Opposite Effects of Starvation on Oxidation of [^{14}C]Adenosine and Adenosine-Induced Insulin Release by Isolated Mouse Pancreatic Islets

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To test further the hypothesis that ribonucleosides stimulate insulin secretion and biosynthesis by producing metabolic signals, the effects of starvation on adenosine-stimulated insulin production and the oxidation of adenosine by isolated mouse pancreatic islets were examined. No direct correlation was found between the metabolic flux and insulin secretion, since the starvation-induced impairment of the adenosine-stimulated insulin secretion was accompanied by an increased rate of adenosine oxidation. Adenosine-stimulated insulin biosynthesis was, however, unaffected by starvation.

In recent years numerous studies have demonstrated that the suitability of different hexoses as substrates for glycolysis closely parallels their abilities to stimulate insulin release, suggesting a key role for this pathway in the control of insulin secretion. These data provide strong support for the substrate-site hypothesis, one of two models proposed to describe the process for signal recognition of the β -cells of the islets of Langerhans (Randle *et al.*, 1968). According to this model the signal compound enters the β -cell where it is metabolized, and it is the formation of some glycolytic intermediate or cofactor that triggers the hormone secretion. Indeed, recent studies have suggested that phosphoenolpyruvate possibly fills this role (Sugden & Ashcroft, 1977).

Further support for the substrate-site hypothesis emerged from the observations that various purine ribonucleosides were potent primary stimulators of insulin secretion (Capito & Hedekov, 1976) and insulin biosynthesis (Jain & Logothetopoulos, 1977, 1978; Andersson & Hellerström, 1977; Ashcroft & Hedekov, 1978). The stimulatory effect is presumably due to the splitting of ribonucleosides into the purine base and ribose 1-phosphate, the ribose moiety being then further metabolized via the pentose phosphate cycle with the formation of glycolytic intermediates. The present paper reports observations on adenosine-stimulated insulin release and biosynthesis from islets isolated from starved or fed mice and the concomitant oxidation of [^{14}C]adenosine.

Experimental

Islet isolation

Male NMRI mice were either fed on a standard laboratory diet *ad libitum* or starved for 65 h with free access to drinking water. Isolated islets obtained by a modified collagenase method (Howell & Taylor,

1968) were washed in Hanks solution (Hanks & Wallace, 1949) before being transferred, in groups of ten, to the different glucose-free incubation media.

Insulin release

The islets were incubated in glass vials (Keen *et al.*, 1963) for 60 min in a water thermostat at 37°C with continuous shaking. The incubation medium consisted of a bicarbonate-buffered medium (Krebs & Henseleit, 1932) supplemented with 2 mg of bovine albumin/ml, 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] and 10 mM-adenosine. Before the experiment each vial was gassed for 30 s with O_2/CO_2 (19:1). At the end of the incubation the medium was removed and frozen and stored at -20°C before insulin assay, which was performed as described by Heding (1972).

Insulin biosynthesis

The incubation device described above was used, but the islets were incubated for 120 min. The medium consisted of a bicarbonate-buffered medium (Gey & Gey, 1936) supplemented with 10 mM-adenosine, 100 μCi of L-[2,4,6- ^3H]phenylalanine/ml (sp. radioactivity 60 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) and 20 μg of each of 12 naturally occurring amino acids/ml as described by Eagle (1959), except that non-radioactive L-phenylalanine was omitted. At the end of the incubation period the islets were washed in Hanks solution containing an excess of non-radioactive L-phenylalanine and then homogenized by sonication. The incorporation of ^3H into (pro)insulin and other trichloroacetic acid-precipitable proteins was determined by a modified immune binding technique as described in detail by Berne (1975).

Adenosine oxidation

The incubation medium of Gey & Gey (1936) was used in these experiments, which were essentially performed as described by Andersson (1974). [$U\text{-}^{14}\text{C}$]Adenosine (sp. radioactivity 567 mCi/mmol; The Radiochemical Centre) was added at a concentration of 5 $\mu\text{Ci/ml}$. The concentration of non-radioactive adenosine was 1 mM, so that the specific radioactivity of the incubation medium became 4.96 mCi/mmol.

Statistical calculations of results

The results are given as means $\pm$ S.E.M., and each experiment represents the mean value of duplicate (biosynthesis) or triplicate (release and oxidation) incubations. The probability (P) of chance differences between paired values was tested by Student's t test.

Results and Discussion

The starvation period markedly impaired the adenosine-stimulated insulin release. Thus the islets from the fed animals released 22.6 ± 3.4 ng of insulin/h per five islets, whereas the islets isolated from the starved mice secreted 9.8 ± 1.9 ng of insulin/h per five islets ($P < 0.01$; $n = 5$). By comparison, islets incubated in the absence of adenosine or other stimulators have previously been shown to release less than 5 ng of insulin/h per five islets (Andersson, 1978). There is at present some controversy about the effects of adenosine on the secretion of insulin. It has thus been shown that 10 mM-adenosine enhances insulin secretion at both low (3.3 mM; Feldman & Jackson, 1974) and high (16.7 mM; Taniguchi *et al.*, 1977) glucose concentrations. Other studies, however, suggest that adenosine and guanosine, when present at a thousandfold lower concentration, inhibit glucose-induced insulin secretion by isolated rat islets (Ismail *et al.*, 1977; Ismail & Montague, 1977). These discrepancies could, however, partly be explained by the great species differences in the response of islets to purine ribonucleosides as reported by Campbell & Taylor (1977) and Jain & Logothetopoulos (1978).

The present results nevertheless indicate that adenosine, in common with inosine (Capito & Hedekov, 1976), stimulates insulin secretion by isolated mouse islets in batch-type experiments in the total absence of glucose. Furthermore they are consistent with the results of Capito & Hedekov (1976), showing that inosine-stimulated insulin secretion is totally abolished by 48 h starvation.

Table 1 shows that starvation affected neither the adenosine-stimulated (pro)insulin biosynthesis nor the total rate of protein synthesis. This conforms with our previous findings on leucine-stimulated insulin biosynthesis in starved animals (Andersson, 1976),

Table 1. Effects of starvation on adenosine-stimulated insulin biosynthesis in isolated pancreatic islets

The results are given as [^3H]phenylalanine incorporation into proinsulin+insulin and trichloroacetic acid-precipitable proteins. The final column gives the mean values of the ratios between the radioactivities in proinsulin+insulin and trichloroacetic acid-precipitable proteins for each individual incubation. Each of the six experiments represents the mean value of three individual incubations.

Islet source	10 ⁻³ Phenylalanine incorporation (c.p.m./2h per ten islets)		
	Proinsulin +insulin (A)	Trichloroacetic acid (B)	(A/B) $\times$ 100
Fed animals	14.1 $\pm$ 3.8	54.4 $\pm$ 11.8	25.2 $\pm$ 2.5
Starved animals	14.6 $\pm$ 4.5	53.8 $\pm$ 12.6	26.3 $\pm$ 2.2

thus suggesting that starvation influences the regulatory mechanisms for insulin biosynthesis to a much smaller extent than those for insulin secretion. It is noteworthy that this also seems to be the case for glucose stimulation of these two processes (Andersson, 1976; Levy *et al.*, 1976; Bone & Howell, 1977). Since cyclic AMP only slightly affects the biosynthetic rate of insulin (Schatz *et al.*, 1973; Andersson, 1976; Bone & Howell, 1977), this is what would be expected, assuming that influences on the cyclic AMP system of the β -cells are responsible for the impairment of glucose- and inosine-stimulated insulin secretion during starvation (Selawry *et al.*, 1973; Howell *et al.*, 1973; Capito & Hedekov, 1974, 1976; Rabinovitch *et al.*, 1976).

The influence of starvation on the cyclic AMP system might, however, be caused by the concomitant impairment of glucose metabolism (Hedekov & Capito, 1974; Levy *et al.*, 1976; M. Welsh & A. Andersson, unpublished work). It was therefore decided to evaluate whether adenosine oxidation in the islets of starved animals was also affected. Surprisingly, with 1 mM-adenosine, the $^{14}\text{CO}_2$ production from [^{14}C]adenosine was higher for the islets from starved mice: 145.2 ± 15.8 as compared with 77.2 ± 14.2 pmol/90 min per 10 islets for the islets from fed mice ($P < 0.001$; $n = 5$).

It is noteworthy that a similar enhancement of leucine oxidation by pancreatic islets isolated from starved mice has been demonstrated (M. Welsh & A. Andersson, unpublished work), suggesting adaptive changes towards oxidation of substrates other than glucose during starvation. Indeed, this has been described for leucine oxidation by diaphragm (Goldberg & Odessey, 1972) and gastrocnemius muscles (Paul & Adibi, 1978) from starved animals. Furthermore there seems to be a parallelism between the influence of starvation on the stimulation of insulin biosynthesis by a given compound and the

oxidative degradation of the latter. Thus the impairment of glucose-stimulated insulin biosynthesis during starvation is accompanied by a decreased rate of glycolysis, whereas adenosine- and leucine-stimulated biosynthesis of insulin is unaffected by starvation, their corresponding oxidation rates even being enhanced.

In conclusion, the results of the present study suggest that there is no direct correlation between the glycolytic metabolic flux below the triose phosphate level and the secretion of insulin during stimulation with adenosine. This is in contrast with the claim by Jain & Logothetopoulos (1978), who found that an ineffective stimulation of insulin secretion by a given nucleoside was reflected in a low rate of oxidation of the latter. The present results, however, add further support to the hypothesis that a metabolic signal of some kind is involved in the regulation of the rate of (pro)insulin biosynthesis.

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