normal mice vary between 5–8 mM, but interferon inhibition only occurs above 10 mM-glucose *in vitro* (Fig. 3). It is therefore only likely to occur under hyperglycaemic states *in vivo*. (c) The effect of interferon on the host-cell protein-synthesizing system is only transient (Lengyel, 1982). (d) The inhibition of (pro)insulin biosynthesis is not absolute, and the total insulin content of the islets is not decreased. As insulin release is not affected by interferons, it appears that this much decreased rate of stimulated (pro)insulin biosynthesis in the presence of interferon is still sufficient to maintain a constant supply of insulin for normal release. However, this may not be the case under non-physiological conditions, as in the prolonged hyperglycaemia of mild diabetes.

Paradoxically, it has been shown that pre-treatment with interferons or an interferon inducer inhibits the induction of diabetes by diabetogenic viruses in animals (Gadzick *et al.*, 1982; Yoon *et al.*, 1983; Giron *et al.*, 1983; Vialettes *et al.*, 1983). However, under these circumstances stimulated (pro)insulin biosynthesis may still be transiently inhibited.

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