2-5A-dependent endoribonuclease activity and effects of A2′p5′A2′p5′A (2-5A core) in mouse pancreatic islets

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Glucose-stimulated (pro)insulin biosynthesis is markedly inhibited is mouse pancreatic islets incubated with micromolar concentrations of 2-5A 'core', (A2′p5′A2′p5′A) Total protein synthesis was also reduced, but to a lesser extent, while insulin release and total insulin content of the islets was untouched by 2-5A 'core' Evidence is given for the presence of a 2-5A-dependent endoribonuclease which mediates these effects

Insulin biosynthesis *Oligoadenylate* *Interferon* *Islets of Langerhans* *Endoribonuclease*

1. INTRODUCTION

As is well known, in many cells interferon is capable of inducing at least two enzyme systems involved in setting up an antiviral state in the cell by inhibiting the initiation of protein synthesis [1–4]. One such enzyme system is a dsRNA-dependent oligoadenylate synthetase, which synthesises 2′-5′-linked oligoadenylates (2-5A) from ATP. The major species of 2-5A is the trimer pppA2′p5′A2′p5′A, which acts as a secondary messenger in activating an otherwise latent 2-5A-dependent endoribonuclease which will degrade cytoplasmic free mRNA and rRNA, to halt new protein synthesis in the cell [5–7]. Here, the induction of 2-5A-dependent endoribonuclease by interferon and dsRNA in isolated mouse pancreatic islets has been studied in vitro.

Inhibition of protein synthesis by 2-5A itself, has been observed in cell-free systems [8,9] at nanomolar concentrations, mediated by 2-5A activation of endoribonuclease [10]. 2-5A analogues (co-precipitated with calcium phosphate to facilitate uptake of 2-5A into cells), have also been shown to inhibit protein synthesis [11,12]. However, a non-triphosphorylated analogue of 2-5A, (A2′p5′A2′p5′A), known as 2-5A 'core' at micromolar concentrations has been shown to be taken up by cells in vitro, and to inhibit protein synthesis in non-permeabilised intact cells [13]. This 2-5A 'core' is probably internally phosphorylated in the cell; it then activates the 2-5A-dependent endoribonuclease, which in turn inhibits protein synthesis [14].

In some cells treated with relatively high concentrations of interferon, inhibition of the synthesis of some inducible host cell proteins, including glucose-stimulated (pro)insulin, has been observed [15–19]. In this study the effect of exogenous 2-5A core on isolated islets in vitro has been investigated, to compare its effects with those of interferon [18,19].

2. EXPERIMENTAL

2.1. *Assay of 2-5A endoribonuclease activity in isolated islets*

Pancreatic islets were isolated from fed male DBA/2 mice, 10–12 wk old, under aseptic conditions by collagenase digestion [20]. Groups of 100 islets were suspended in 1 ml of RPMI 1640 tissue culture medium containing glucose (2 mM), penicillin (100 U/ml), streptomycin (0.1 mg/ml) and 10% (v/v) heat inactivated foetal calf serum.

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Islets were incubated in this medium in the presence of either 1000 U/ml β-interferon (Enzo Biochem., New York) or 10 μg/ml poly(I)·poly(C) (PL Biochemicals, Northampton, England) or no additions for control islets, for 24 h, at 37°C in a CO_2-incubator. The islets were then washed in 20 mM Hepes buffer, and the islet cells broken open using a Dounce homogeniser as described in [21] to produce a cytoplasmic extract. 2-5A-Dependent endoribonuclease activity in these islet cell extracts was monitored as described elsewhere [21], over a range of 2-5A trimer (pppA2'p5'A2'p5'A, PL Biochemicals) concentrations. Ribonuclease digestion of [8-^{3}H]poly(A) (Miles Laboratories, Slough, England) was monitored in the presence and absence of 2-5A. The remaining [8-^{3}H]poly(A) after digestion was extracted by oligo(dT) cellulose chromatography, and counted. The ratio of counts in the presence of 2-5A to the counts in the absence of 2-5A was calculated. If this ratio was below unity, 2-5A-dependent endoribonuclease activity was present.

2.2. *Incubation of 2-5A 'core' with isolated islets*

Pancreatic islets were obtained as previously described [20], and washed in bicarbonate buffered medium [22] previously equilibrated with 95:5% O_2:CO_2, and containing glucose (2 mM) and bovine serum albumin (1 mg/ml). Groups of 15 islets were placed in microfuge tubes suspended in 150 μl of this medium containing 2 mM glucose and varying concentrations of 2-5A 'core' (Calbiochem-Behring, Bishops Stortford, England) and incubated at 37°C in a shaking water bath for 30 min, with no 2-5A 'core' for controls. At the end of this incubation, the islets were centrifuged down at 1500 × g for 30 s and the medium removed. The islets were then resuspended in 150 μl of fresh bicarbonate buffered medium, containing the same concentration of 2-5A 'core' as in the previous pre-incubation and varying concentrations of glucose between 2–20 mM. Also present was 0.1 mCi/ml L-[4,5-^{3}H]leucine (Amersham International) to monitor incorporation into protein. The islets were then incubated a second time at 37°C between 15–210 min. After this incubation the islets were centrifuged down and the supernatant removed for assay of insulin released by radioimmunoassay [23]. (Residual [^{3}H]leucine present did not significantly interfere with this assay.) The islets were washed and then disrupted by sonication, in 100 μl of phosphate buffered saline. Aliquots of this sonicate were then assayed for [^{3}H]leucine incorporation into trichloroacetic acid-precipitable protein (total protein) and into immunoextractable (pro)insulin [24]. No attempt was made to distinguish between proinsulin and insulin. Total insulin content [23] and DNA content [25] were also determined in the sonicate. Results are expressed in terms of islet DNA.

Statistically significant differences between groups of results were analysed by Student's t-test.

3. RESULTS

3.1. *2-5A-dependent endoribonuclease activity in isolated islets*

Significant 2-5A-dependent endoribonuclease activity was detected in extracts of interferon

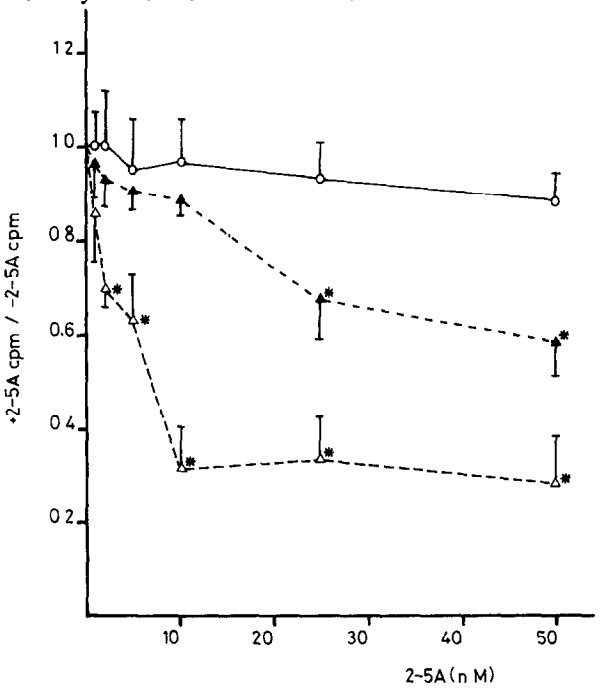

Fig.1. 2-5A-dependent endoribonuclease activity in control islets (○—○); 10 μg/ml poly(I)·poly(C) treated islets (▲---▲); and 1000 U/ml β-interferon treated islets (△---△). Cytoplasmic extracts of the treated islets were made and assayed for 2-5A-dependent endoribonuclease (see section 2). Each point represents the mean ± SE of at least 5 observations. * Significance from the equivalent control with at least $p < 0.05$ 2-5A-dependent endoribonuclease activity is expressed as a ratio of residual radioactivity in tritiated poly A in the presence and absence of 2-5A (see section 2).

treated islets at 2 nM 2-5A concentrations and above (fig.1). This activity appeared to reach a maximum at 10 nM 2-5A. In dsRNA (poly(I)·poly(C)) treated islets significant activity was present at 25 nM 2-5A and above, but not as much as seen in interferon treated islets. Negligible activity was observed in control islets over the range of 2-5A concentrations assayed.

3.2. *Effects of 2-5A 'core' on isolated islets*

At concentrations of 1.0 μM 2-5A 'core' and above, 20 mM glucose-induced (pro)insulin biosynthesis rate was significantly inhibited with no effect on the basal rate at 2 mM-glucose (table 1) At these 2-5A 'core' concentrations, total protein biosynthesis was also reduced, but to a lesser extent than that of glucose-induced (pro)insulin biosynthesis. The rate of insulin release at basal or stimulatory glucose concentrations was unaffected at any concentration of 2-5A 'core' used, as was total insulin content, within the 2 h assay incubation period (table 1).

At stimulatory glucose concentrations (7 mM and above), 10 μM 2-5A 'core' treatment of islets inhibited glucose induced (pro)insulin biosynthesis (fig.2), with no significant effect at basal glucose concentrations (2 and 4 mM). In further experiments (not illustrated), the inhibitory effect was evident in 10 μM 2-5A 'core' treated islets after 15 min exposure to 20 mM glucose and this effect continued for 210 min.

Only a slight reduction in the rate of total protein synthesis in 10 μM 2-5A 'core' treated islets was observed after 60 min at glucose concentrations above 10 mM. However, 2-5A 'core' had no effect on the rate of insulin release at any glucose concentration or incubation time.

4. DISCUSSION

Interferon is able to induce the 2-5A enzyme system [1,4] in isolated islets from DBA/2 male mice, since 2-5A-dependent activity was detectable at nanomolar concentrations of 2-5A. It is uncer-

Table 1

Islet function after exposure to varying concentrations of 2-5A 'core'

Concentration of 2-5A 'core' (μM)	Glucose (mM)	Total protein synthesis (10^3 cpm/μg DNA per h)	(Pro)insulin biosynthesis (10^3 cpm/μg DNA per h)	Insulin release (pg/μg DNA per min)	Total insulin content (ng/μg DNA)
0	2	132.2 ± 11.1 (5)	1.2 ± 0.2 (5)	119.7 ± 22.5 (5)	298.2 ± 41.6 (5)
	20	152.8 ± 19.2 (5)	8 3 ± 1.2 (5)	336 1 ± 29.5 (5)	322.1 ± 48.7 (5)
0.01	2	120.6 ± 13 0 (5)	1.0 ± 0.2 (4)	118.6 ± 17.6 (5)	289.3 ± 30.2 (5)
	20	152.9 ± 6.4 (5)	8.1 ± 1.3 (5)	300.2 ± 23.4 (5)	271.4 ± 36 3 (5)
0 1	2	117.1 ± 7.9 (5)	1.1 ± 0 2 (5)	113 5 ± 15.8 (5)	332.1 ± 48.6 (5)
	20	129.9 ± 12.4 (5)	6.6 ± 1 2 (5)	342.7 ± 40.4 (5)	309.7 ± 38.3 (5)
1 0	2	124.8 ± 7 8 (5)	1.2 ± 0.3 (4)	96.3 ± 16 0 (5)	318 6 ± 42.9 (5)
	20	118.6 ± 11.0 (5)[a]	4.1 ± 1.1 (5)[a]	311.8 ± 51.2 (5)	326.9 ± 50.7 (5)
10 0	2	93.2 ± 5 0 (5)[a]	1.1 ± 0.2 (5)	101.5 ± 8.5 (5)	284.3 ± 36.5 (5)
	20	112 6 ± 9.2 (5)[a]	4.0 ± 0.8 (5)[a]	290.4 ± 31.3 (5)	297.6 ± 30.4 (5)

[a] Significant difference from zero 2-5A 'core' concentration, with at least $p < 0.05$

Islets were pre-incubated with varying concentrations of 2-5A 'core' for 30 min at 2 mM glucose, then assayed for a further 2 h incubation at 2 and 20 mM glucose in the presence of the same concentration of 2-5A 'core'. Total protein synthesis, (pro)insulin biosynthesis, insulin release and total insulin content were determined as decsribed in section 2

The results represent the mean ± SE with the number of observations in parentheses

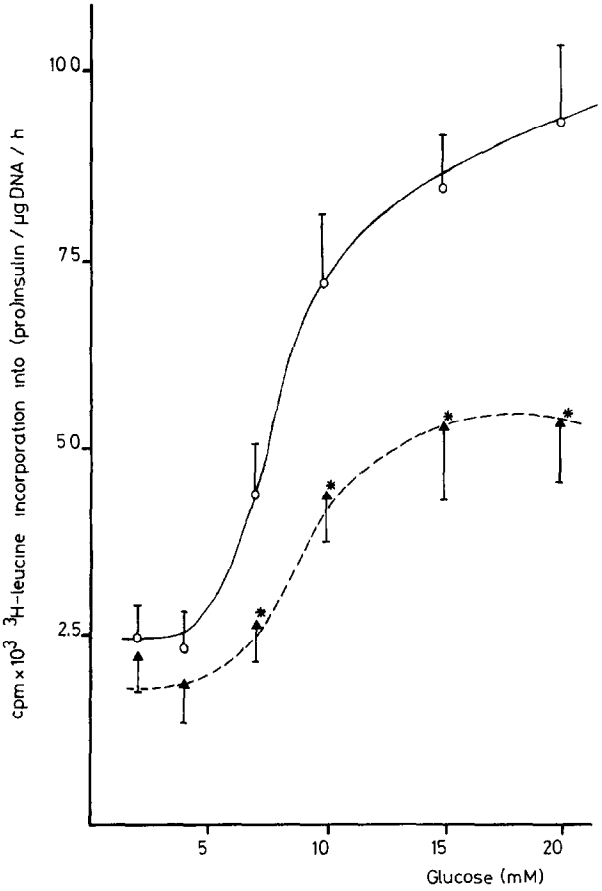

Fig.2. (Pro)insulin biosynthesis in islets incubated with 10 μM 2-5A 'core'. Islets were preincubated with 10 μM 2-5A 'core' for 30 min at 2 mM glucose, then assayed over a 2 h incubation at 2,4,7,10,15 and 20 mM glucose concentrations (see section 2) The results of [^{3}H]leucine incorporation into (pro)insulin represent the mean ± SE of 5 observations. Control islets (○—○); 10 μM 2-5A 'core' treated islets (▲—▲). * Significant difference from the equivalent control, with $p < 0.05$.

tain whether poly(I)·poly(C) induces this enzyme system in its own right, or whether it is inducing islets to produce interferon [26], which in turn is activating the 2-5A enzyme system. The substrate specificity of this 2-5A-dependent endoribonuclease induced in islets in digesting poly(A) is different to that observed in other cells, where only poly(U) was digested [27].

Intact isolated islet cells are able to take up 2-5A 'core' at micromolar concentrations. The 2-5A 'core' alone is unable to activate the 2-5A-dependent endoribonuclease [12], but it has been suggested that once taken up, 2-5A 'core' is phosphorylated and then can activate the endoribonuclease [14]. It has been shown that at any early phase of glucose stimulation of isolated islets, net synthesis of proinsulin-mRNA is not required for the stimulation of (pro)insulin biosynthesis [28,29]. However, there is an increased removal of cytoplasmic free proinsulin-mRNA to membrane-bound polysomes, where proinsulin is actively synthesised [28,29]. In isolated islets treated with exogenous 2-5A 'core', the 2-5A-dependent endoribonuclease may be activated [14]. This would then digest cytoplasmic free proinsulin-mRNA, thereby reducing the glucose stimulation of insulin biosynthesis.

As no new net protein synthesis is required for normal glucose stimulated insulin release [30], 2-5A 'core' or 2-5A-dependent endoribonuclease activity would not directly affect insulin release. This endoribonuclease activity, in digesting RNA slightly inhibits total protein synthesis in 2-5A 'core' treated islets, but this does not significantly lower the total insulin content of the islets within this limited time period.

In some islet cell tissue cultures treated with interferon, the replication of a diabetogenic virus was inhibited [31]. It is most likely that an interferon-induced 2-5A-dependent endoribonuclease activity is involved in this inhibition. Such an activity would degrade viral RNA halting the synthesis of more virus particles, and may be important in the protection against virus-induced diabetes

We have already shown that interferon inhibits insulin synthesis in islets [18,19]. The detection of an endoribonuclease and the effects of 2-5A 'core' suggest that interferon may function through this enzyme.

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