Stimulation of Proinsulin Biosynthesis and Insulin Release by Pyruvate and Lactate

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Increasing concentrations of pyruvate failed to stimulate proinsulin biosynthesis and insulin release in freshly isolated islets. Glycolytic flux ($^3\text{H}_2\text{O}$ from $[5\text{-}^3\text{H}]\text{glucose}$) decreased by 80–85%, but decarboxylation of $[1\text{-}^{14}\text{C}]\text{pyruvate}$ was unaffected in islets tested immediately after alloxan exposure. This strongly suggested that in freshly isolated islets, β -cells, in relation to other islet cells, hardly contribute to the decarboxylation of pyruvate. Non-alloxan-treated cultured islets decarboxylated 2–2.5 times as much pyruvate as did alloxan-treated islets cultured for 15–18 h. Thus the contribution of β -cells to the metabolism of pyruvate after culturing markedly increased. Concomitantly β -cells became responsive to pyruvate. At 20 mM-pyruvate, release of prelabelled proinsulin and insulin and incorporation of $[^3\text{H}]\text{leucine}$ into proinsulin reached values approximately half of those obtained with 20 mM-glucose. Lactate was as effective as pyruvate in inducing responses in cultured islets. The experiments indicate that a critical degree of substrate utilization is necessary for the generation of signals for insulin release and proinsulin biosynthesis.

The molecular mechanism by which pancreatic cells recognize glucose and respond with increased proinsulin biosynthesis and insulin secretion are not yet well understood. The correlations that have been demonstrated between the rate of glucose metabolism and the rates of insulin release and proinsulin biosynthesis strongly support the hypothesis that intermediates of glycolysis are important primary signals for β -cell activation (Grodsky *et al.*, 1963; Coore & Randle, 1964; Ashcroft *et al.*, 1970; Lin & Haist, 1971). Models have also been proposed or discussed (Cerasi & Luft, 1970; Matschinsky *et al.*, 1972; Matchinsky & Ellerman, 1973) which imply primary interactions of intact glucose molecules with cell receptors triggering activation of the β -cell. The activation of β -cell functions would in turn increase the rate of glucose metabolism. The experimental findings that D-glyceraldehyde and purine nucleosides stimulate proinsulin biosynthesis and insulin secretion (Ashcroft *et al.*, 1973; Hellman *et al.*, 1974; Jain *et al.*, 1975; Capito & Hedeskov, 1976; Jain & Logothetopoulos, 1977, 1978) strongly reinforced the view that the triggering signals for proinsulin biosynthesis and insulin secretion are of metabolic origin. In the search for the crucial glycolytic intermediates which would fulfil the role of triggering signals, our conceptual models have been influenced by the experimental fact that pyruvate, in spite of being relatively well metabolized by pancreatic islets (Humbel & Renold, 1963; Lin & Haist, 1971;

Hedeskov *et al.*, 1972), does not induce insulin release (Grodsky *et al.*, 1963; Coore & Randle, 1964; Burr *et al.*, 1970; Georg *et al.*, 1971) or stimulate proinsulin biosynthesis (Lin & Haist, 1971; Parry & Taylor, 1974).

We report in the present paper experiments that indicate that pyruvate may not in fact be an effective metabolic substrate of the β -cells of freshly isolated pancreatic islets of the rat. After a short period (15–18 h) of culturing these islets in an enriched medium, however, pyruvate metabolism is enhanced and β -cells respond to pyruvate with increased rates of insulin release and proinsulin biosynthesis.

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, Elkhart, IN, U.S.A.; D-[5- ^3H]glucose, [1- ^{14}C]pyruvic acid (sodium salt), $\text{NaH}^{14}\text{CO}_3$, PPO (2,5-diphenyloxazole) and POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] were from Amersham-Searle Corp., Don Mills, Ont., Canada; L-[4,5- ^3H]leucine, L-[4,5- ^3H]lysine, $^3\text{H}_2\text{O}$, Aquasol and Hyamine were from New England Nuclear Corp., Boston, MA, U.S.A.; Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and all ingredients

of the F12K tissue culture medium were obtained from Calbiochem, La Jolla, CA, U.S.A.; heat-inactivated foetal calf serum and sodium penicillin G were obtained from Grand Island Biological Co., New York, NY, U.S.A.; xylene, Triton X-100, Triton X-114, L(+)-lactic acid and sodium pyruvate were from Sigma, St. Louis, MO, U.S.A.; guinea-pig anti-insulin antibodies and rabbit anti-(guinea-pig) γ -globulins were prepared as previously described (Zucker & Logothetopoulos, 1975). All other reagents were of the finest grade available commercially.

Experimental methods

Isolation of pancreatic islets and maintenance of islets in culture. Pancreatic islets were isolated by the collagenase technique (Lacy & Kostianovsky, 1967) from fed male Wistar rats weighing 350–400 g. The sediment of the collagenase digest was washed six to seven times with cold (4°C) Krebs–Ringer bicarbonate buffer (KRB) (Umbreit *et al.*, 1964) containing 4 mM-glucose and 7.5 mM-Hepes (KRBM), and then the islets were collected with braking pipettes into Petri dishes containing cold (4°C) KRBM buffer. These islets were either used immediately (freshly isolated islets) or were cultured for 15–18 h before being used for the metabolic and functional studies. For the tissue culture 400–500 islets were suspended in 5–6 ml of F12K tissue-culture medium (pH 7.35) (Kaighn, 1973) containing 12.5% heat-inactivated foetal calf serum, 10 mM-glucose and 35 μ g of penicillin G/ml. The suspended islets were placed in a 60 mm-diameter Falcon Petri culture dish and were maintained at 37°C in a humidified incubator gassed with air/CO₂ (19:1). Cultured islets were washed three times with cold (4°C) KRBM buffer before being distributed in tubes for metabolic and functional studies.

Alloxan-treatment of islets. The cold KRBM solution in Petri plates containing 300–400 freshly isolated islets was replaced with a freshly prepared solution of alloxan (0.5 mg/ml) which was added to a warm (37°C) oxygenated KRB solution containing 7.5 mM-Hepes; the dish was then placed in the tissue-culture incubator at 37°C for 5 min. At the end of 5 min the buffer containing alloxan was removed and the islets were washed three times in cold (4°C) KRBM solution. The alloxan-treated islets were either used directly or after culturing for 15–18 h as described above.

Determination of the rate of pyruvate decarboxylation. [1-¹⁴C]Pyruvate was used. The freeze-dried material received in solid ice from the supplier was dissolved in 0.01 M-HCl and distributed in 0.1 ml batches containing 20 μ Ci of [1-¹⁴C]pyruvate. The tubes were kept at –80°C. Before experimental use, a tube was thawed at room temperature, and CO₂

was blown for 3–4 min over the solution, which was then dried with a gentle jet of filtered N₂. The dry residue was used to make the desired solution for incubating pancreatic islets. Groups of 10 pancreatic islets were incubated in the micro-spirometric devices described by Cole & Logothetopoulos (1974) in 50 μ l of KRB buffer, pH 7.35, containing no glucose, bovine albumin (1 mg/ml), 7.5 mM-Hepes and 0.5 μ Ci of [1-¹⁴C]pyruvate at pyruvate concentrations ranging from 2.5 to 20 mM. The gas phase was O₂/CO₂ (19:1) and the vials were kept at 37°C under continuous shaking. At the end of a 2 h incubation period 0.5 ml of Hyamine was injected into the outer vial and CO₂ was released by injecting into the inner vessel 100 μ l of 0.2 M-phosphoric acid/citrate buffer (pH 3.8) containing 25% (v/v) methanol. After 2½ h at room temperature, the radioactivity trapped in Hyamine was determined by liquid-scintillation counting in a scintillation fluid composed of toluene (800 ml), methanol (200 ml), PPO (4 g) and POPOP (0.1 g). Blank values of incubations without islets were subtracted from experimental values. The recovery of ¹⁴CO₂ from NaH¹⁴CO₃ was 97–100% with this method under identical conditions of incubation.

Determination of glycolytic flux. The release of ³H₂O from D-[5-³H]glucose was used to measure glucose utilization (Ashcroft *et al.*, 1972). The same micro-spirometric device used for measuring ¹⁴CO₂ output from [1-¹⁴C]pyruvate was used to determine the amount of ³H₂O release from [5-³H]glucose. Ten islets were incubated in a 7 mm × 25 mm conical tube with 25 μ l of KRB solution containing 7.5 mM-Hepes, 1 mg of albumin/ml, 1 μ Ci of D-[5-³H]glucose and various glucose concentrations. The tubes were enclosed in scintillation vials capped with rubber stoppers. They were then gassed for 10 min with O₂/CO₂ (19:1), saturated with water vapour at 37°C. After 2 h of incubation at 37°C the stoppers were removed, 0.5 ml of water was added to the floor of the vial and 10 μ l of 1 M-HCl saturated with ethylmercurithiosalicylate was added to the incubation tube. A small triangular piece of filter paper was also placed in the bottom of the tube to increase the evaporating surface. The vials were resealed with their plastic scintillation caps and placed in an incubator at 40°C for 24 h. At the end of this period, after cooling briefly at room temperature, the outside of each incubation tube was washed in its companion scintillation vial with 10 ml of scintillator fluid (Aquasol). Equilibration of ³H₂O between the buffer in the incubation tube (0.035 ml) and the water in the vial (0.5 ml) was tested with ³H₂O. For this, 1 μ Ci of ³H₂O in 25 μ l was added to each of the 10 incubation tubes, which were then processed as described above for tubes containing islets: 90% of the radioactivity added to the tubes was recovered in the vials. Experimental values were corrected accordingly.

Determination of proinsulin biosynthesis and insulin secretion. The procedure used to measure incorporation of [³H]leucine or [³H]lysine into proinsulin and other islet proteins, and the method used to measure insulin secretion, are described in detail in previous publications (Zucker & Logothetopoulos, 1975; Jain & Logothetopoulos, 1978).

Results

The rates of decarboxylation of [1-¹⁴C]pyruvate in pancreatic islets either freshly isolated or cultured for 15–16h in F12K culture medium after the enzymic isolation procedures are shown in Fig. 1. Cultured islets metabolized 2–2.5 times as much pyruvate as did freshly isolated islets at low and high concentrations of substrate.

The effect of alloxan treatment on the rate of pyruvate decarboxylation and glucose utilization are shown in Table 1. When pancreatic islets exposed to alloxan were compared with control islets immediately after alloxan treatment, it was found that the rates of pyruvate decarboxylation were similar in both groups, but that the alloxan-treated group metabolized one-fifth to one-quarter as much glucose as the control group. When alloxan-treated and non-*alloxan*-treated islets were tested metabolically after a period of culturing in F12K medium containing 10mM-glucose, large differences in the rate of pyruvate decarboxylation between the two groups emerged. Cultured non-*alloxan*-treated islets decarboxylated 2–2.5 times as much pyruvate as did *alloxan*-treated islets. Glycolytic flux increased in both non-*alloxan*-treated and *alloxan*-treated islets, but the ratio of glycolytic fluxes between the two groups was not significantly affected, the cultured controls utilizing approx. 4–5 times as much glucose as the cultured *alloxan*-treated islets (Table 1).

Table 2 shows the effect of increasing concentrations of pyruvate on the rate of incorporation of [³H]leucine into proinsulin and insulin and into other islet proteins in freshly isolated pancreatic islets and in those cultured for 16h. Values for 20mM-glucose are also given for comparison. The striking increase

Fig. 1. Rates of pyruvate decarboxylation by cultured and non-cultured pancreatic islets of the rat

Groups of freshly isolated or cultured rat islets were incubated at 37°C in 50µl of KRB buffer containing 1 mg of bovine albumin/ml, 7.5 mM-Hepes and 0.5 µCi of [1-¹⁴C]pyruvate at pyruvate concentrations shown on the abscissa. Decarboxylation rates of pyruvate were calculated from the formation of ¹⁴CO₂ and the specific radioactivity of pyruvate in the buffer. Each value is the mean ± S.E.M. of ten replicates from two experiments. □, Freshly isolated islets; ▨, cultured islets.

Table 1. Effect of alloxan treatment on the rates of pyruvate decarboxylation and glucose utilization

Alloxan-treated islets were compared with control islets either immediately after exposure *in vitro* to *alloxan* (0.5 mg/ml) for 5 min or after culturing for 16h in F12K culture medium. Islets were incubated for 2h at 37°C in KRB solution containing bovine albumin (1 mg/ml), 7.5 mM-Hepes and either [1-¹⁴C]pyruvate (2.5 mM) or D-[5-³H]glucose (10 mM). The rate of pyruvate decarboxylation was calculated from released ¹⁴CO₂ and the specific radioactivity of [1-¹⁴C]pyruvate in the incubation buffer. Glycolytic flux was calculated from the formation of ³H₂O and the specific radioactivity of D-[5-³H]glucose in the buffer. Results are given as pmol of substrate metabolized/h per 10 islets. Means ± S.E.M. from ten replicates of two experiments.

Glucose utilization				Pyruvate decarboxylation			
Freshly isolated islets		Cultured islets		Freshly isolated islets		Cultured islets	
Alloxan-treated	Controls	Alloxan-treated	Controls	Alloxan-treated	Controls	Alloxan-treated	Controls
210±17	1004±65	370±32	1924±104	510±25	540±36	585±29	1512±44

Fig. 2. Effect of extracellular concentration of pyruvate on the secretion of $[^3\text{H}]$ proinsulin + $[^3\text{H}]$ insulin by prelabelled cultured and non-cultured rat pancreatic islets

Freshly isolated islets or islets incubated for 16 h in F12K tissue-culture medium were incubated for 2½ h in leucine and serum-free F12K tissue-culture medium (2 ml) containing 15 μCi of L-[4,5- ^3H]leucine (40 Ci/mmol), 1 mg of albumin/ml, 2 mg of glucose/ml and penicillin (100 i.u./ml). At the end of the incubation period, the radioactive medium was removed, and the islets were washed three or four times with cold KRBM buffer containing 2 mM-leucine. Groups of seven to ten prelabelled islets were placed in individual tubes containing cold (4°C) KRBM solution supplemented with 1 mg of albumin/ml. These islets were then preincubated for 45 min in 150 μl of KRB buffer containing 5 mg of albumin/ml, and 7.5 mM-Hepes. At the end of 45 min another 150 μl of KRB buffer was added containing 5 mg of albumin/ml, 7.5 mM-Hepes and the appropriate concentration of sodium pyruvate. Tubes were further incubated for 90 min. All incubations were conducted in a Dubnoff metabolic shaker under a constant flow of O_2/CO_2 (19:1), at a shaking frequency of 70 cycles/min. Labelled proinsulin + insulin secreted in the medium and remaining in the islets were measured by a double-immunoprecipitation technique (Zucker & Logothetopoulos, 1975). The secretory response was expressed as immunoprecipitable radioactivity secreted in the medium relative to the sum of immunoreactivity retained within the islets and secreted in the medium at the end of the incubation period. ■, Cultured islets; □, freshly isolated islets. Results are means $\pm$ S.E.M. of ten replicates from two experiments.

in the basal incorporation of $[^3\text{H}]$ leucine into proinsulin and insulin and the decrease in the difference between rates of incorporation at 0 and 20 mM-glucose are characteristic effects of culturing pancreatic islets (for 15–18 h) in enriched culture media containing glucose (K. Jain, S. Asina & J. Logothetopoulos, unpublished work).

In freshly isolated islets pyruvate did not exert a significant stimulatory effect on the rate of $[^3\text{H}]$ -leucine incorporation into proinsulin or other islet proteins. In cultured islets a response significantly

different from the basal value was already observed at 2.5 mM. Incorporation of $[^3\text{H}]$ leucine progressively increased with an increase in the concentration of pyruvate.

The relationship between the concentration of pyruvate and the release of ^3H -labelled proinsulin + insulin from prelabelled islets is given in Fig. 2. No release of labelled hormones was found in freshly isolated pancreatic islets. In contrast, at 20 mM-pyruvate secretory responses in cultured islets were obtained which were approx. 12–15 times the basal secretory response and about 0.5–0.7 times that obtained with 20 mM-glucose. Since pyruvate was used as sodium salt, it was essential to test the effect of excess Na^+ on the basal rates of insulin secretion and on the incorporation of $[^3\text{H}]$ leucine into proinsulin: 20 mM- NaCl did not modify the basal activity of islets (Table 3). Adrenaline (5 μM) and $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free medium completely abolished the stimulatory effects by induced pyruvate in cultured islets, whereas D-mannoheptulose only partly inhibited the stimulatory effects of pyruvate (Table 3).

Results given in Table 4 show that sodium lactate was on the whole as effective as pyruvate in stimulating both proinsulin biosynthesis and release of labelled hormones in cultured islets.

Data presented in Table 5 shows that L-leucine and 4-methyl-2-oxopentanoate were very effective stimulants of insulin secretion and proinsulin biosynthesis ($[^3\text{H}]$ lysine incorporation) in cultured islets.

Discussion

We investigated the responses of cultured islets to pyruvate, since, as other investigators have already reported, we found that pyruvate did not exert any significant insulinotropic effect in freshly isolated rat islets. Our experiments have shown that, in cultured islets, pyruvate in the complete absence of exogenous glucose is a very effective insulin secretagogue and stimulator of proinsulin biosynthesis. The functional responses of cultured islets to pyruvate were accompanied with an increase in the ability of the cultured islets to metabolize $[^{14}\text{C}]$ pyruvate.

A possible explanation for the failure of freshly isolated islets to respond to pyruvate was provided by experiments with alloxan, a specific cytotoxic compound for β -cells (Dunn *et al.*, 1943). Alloxan treatment of pancreatic islets *in vitro* hardly affected the rate of decarboxylation of $[1\text{-}^{14}\text{C}]$ pyruvate, but decreased glycolytic flux to 20–25% of that of control islets. This indicated either that only α - and connective-tissue cells, but not β -cells, metabolize exogenous pyruvate, or that alloxan treatment affected glycolytic steps, but left intact pyruvate entry into the β -cell and pyruvate dehydrogenase activity. After culturing for 15–16 h, the rate of $[1\text{-}^{14}\text{C}]$ -

Table 2. *Effect of pyruvate on the incorporation of [³H]leucine into proinsulin+insulin and into 'other' islet-cell proteins*
The freshly isolated or cultured islets (16h in F12K tissue-culture medium containing 1.8mg of glucose/ml) were incubated for 50min in KRB buffer containing 1mg of bovine albumin/ml, 7.5mM-Hepes and [³H]leucine in the presence of varying concentrations of pyruvate and no glucose. Labelled proinsulin + insulin was extracted from the islets and quantitatively precipitated by a double-antibody procedure. Radioactivity in other proteins was measured after trichloroacetic acid precipitation of the digest of extracted islets (see Jain & Logothetopoulos, 1977). Results are given as the means±S.E.M. from ten replicates of two experiments.

Pyruvate concentration in medium (mM)	10 ⁻² × [³ H]Leucine incorporated (c.p.m./50 min per 10 islets)			
	Proinsulin and insulin		Other proteins	
	Freshly isolated islets	Cultured islets	Freshly isolated islets	Cultured islets
0.0	16±1	392±15	267±28	747± 51
2.5	17±1	693±45	288±25	1134± 91
5.0	19±1	802±34	296±23	1060± 97
10	21±1	900±44	317±28	1113±101
20	22±1	986±35	330±35	1297±123
20mM-Glucose*	526±9	1574±34	522±19	1579±147

* Incorporation of [³H]leucine in the two protein fractions in the presence of 20mM-D-glucose.

Table 3. *Effect of NaCl, D-mannoheptulose, adrenaline and Ca²⁺+Mg²⁺-free medium on proinsulin biosynthesis and insulin release induced by pyruvate in cultured rat pancreatic islets*

See legends of Fig. 2 and Table 2 for experimental details. Results are means±S.E.M. from five replicates.

Pyruvate (mM)	Additions	10 ⁻² × [³ H]Leucine incorporated (c.p.m./50 min per 10 islets)	Percentage of [³ H]proinsulin+ [³ H]insulin released in 90min
—	—	363±25	1.1±0.1
—	NaCl (20mM)	375±28	1.0±0.3
20	—	843±10	17.4±1.6
—	—	275±32	1.1±0.1
10	—	888±36	12.7±0.5
10	D-Mannoheptulose (10mM)	446±18	4.7±0.3
10	—	—	9.5±0.5
10	Adrenaline (15 μM)	—	0.9±0.1
10	Ca ²⁺ /Mg ²⁺ -free medium	—	0.8±0.1

Table 4. *Effect of L(+)-lactic acid on proinsulin biosynthesis and insulin release in cultured pancreatic rat islets*
Results are given as the means±S.E.M. from ten replicates of two experiments. For experimental details, see the legends of Fig. 2 and Table 2.

Lactate concentration (mM)	10 ⁻² × [³ H]Leucine incorporated (c.p.m./50 min per 10 islets)		Percentage of [³ H]proinsulin+ [³ H]insulin released in 90min
	Proinsulin+insulin	Other islet proteins	
0.0	352±25	1121± 77	1.4±0.1
2.5	793±85	1701±187	5.9±0.4
5.0	902±34	1789±116	9.3±0.5
10	1013±77	1769±111	15.3±1.1
20	1009±35	1964±140	19.9±1.6

pyruvate decarboxylation increased 2-2½-fold in control islets, but showed an insignificant increase in alloxan-treated islets. Histological examination of sections of alloxan-treated islets after culturing showed nearly all β-cells separating from each other

with heavily pycnotic nuclei. The necrotic β-cells were surrounded by crescents of healthy looking α-cells and enclosed healthy looking endothelial cells. It was therefore difficult to attribute to these cultured necrotic β-cells the ability to decarboxylate pyruvate.

Table 5. Effect of 4-methyl-2-oxopentanoate, D-glucose, L-leucine and pyruvate on the incorporation of [3 H]lysine into proinsulin+insulin and into 'other' islet proteins and on insulin release by cultured pancreatic rat islets

For insulin release all compounds were tested at the concentration of 20 mM. For experimental details see the legend of Fig. 2. For proinsulin biosynthesis islets cultured in F12K tissue culture medium containing 1.0 mg of glucose/ml were incubated in groups of ten for 50 min in glucose-free KRB buffer containing 1 mg of albumin/ml, 7.5 mM-Hepes and [3 H]lysine in the presence of 4-methyl-2-oxopentanoate (10 mM), glucose (20 mM) or L-leucine (1.5 mM). Labelled proinsulin and insulin extracted from the islets were quantitatively precipitated by a double-antibody procedure. Radioactivity in other islet proteins was measured after trichloroacetic acid precipitation of digests of extracted islets (see Jain & Logothetopoulos, 1977). Results are given as the means $\pm$ S.E.M. from five replicates.

Compounds	$10^{-2} \times$ [3 H]Lysine incorporated (c.p.m./50 min per 10 islets)		Percentage of [3 H]proinsulin+ [3 H]insulin released in 90 min
	Proinsulin+insulin	Other islet proteins	
None	23 $\pm$ 1	416 $\pm$ 50	1.0 $\pm$ 0.1
D-Glucose	151 $\pm$ 8	851 $\pm$ 28	24.5 $\pm$ 1.0
4-Methyl-2-oxopentanoate	104 $\pm$ 2	698 $\pm$ 40	29.4 $\pm$ 0.9
L-Leucine	66 $\pm$ 5	649 $\pm$ 10	6.5 $\pm$ 0.1
Pyruvate	—	—	12.9 $\pm$ 0.5

It appears plausible that β -cells in freshly isolated islets, owing possibly to adverse effects of the isolation procedure, metabolize little pyruvate compared with non- β -cells and that generation of signals from pyruvate for insulin secretion and proinsulin biosynthesis does not reach threshold values under these conditions. Culturing of islets, possibly by promoting pyruvate transport across the cell membrane of the β -cell, could make pyruvate metabolically available to the β -cell, and thus permit the generation of signals above threshold. Whether endogenous fuels stored during the culturing period (glycogen, lipids) or an enhanced response of the β -cell adenylate cyclase (Howell *et al.*, 1973) also contribute to the responsiveness of pyruvate remains to be investigated.

An insulinotropic effect of pyruvate in the absence of any exogenous substrate or agent has been described by Lambert *et al.* (1969) in foetal rat pancreatic explants maintained in culture for 4 days, and has also been observed in isolated islets from pregnant rats (Green & Taylor, 1975). In the light of our observations it is reasonable to assume that the response of the foetal rat pancreas is not an intrinsic characteristic of foetal β -cells, but is shared by adult rat islets after brief maintenance in culture. A moderate insulinogenic effect of L(+)-lactate has also been reported by Loubatieres-Mariani *et al.* (1976) in the isolated perfused rat pancreas. Pieces of adult rat pancreas perfused with 2.4 mM-pyruvate, however, failed to release insulin (Burr *et al.*, 1970). It is possible that 2.4 mM-pyruvate may not have been sufficient to produce signals above the threshold in this preparation.

What are the signals arising from pyruvate which stimulate insulin release and proinsulin biosynthesis?

It is uncertain whether β -cells possess effective pyruvate carboxylase and phosphoenolpyruvate

carboxylase activity and are able to synthesize phosphoenolpyruvate from pyruvate. Sugden & Ashcroft (1977) reported that in isolated rat islets the concentration of phosphoenolpyruvate increases with increasing concentrations of glucose, and Capito & Hedekov (1977) showed an effect of phosphoenolpyruvate on adenylate cyclase activity.

The insulinotropic effects of L-leucine and 4-methyl-2-oxopentanoate reported by Panten *et al.* (1972), Panten (1975), Anderson (1976) and Hutton & Malaisse (1977) in freshly isolated islets and confirmed in the present investigation with cultured islets are difficult to reconcile with a 'phosphoenolpyruvate signal' hypothesis. The terminal catabolic products of 4-methyl-2-oxopentanoate acid are acetoacetate and acetyl-CoA.

Existing experimental data do not as yet clearly suggest a unified model of how the β -cell recognizes metabolizable substrates. It appears attractive, however, to speculate that important functional signals may be related to the energy charge (Atkinson & Walton, 1967) of the β -cell.

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