Metabolic Signals Produced by Purine Ribonucleosides Stimulate Proinsulin Biosynthesis and Insulin Secretion

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Inosine, guanosine and adenosine strongly stimulated proinsulin biosynthesis and insulin secretion in isolated mouse pancreatic islets. None of the purine ribonucleosides stimulated insulin secretion in rat islets, although as reported [Jain & Logothetopoulos (1977) *Endocrinology* 100, 923–927] inosine and guanosine, but not adenosine, were potent stimulants of proinsulin biosynthesis in this species. The purine bases had no effect in either species. D-Ribose, which enhanced proinsulin biosynthesis at 0.3 and 0.6 mM but not at 5 mM in rat pancreatic islets [Jain & Logothetopoulos (1977) *Endocrinology* 100, 923–927], produced no secretory signals in rat islets and was without any effect on proinsulin biosynthesis and insulin secretion in mouse islets. The rates of oxidation of ^{14}C -labelled purine ribonucleosides and D-ribose in islets of the two species correlated well with their effectiveness as inducers of insulin secretion and proinsulin biosynthesis. Specific inhibitors of purine ribonucleoside phosphorylase, adenosine deaminase and of purine ribonucleoside transport suppressed the stimulatory effects of nucleosides in pancreatic islets without altering the effect of D-glucose. The same inhibitors also markedly diminished the oxidation rates of the labelled purine ribonucleosides. The experiments clearly indicate that proinsulin biosynthesis and insulin secretion are modulated through metabolic signals and not through interactions of intact substrate molecules with cell receptors.

The molecular mechanisms underlying the stimulation of proinsulin biosynthesis and insulin secretion in pancreatic β -cells by glucose are not yet well understood. Two hypotheses have been advanced to explain the generation of stimulating signals by glucose. According to the 'metabolic' or 'substrate-site' hypothesis (Grodsky *et al.*, 1963; Coore & Randle, 1964; Malaisse *et al.*, 1976), the signals triggering proinsulin biosynthesis and insulin secretion arise from glucose metabolism. On the other hand, according to the 'glucose receptor' or 'intact glucose' model an interaction of glucose with a membrane or cytosol receptor generates the triggering signals (Matschinsky *et al.*, 1972; Ashcroft *et al.*, 1973; Niki *et al.*, 1974). Each of these hypotheses can be supported independently by a great number of experimental findings.

Data on the potent stimulation of insulin secretion and proinsulin biosynthesis by D-glyceraldehyde could not be easily reconciled with the 'intact glucose model' (Ashcroft *et al.*, 1973; Hellman *et al.*, 1974; Jain *et al.*, 1975). Capito & Hedekov (1976) showed that inosine, which effectively maintains glycolysis (Whittam & Ager, 1965) in the erythrocyte,

induced a strong insulin secretion in mouse islets. Furthermore, we (Jain & Logothetopoulos, 1977) have shown that purine ribonucleosides, inosine, guanosine and D-ribose stimulate proinsulin biosynthesis in rat islets. The stimulatory effect of the two nucleosides on proinsulin biosynthesis was nearly abolished by Formycin B, an inhibitor of purine ribonucleoside phosphorylase (EC 2.4.2.1) (Jain & Logothetopoulos, 1977).

The present paper reports further observations of the metabolism of the purine ribonucleosides and D-ribose by the pancreatic islets of the rat and the mouse in relation to their effects on proinsulin biosynthesis and insulin secretion. The experimental results provide substantial support for the metabolic origin of the primary signals initiating proinsulin biosynthesis and insulin secretion.

Materials and Methods

Materials

Collagenase and hyaluronidase were obtained from Worthington Biochemical Co., Freehold, NJ, U.S.A.; crystallized fat-free bovine albumin was from Miles Laboratories; L-[4,5- ^3H]leucine was from New England Nuclear Corp., Boston, MA, U.S.A.; D-[U- ^{14}C]ribose, [U- ^{14}C]inosine and [U- ^{14}C]adeno-

Abbreviation used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

sine were from Amersham/Searle Corp., Oakville, Ont., Canada; rat insulin was a gift from Dr. Schlichtkrull of the Novo Laboratories, Bagsvaerd, Denmark; Formycin B (7-hydroxy-3 β -D-ribofuranosylpyrazolo[4,3-d]pyrimidine) and polymerized calf thymus DNA were obtained from Sigma, St. Louis, MO, U.S.A.; Coformycin {3 β -D-ribofuranosyl-6,7,8-trihydroimidazo[4,5-d]-[1,3]-diazepin-8(R)-ol} was generously supplied by Professor H. Umezawa of the Institute of Microbial Chemistry, Tokyo, Japan; *p*-nitrobenzylthioguanosine {2-amino-6-[(4-nitrobenzyl)thio]-9 β -D-ribofuranosylpurine} was supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute Bethesda, MD, U.S.A. Heat-inactivated foetal calf serum, sodium penicillin G, kanamycin and fungizone were obtained from the Grand Island Biological Co., New York, NY, U.S.A.

Preparation of pancreatic islets

Adult male Wistar rats and adult female mice of the Swiss strain were used. Animals with free access to food were killed by decapitation. The islets were isolated by a modification (Jain & Logothetopoulos, 1977) of the collagenase method of Lacy & Kostianovsky (1967). Four to five pancreases were processed simultaneously.

Proinsulin biosynthesis

The methods used for the measurement of the rate of proinsulin biosynthesis were identical with those reported in detail in our previous publication (Jain & Logothetopoulos, 1977).

Insulin secretion

A novel approach was used for the study of insulin secretion. This technique has advantages over standard measurements of secreted insulin by immunoassay, e.g.: (i) a low basal (without any substrate) secretion rate; (ii) steeper differences with increasing concentrations of glucose; (iii) low standard deviations within groups of islets exposed to the same secretory stimulus. After isolation, pancreatic islets were incubated in a 60mm $\times$ 15mm Falcon plastic Petri dish in a water-saturated atmosphere of air/CO₂ (19:1) at 37°C for 12–14 h in 4 ml of F12K tissue-culture medium (Kaighn, 1973) containing 10mM-D-glucose, 12.5% (v/v) foetal calf serum, fungizone (6.25 μ g/ml), kanamycin (100 μ g/ml) and penicillin sulphate (35 μ g/ml). The islets were then incubated for 150 min in a leucine-free F12K medium to which was added [³H]leucine, 10mM-glucose, 1mg of albumin/ml and the above-mentioned antibiotics. The radioactive medium was removed completely and the islets were washed with 3 $\times$ 6 ml of cold Krebs–Ringer bicarbonate buffer (Umbreit *et al.*, 1964), pH 7.4, containing 3mM-leucine. Groups of eight to ten islets were then placed in individual

tubes. Five to seven tubes were randomly selected for a particular treatment. Islets were incubated in 0.3 ml of Krebs–Ringer bicarbonate buffer containing 10mM-Hepes, 5mg of albumin/ml and the substrate to be tested under continuous gassing with O₂/CO₂ (19:1) in a Dubnoff metabolic shaker for 90 min. At the end of this period the medium was separated from the islets and transferred directly to a tube containing anti-insulin γ -globulin (guinea pig) in 0.1M-sodium phosphate buffer, pH 7.5, with 0.05M-NaCl, 0.05% sodium dodecyl sulphate and 0.5% Triton X-100. The islets were extracted with 1 ml of cold acid alcohol for 48 h at 4°C. Labelled proinsulin and insulin in the medium and in the extract were measured quantitatively by a double-immunoprecipitation technique (Zucker & Logothetopoulos, 1975).

Oxidation of labelled substrates

The amount of ¹⁴CO₂ released from ¹⁴C-labelled substrates was measured by a specially adapted radiospirometric device (Cole & Logothetopoulos, 1974).

Expression of results

In the experiments involving insulin biosynthesis the volume of each islet was measured by a method described earlier (Jain & Logothetopoulos, 1977). Incorporation of [³H]leucine was expressed per 10⁷ μ m³ of islet volume. This volume corresponds approximately to a medium-sized rat islet or to a large-sized mouse islet. In the experiments on insulin secretion the secretory response (immunoprecipitable radioactivity in the medium) was expressed as a percentage of the immunoprecipitable radioactivity present in the islets and the medium at the end of the incubation. The output of ¹⁴CO₂ was expressed per μ g of DNA. DNA was measured by the Perman (1975) method, with highly polymerized calf thymus DNA as a standard.

Results

Table 1 shows the effects of inosine, guanosine, adenosine, D-ribose and D-glucose on the incorporation of [³H]leucine into proinsulin and insulin by mouse pancreatic islets. The effects of purine ribonucleosides and D-ribose on proinsulin biosynthesis in rat islets have already been reported (Jain & Logothetopoulos, 1977). Both mouse and rat islets responded to concentrations of nucleosides that were lower than the threshold for glucose. Nearly maximal effects were reached at 2.5 mM. With mouse islets all three ribose nucleosides were equally effective, and responses at 20 mM were close to those obtained with glucose. D-Ribose had no stimulatory effect on mouse islets, but, as reported previously

Table 1. *Effects of purine nucleosides, purine bases, D-ribose and D-glucose on proinsulin biosynthesis in pancreatic islets of the mouse*

Eight to twelve pancreatic islets were incubated for 45 min in buffer containing [^3H]leucine in the presence of various compounds. Labelled proinsulin and insulin was extracted from the islets and quantitatively precipitated by a double-antibody procedure (for experimental details see Jain & Logothetopoulos, 1977). Results are given as the means $\pm$ S.E.M. from six replicates.

Compounds	Concn. in medium (mM)	...	$10^{-1} \times [^3\text{H}]\text{Leucine incorporated into proinsulin and insulin}$ (c.p.m./ $10^7 \mu\text{m}^3$ of islet volume)				
			0.0	0.3	0.6	2.5	20
Adenosine			26 $\pm$ 2	172 $\pm$ 9	288 $\pm$ 10	481 $\pm$ 31	577 $\pm$ 19
Guanosine			26 $\pm$ 2	178 $\pm$ 9	314 $\pm$ 10	615 $\pm$ 27	—
Inosine			26 $\pm$ 1	201 $\pm$ 3	485 $\pm$ 32	525 $\pm$ 27	580 $\pm$ 21
D-Ribose			16 $\pm$ 2	16 $\pm$ 1	19 $\pm$ 2	16 $\pm$ 2	19 $\pm$ 2
Adenine			19 $\pm$ 1	21 $\pm$ 1	—	20 $\pm$ 1	—
Guanine			19 $\pm$ 1	20 $\pm$ 1	—	21 $\pm$ 1	—
Hypoxanthine			17 $\pm$ 4	29 $\pm$ 2	—	20 $\pm$ 1	—
D-Glucose			21 $\pm$ 2	22 $\pm$ 2	22 $\pm$ 1	60 $\pm$ 3	591 $\pm$ 22

Table 2. *Effects of purine ribonucleosides, purine bases, D-ribose and D-glucose on the release of [^3H]proinsulin + [^3H]insulin from prelabelled pancreatic islets*

Results are given as the means $\pm$ S.E.M. from six replicates. See the Materials and Methods section for details.

Compound	Species	Concn. (mM)	...	$[^3\text{H}]\text{Proinsulin} + [^3\text{H}]\text{insulin released (\% / 90 min)}$				
				0.0	0.3	1.25	2.5	20
Adenosine	Mouse			1.1 $\pm$ 0.2	3.1 $\pm$ 0.1	5.9 $\pm$ 0.2	9.2 $\pm$ 0.8	9.0 $\pm$ 0.9
	Rat			1.5 $\pm$ 0.1	1.6 $\pm$ 0.2	1.6 $\pm$ 0.1	1.6 $\pm$ 0.1	1.4 $\pm$ 0.1
Guanosine	Mouse			1.1 $\pm$ 0.1	2.4 $\pm$ 0.1	6.3 $\pm$ 0.1	9.1 $\pm$ 0.4	—
	Rat			1.2 $\pm$ 0.1	1.4 $\pm$ 0.1	1.5 $\pm$ 0.1	2.4 $\pm$ 0.1	—
Adenine	Mouse			0.8 $\pm$ 0.3	0.9 $\pm$ 0.1	1.0 $\pm$ 0.2	—	—
	Rat			1.1 $\pm$ 0.1	1.2 $\pm$ 0.2	1.2 $\pm$ 0.1	—	—
Guanine	Mouse			0.8 $\pm$ 0.3	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	—	—
	Rat			1.1 $\pm$ 0.1	1.1 $\pm$ 0.1	1.1 $\pm$ 0.2	—	—
Hypoxanthine	Mouse			1.0 $\pm$ 0.1	1.5 $\pm$ 0.1	1.5 $\pm$ 0.2	—	—
	Rat			1.2 $\pm$ 0.1	1.5 $\pm$ 0.2	1.6 $\pm$ 0.2	—	—
D-Ribose	Mouse			1.2 $\pm$ 0.1	1.2 $\pm$ 0.1	1.0 $\pm$ 0.1	—	1.2 $\pm$ 0.1
	Rat			1.1 $\pm$ 0.1	1.5 $\pm$ 0.1	1.0 $\pm$ 0.1	—	1.0 $\pm$ 0.1
D-Glucose	Mouse			0.7 $\pm$ 0.1	—	—	1.0 $\pm$ 0.1	9.3 $\pm$ 0.4
	Rat			1.2 $\pm$ 0.1	—	—	1.3 $\pm$ 0.1	20.1 $\pm$ 1.2

(Jain & Logothetopoulos, 1977), produced a strong stimulatory effect at 0.3 and 0.6 mM on rat islets, which declined to basal values at high concentrations. The corresponding free purine bases exerted no effect on proinsulin biosynthesis, in either rat or mouse islets. As previously reported (Jain & Logothetopoulos, 1977), the effect of nucleosides was highly specific for proinsulin biosynthesis, since incorporation of [^3H]leucine into other islet proteins was not significantly stimulated.

The secretory responses of mouse and rat islets to purine ribonucleosides are shown in Table 2 and Fig. 1. All three nucleosides produced strong secretory responses in mouse islets at concentrations that were much lower than the threshold for glucose. Maximal secretory responses obtained with mouse islets were close to those obtained with glucose.

D-Ribose was, however, completely ineffective. All three purine nucleosides and D-ribose did not produce a significant secretory response in rat islets (Table 2 and Fig. 1). The free purine bases exerted no stimulatory effect on insulin secretion in rat or mouse islets (Table 2).

The effects of inhibitors of purine nucleoside phosphorylase (Formycin B) (Sheen *et al.*, 1968), of adenosine deaminase (Coformycin) (Sawa *et al.*, 1967) and of nucleoside transport *p*-nitrobenzylthioguanosine (Paterson & Oliver, 1971) on proinsulin biosynthesis and insulin secretion induced by purine ribonucleosides or glucose are given in Tables 3, 4 and 5. *p*-Nitrobenzylthioguanosine abolished the stimulatory effects of all three nucleosides (Table 3). Formycin B inhibited the effects of inosine and adenosine, whereas Coformycin abolished only the

Fig. 1. Effect of inosine on release of $[^3\text{H}]$ proinsulin + $[^3\text{H}]$ insulin

Results are given as means $\pm$ S.E.M. for six replicates. See the Materials and Methods section for details. $\square$, Rat; $\blacksquare$, mouse.

Table 3. Effects of $20\mu\text{M}$ -*p*-nitrobenzylthioguanosine on proinsulin biosynthesis and on $[^3\text{H}]$ proinsulin + $[^3\text{H}]$ insulin release induced by adenosine (0.6 mM), guanosine (0.6 mM), inosine (0.6 mM) or D-glucose (20 mM)

Results are given as the means $\pm$ S.E.M. from six replicates. See the section on Materials and Methods and the legend of Table 2 for details.

Compound	P Nitro- benzyl- thiogua- nosine	$10^{-1} \times [^3\text{H}]$ Leucine incorporated into proinsulin and insulin (c.p.m./ $10^7\mu\text{m}^3$ of islet volume)		$[^3\text{H}]$ Pro- insulin + $[^3\text{H}]$ insulin released (%/90 min) Mouse
		Rat	Mouse	
Adenosine	—		209 ± 23	5.7 ± 0.6
	+		36 ± 2	1.5 ± 0.2
Guanosine	—	140 ± 5	205 ± 19	6.3 ± 0.1
	+	37 ± 2	29 ± 5	1.4 ± 0.1
Inosine	—	150 ± 18	220 ± 16	5.3 ± 0.3
	+	53 ± 3	31 ± 4	1.6 ± 0.1
D-Glucose	—	639 ± 34	554 ± 24	11.6 ± 0.5
	+	712 ± 30	585 ± 20	14.8 ± 0.6
<i>p</i> -Nitrobenzyl- thioguanosine	+	24 ± 2	28 ± 4	1.4 ± 0.1

effects of adenosine (Table 5). The inhibitors of nucleoside metabolism or transport did not affect the functional stimulation of rat and mouse pancreatic islets by glucose (Tables 3, 4 and 5). D-Manno-

Table 4. Effects of Formycin B (4.5 mM) on proinsulin biosynthesis and on $[^3\text{H}]$ proinsulin + $[^3\text{H}]$ insulin release induced by adenosine (0.6 mM), inosine (0.6 mM) or D-glucose in (20 mM) pancreatic islets of the mouse. Results are given as the means $\pm$ S.E.M. from six replicates. See the Materials and Methods section and the legend of Table 1 for details.

Compound	Formycin B	$10^{-1} \times [^3\text{H}]$ Leu- cine incorpo- rated into pro- insulin and insulin (c.p.m./ $10^7\mu\text{m}^3$ of islet volume)	$[^3\text{H}]$ Proinsulin + $[^3\text{H}]$ insulin released (%/90 min)
Adenosine	—	291 ± 15	5.7 ± 0.6
	+	185 ± 14	0.9 ± 0.1
Inosine	—	320 ± 10	7.5 ± 0.1
	+	198 ± 11	1.1 ± 0.1
D-Glucose	—	525 ± 24	9.8 ± 0.2
	+	568 ± 25	13.2 ± 1.2
—	—	23 ± 4	0.9 ± 0.1
	+	25 ± 3	1.0 ± 0.2

Table 5. Effects of Coformycin (2 $\mu\text{g}/\text{ml}$) on proinsulin biosynthesis and on $[^3\text{H}]$ insulin release induced by adenosine (0.6 mM), inosine (0.6 mM) or D-glucose (20 mM) in the pancreatic islets of the mouse

Results are given as the means $\pm$ S.E.M. from six replicates. See the Materials and Methods section and the legend of Table 1 for details.

Compound	Cofor- mycin	$10^{-1} \times [^3\text{H}]$ Leu- cine incorpo- rated into pro- insulin and insulin (c.p.m./ $10^7\mu\text{m}^3$ of islet volume)	$[^3\text{H}]$ Proinsulin + $[^3\text{H}]$ insulin released (%/90 min)
Adenosine	—	297 ± 14	5.7 ± 0.1
	+	38 ± 4	1.5 ± 0.2
Inosine	—	204 ± 11	5.3 ± 0.3
	+	217 ± 12	6.6 ± 0.2
D-Glucose	—	526 ± 41	11.6 ± 0.5
	+	574 ± 27	14.8 ± 0.8

heptulose did not inhibit the stimulatory effects of purine ribonucleosides and D-ribose (Table 6).

Fig. 2 shows the rates of decarboxylation of $[\text{U}-^{14}\text{C}]$ inosine, $[\text{U}-^{14}\text{C}]$ adenosine and D- $[\text{U}-^{14}\text{C}]$ -ribose by mouse and rat islets. $[\text{U}-^{14}\text{C}]$ inosine was metabolized by mouse islets 2–3 times as fast as by rat islets. The rate of $[\text{U}-^{14}\text{C}]$ adenosine decarboxylation by mouse islets was 5 times that obtained with rat islets. Conversely, rat islets metabolized D- $[\text{U}-^{14}\text{C}]$ -ribose (0.6 mM) at a rate 3 times that of mouse islets.

Ribose at 5 mM inhibited its own metabolism in rat islets. One-third as much CO_2 was released when

rat islets were incubated with 5 mM-D-ribose as with 0.6 mM (Fig. 2).

Table 7 shows the effects of metabolic and transport inhibitors of purine nucleosides on the rate of oxidation of [U-¹⁴C]inosine and [U-¹⁴C]adenosine. Coformycin, the inhibitor of adenosine deaminase, affected the oxidation of [¹⁴C]adenosine but not of inosine. Formycin B, the inhibitor of nucleoside phosphorylase, and the transport inhibitor *p*-nitrobenzylthioguanosine inhibited the oxidation of inosine. *p*-Nitrobenzylthioguanosine had a far stronger effect than Formycin B.

Discussion

Our experiments revealed great differences in the responses of rat and mouse pancreatic islets to purine

ribonucleosides and to D-ribose. The metabolism of these compounds by the islets of these species also showed marked quantitative differences. The high rates of decarboxylation of inosine by mouse and rat islets and of adenosine by mouse islets indicate that pancreatic-islet cells possess transport systems for the nucleosides and enzymes for their catabolism. The presence of nucleoside phosphorylase (EC 2.4.2.1) in pancreatic islets of the mouse has been reported by Capito & Hedekov (1976). This enzyme in mammalian tissues has a higher intrinsic activity for inosine and guanosine than for adenosine (Zimmerman *et al.*, 1971). Adenosine has to be deaminated by adenosine deaminase (EC 3.5.4.4) before being split by the phosphorylase. Mouse

Table 6. Effect of D-mannoheptulose (10 mM) on proinsulin biosynthesis and release of [³H]proinsulin + [³H]insulin induced by adenosine (1.5 mM), inosine (1.5 mM) or D-glucose (20 mM) in the pancreatic islets of the mouse. Results are given as the means ± S.E.M. from six replicates. See the Materials and Methods section and the legend of Table 1 for details.

Compound	D-Mannoheptulose	10 ⁻¹ × [³ H]Leucine incorporated into proinsulin and insulin + [³ H]insulin released (c.p.m./10 ⁷ μm ³ of islet volume)	
		[³ H]Proinsulin released (%/90 min)	
Adenosine	—	286 ± 11	7.5 ± 0.1
	+	299 ± 20	7.8 ± 0.2
Inosine	—	297 ± 18	8.2 ± 0.7
	+	316 ± 21	9.2 ± 0.5
D-Glucose	—	520 ± 26	9.8 ± 0.2
	+	75 ± 10	1.2 ± 0.1
—	+	30 ± 2	1.8 ± 0.1

Fig. 2. Production of ¹⁴CO₂ from D-[U-¹⁴C]ribose, [U-¹⁴C]inosine and [U-¹⁴C]adenosine by rat and mouse islets. The labelled substrates used are as follows: D-[U-¹⁴C]ribose 0.6 mM (A); D-[U-¹⁴C]ribose 5.0 mM (B); [U-¹⁴C]inosine (C); [U-¹⁴C]adenosine (D). See the section on Materials and Methods for details. Results are given as the means ± S.E.M. from six replicates. ···, Rat; ▨, mouse.

Table 7. Effects of Formycin B (4.5 mM), Coformycin (2 μg/ml) and *p*-nitrobenzylthioguanosine (20 μM) on CO₂ production from [U-¹⁴C]adenosine and [U-¹⁴C]inosine in pancreatic islets of the rat and the mouse. Results are given as the means ± S.E.M. from six replicates. For details, see the Materials and Methods section.

Concentration (mm) of:		Presence of:			CO ₂ formation (pmol of substrate/h per μg of DNA)	
Inosine	Adenosine	Formycin B	Coformycin	<i>p</i> -Nitrobenzylthioguanosine	Mouse	Rat
1.5	—	—	—	—	403 ± 24	185 ± 11
1.5	—	+	—	—	265 ± 18	95 ± 8
—	2.0	—	—	—	300 ± 12	—
—	2.0	—	+	—	26 ± 3	—
1.5	—	—	—	—	408 ± 15	—
1.5	—	—	+	—	424 ± 22	—
1.5	—	—	—	—	415 ± 12	180 ± 8
1.5	—	—	—	+	49 ± 6	57 ± 14

pancreatic islets probably have a high activity of adenosine deaminase or a much more effective transport system for adenosine than rat islets. Coformycin, an inhibitor of adenosine deaminase, strongly inhibited the rate of oxidation of adenosine in mouse islets without affecting the oxidation of inosine.

The nucleosides are split by nucleoside phosphorylase into purine base and 1-phosphoribose. The latter can enter pathways of carbohydrate metabolism after conversion into 5-phosphoribose by phosphoribose mutase. The presence of this enzyme in pancreatic islets remains to be shown. The nucleosides generated strong signals, stimulating proinsulin biosynthesis and insulin secretion in mouse islets and proinsulin biosynthesis in rat islets. Several facts suggest that these signals arise from further metabolism of the phosphoriboses and are not due to the interaction of intact nucleosides with membrane or cytosol receptors. (a) Purine bases had no effect. (b) The responses to purine ribonucleosides were nearly completely inhibited by inhibitors of nucleoside metabolism or nucleoside transport. (c) D-Ribose in the rat produced a strong stimulation of proinsulin biosynthesis at 0.6mM but not at 5mM. (d) D-Mannoheptulose did not inhibit the stimulatory effect of purine ribonucleosides. By way of objection to the above conclusions, the hypothesis may be advanced that free glucose arises from the metabolism of phosphoribose moieties within the β -cells. The generation of functional signals is then the result of glucose interactions with cell receptors. This view cannot be defended, because, as Capito & Hedeskov (1976) have shown, glucose that appeared in mouse islets incubated in inosine reached a concentration of only 10 μ M. The threshold for glucose stimulation is, however, 3–4mM, and glucose equilibrates across the β -cell membrane within minutes (Idahl & Hellman, 1968; Matschinsky & Ellerman, 1968).

The oxidation experiments with 14 C-labelled substrates provided explanations of the different response patterns to purine nucleosides and D-ribose obtained with pancreatic islets of the two species and also of the paradoxical effect of D-ribose on proinsulin biosynthesis in rat islets. Furthermore they strongly supported the evidence that secretory and biosynthetic signals in the β -cell are of metabolic origin. An ineffective stimulation of insulin secretion or biosynthesis by a given nucleoside or ribose was reflected in the low rate of oxidation of the compound. The islets of the mouse oxidized adenosine 6 times as fast and D-ribose one-third as fast as rat islets. Consistent with the metabolic origin of the signals, adenosine strongly stimulated proinsulin biosynthesis and insulin secretion in mouse islets but had no effect in rat islets. On the other hand, D-ribose stimulated proinsulin biosynthesis in rat islets but not in mouse islets.

The paradoxical pattern of stimulation of proinsulin biosynthesis by D-ribose could also be explained on the basis of metabolic flux. At 0.6mM-ribose the rate of decarboxylation of D- 14 C]ribose was 3 times that at 5mM (Fig. 2). D-Ribose at 0.6mM maximally stimulated proinsulin biosynthesis, but at 5mM was ineffective as a stimulus (Jain & Logothetopoulos, 1977). It should be mentioned that in erythrocytes a high concentration of ribose inhibits its own metabolism (Lachhein & Mathies, 1959; Kim & McManus, 1971).

Further strong support for the metabolic model for the origin of the triggering primary signals was supplied by the experiments with the inhibitors of purine nucleoside phosphorylase, adenosine deaminase and the inhibitor of purine nucleoside transport. *p*-Nitrobenzylthioguanosine almost completely abolished the effects of the nucleosides on proinsulin biosynthesis and insulin secretion (Table 3). Coformycin, the inhibitor of adenosine deaminase, abolished the effect of adenosine, but not of inosine (Table 5). Formycin B, the inhibitor of purine nucleoside phosphorylase, suppressed the effects of the nucleosides on proinsulin biosynthesis in rat and mouse islets and insulin secretion in mouse islets. A toxic effect of the three inhibitors is excluded, as they did not diminish the effect of glucose on proinsulin biosynthesis and secretion (Tables 3, 4 and 5). The rates of oxidation of 14 C]inosine and 14 C]adenosine were affected by the inhibitors (Table 7).

Our experiments do not permit a definite conclusion about the metabolic intermediates that would constitute metabolic signals. 5-Phosphoribose through the later enzymic steps of the hexose monophosphate shunt will give rise to fructose 6-phosphate, fructose 1,6-bisphosphate and glyceraldehyde 3-phosphate. Concentrations of many glycolytic intermediates can be expected to change. Any of these intermediates could be triggering signals for stimulating biosynthesis and insulin secretion. Pyruvate has been shown by many researchers to be ineffective in stimulating insulin biosynthesis or secretion (Grodsky *et al.*, 1963; Coore & Randle, 1964; Kilo *et al.*, 1967; Lin & Haist, 1969). Tricarboxylic acid-cycle intermediates and pyruvate itself appear to be unlikely candidates. The effectiveness of nucleosides in inducing proinsulin biosynthesis and insulin secretion leads us to conclude that the signals arising from glucose, the physiological stimulus for β -cells, are probably of a similar nature. An intracellular metabolic signal should not be considered incompatible with the well-documented changes of β -cell membrane activity and permeability on exposure to glucose (Dean & Matthews, 1970; Malaisse *et al.*, 1973; Bukowiecki & Freinkel, 1976). Plasmalemmal modifications can be induced from the cell interior as well as from the cell exterior.

The identification of the metabolic signals and

their coupling to proinsulin biosynthesis and insulin secretion remain intriguing problems to solve. The availability of substrates, such as the purine ribonucleosides which strongly stimulate β -cells but enter metabolic pathways through initial enzymic steps different from those for glucose, will undoubtedly help in the solution of the problem.

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References

- Ashcroft, S. J. H., Weerasinghe, L. C. C. & Randle, P. J. (1973) *Biochem. J.* **132**, 223–231
- Bukowiecki, L. & Freinkel, N. (1976) *Biochim. Biophys. Acta* **436**, 190–198
- Capito, K. & Hedekov, C. J. (1976) *Biochem. J.* **158**, 335–340
- Cole, E. H. & Logothetopoulos, J. (1974) *Diabetes* **23**, 469–473
- Coore, H. G. & Randle, P. J. (1964) *Biochem. J.* **93**, 66–78
- Dean, P. M. & Matthews, E. K. (1970) *J. Physiol. (London)* **210**, 225–264
- Grodsky, G. M., Batts, A. A., Bennett, L. L., Voella, C., McWilliams, N. B. & Smith, D. (1963) *Am. J. Physiol.* **205**, 638–644
- Hellman, B., Idahl, L. A., Lernmark, A., Sehlin, J. & Taljedal, I. B. (1974) *Arch. Biochem. Biophys.* **162**, 448–457
- Idahl, L. A. & Hellman, B. (1968) *Acta Endocrinol.* **59**, 479–486
- Jain, K. & Logothetopoulos, J. (1977) *Endocrinology* **100**, 923–927
- Jain, K., Logothetopoulos, J. & Zucker, P. (1975) *Biochim. Biophys. Acta* **399**, 384–394
- Kaighn, M. E. (1973) in *Tissue Culture: Methods and Applications*, (Kruse, P. F., Jr. & Patterson, M. K., Jr., eds.), pp. 54–58, Academic Press, New York
- Kilo, C., Devrim, S., Bailey, R. & Recant, L. (1967) *Diabetes* **16**, 377–385
- Kim, H. D. & McManus, T. J. (1971) *Biochim. Biophys. Acta* **230**, 12–19
- Lachhein, L. & Mathies, H. (1959) *Acta Biol. Med. Germ.* **2**, 446–450
- Lacy, P. E. & Kostianovsky, M. (1967) *Diabetes* **16**, 35–39
- Lin, B. J. & Haist, R. E. (1969) *Can. J. Physiol. Pharmacol.* **47**, 791–801
- Malaisse, W. J., Brisson, G. R. & Baird, L. E. (1973) *Am. J. Physiol.* **244**, 389–394
- Malaisse, W. J., Sener, A. & Levy, J. (1976) *J. Biol. Chem.* **251**, 1731–1737
- Matschinsky, F. M. & Ellerman, J. (1968) *J. Biol. Chem.* **243**, 2730–2736
- Matschinsky, F. M., Landgraf, R., Ellerman, J. & Kotler-Grajburg, J. (1972) *Diabetes* **21**, 555–569
- Niki, A., Niki, M., Miwa, I. & Okuda, J. (1974) *Science* **186**, 150–151
- Paterson, A. R. P. & Oliver, J. M. (1971) *Can. J. Biochem.* **49**, 271–274
- Perman, A. U. (1975) *J. Histochem. Cytochem.* **23**, 187–193
- Sawa, T., Fukagawa, Y., Homma, I., Takeuchi, T. & Umezawa, H. (1967) *J. Antibiot. Ser. A* **20**, 227–231
- Sheen, M. R., Kim, B. K. & Parks, R. E., Jr. (1968) *Mol. Pharmacol.* **4**, 293–299
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964) *Manometric Techniques*, 4th edn., p. 132, Burgess Publishing Co., Minneapolis
- Whittam, R. & Ager, M. E. (1965) *Biochem. J.* **97**, 214–227
- Zimmerman, T. P., Gersten, N. B., Ross, A. F. & Miech, R. P. (1971) *Can. J. Biochem.* **49**, 1050–1054
- Zucker, P. & Logothetopoulos, J. (1975) *Diabetes* **24**, 194–200