Rapid Papers

Long-Term Effects of a High Glucose Concentration on Cyclic Nucleotide Phosphodiesterase Activity in Mouse Pancreatic Islets Maintained in Tissue Culture

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It has been suggested that the stimulatory effect of glucose on insulin release may be mediated by the adenylate cyclase–cyclic AMP phosphodiesterase system. In this study it was found that exposure of isolated pancreatic islets to an elevated extracellular glucose concentration for 1 week *in vitro* caused an increase of the cyclic AMP phosphodiesterase activity in the islet cells. These and previous data indicate that there is an increased turnover of cyclic AMP in B-cells exposed for a prolonged time to a high extracellular glucose concentration, which also causes an increased turnover rate of insulin.

The functional activity of many endocrine cells is affected by their concentrations of cyclic AMP. Thus the pancreatic islets are equipped with an adenylate cyclase–cyclic AMP phosphodiesterase system, the activity of which influences the insulin secretion rate. Much attention has been focused on the problem of whether or not the stimulating effect of glucose on the insulin release is mediated via cyclic AMP. For a detailed review see Montague & Howell (1975). In an attempt to explore the long-term effects of extreme variations of the extracellular glucose concentration on the function of the pancreatic B-cells we have investigated the structure and metabolism of isolated pancreatic islets maintained in tissue culture (Andersson & Hellerström, 1972; Andersson, 1974; Andersson *et al.*, 1974). In the present study the cyclic AMP phosphodiesterase activity was determined in intact freeze-dried pancreatic islets, which had been cultured for 6 days at widely different glucose concentrations. The enzyme was assayed by measuring the ATP formed by photokinetic analyses of the bioluminescence obtained from firefly luciferase.

Materials and Methods

Chemicals

Firefly lantern extract (code: FLE-50) was from Sigma Chemical Company, St. Louis, MO, U.S.A. Other enzymes used in the assay, nucleotides and enzyme substrates were from C. F. Boehringer, Mannheim, Germany. Crude collagenase (code: CLS) for islet isolation was from Worthington Biochemical Corporation, Freehold, NJ, U.S.A. Inorganic salts were of analytical grade and were from E. Merck A.G., Darmstadt, Germany. Deionized and double-distilled water was used in the experiments.

Tissue-culture technique

Pancreatic islets, isolated by a collagenase method (Howell & Taylor, 1968), were obtained from adult male NMRI mice, which were starved overnight before the experiments. The islets were kept in tissue culture for 1 week as previously described in detail (Andersson & Hellerström, 1972; Andersson, 1974). The culture medium consisted of TCM 199 (Statens Bakteriologiska Laboratorium, Stockholm, Sweden) supplemented with 10% (v/v) calf serum, and 100 units of penicillin and 0.1 mg of streptomycin/ml; the glucose concentration was adjusted to either 3.3 or 16.7 mM.

Preparation of islets

After 6 days' culture the islets were harvested from the culture dishes by a brief trypsinization (Andersson, 1974) and subsequently rapidly washed in water before they were plunged into isopentane (m.p. -160°C) chilled with liquid N_2 . After freeze-drying (0.08 Pa; -30°C) the islets were weighed with a quartz-fibre balance and 1–3 μg of islet tissue was used for the analyses.

Enzyme assay

Cyclic AMP phosphodiesterase (EC 3.1.4.17) activity was measured with a modified bioluminescence technique (Ashcroft *et al.*, 1972). The assay medium was composed of 0.1 M-triethanolamine hydrochloride, pH 8.0, 10 mM- MgCl_2 , 0.3 mg of bovine serum albumin/ml and either 0.015 mM- or 0.5 mM-cyclic AMP. Blanks were prepared without cyclic AMP. The islets were incubated in 25 μl of the reaction medium for 30 min at 38°C . The enzyme reaction was stopped by a 4 min incubation at 80°C . In order to convert AMP formed in the reaction into ATP, 10 μl samples of the incubation media were mixed with 0.1 M-triethanolamine hydrochloride,

pH 8.0, 10 mM-phosphoenolpyruvate (cyclohexylammonium salt), 0.1 mM-ATP, 5 mM-KCl, and 0.3 mg of bovine serum albumin, 40 units of adenylate kinase (EC 2.7.4.3) and 4 units of pyruvate kinase (EC 2.7.1.40)/ml, and were incubated for another 45 min at 38°C. After dilution (1:5) with 0.1 M-NaHCO₃ buffer, pH 9.5, 10 µl samples were rapidly injected into a cuvette containing a firefly luciferase extract for the determination of ATP. The cuvette containing the light-yielding solution was kept inside a carefully sealed dark chamber in front of a photo-multiplier tube, connected to an oscilloscope. The light-emission profile from each sample was photographed from the oscilloscope screen and the peak height (expressed in mV), which was directly proportional to the amount of ATP injected, was measured (Wettermark *et al.*, 1970; Brodin *et al.*, 1971). Standards were prepared with AMP, which was quantified spectrophotometrically at E_{260} .

Methodological considerations

The present method for assay of cyclic AMP phosphodiesterase activity represents a slight modification of previously used techniques. By using freeze-dried islets, which had not been dialysed before the analyses, several independent observations could be made, which is not the case when a homogenate prepared from the total number of islets isolated from one mouse is used. The omission of the dialysis step means that the adenylate kinase-pyruvate kinase system will convert all the islet adenine nucleotides into ATP, which will then add to the blank in the luciferase assay. One or two freeze-dried islets (dry weight 1–3 µg) were, however, sufficient for an acceptable sample-blank relationship in this system (blank without islets, approx. 50 mV; background of adenine nucleotides in the islets measured in the absence of cyclic AMP from the medium, approx. 500 mV/µg of islet dry wt.; readings with low and high cyclic AMP concentrations, approx. 900 mV/µg and approx. 1500 mV/µg respectively).

Results

In mouse pancreatic islets cultured for 6 days in the high- (16.7 mM) or low- (3.3 mM) glucose-containing media the differences in the measurements of cyclic AMP phosphodiesterase activity depended on the cyclic nucleotide concentration (Table 1). If the latter was low (0.015 mM), the enzyme activity was nearly twice as great with 16.7 mM-glucose, whereas with 0.5 mM-nucleotide the difference was only about 10%. It should be noted, however, that the absolute differences between the means at the low and the high cyclic AMP concentration were roughly the same.

The rationale for assaying the enzyme activity at two widely different concentrations of cyclic AMP is

that the pancreatic islets, in common with most other organs, possess two cyclic AMP-degrading enzyme activities, with low and high K_m values for cyclic AMP (Ashcroft *et al.*, 1972; Sams & Montague, 1972; Capito & Hedeskov, 1974). In the mouse islet the K_m values have been estimated as 0.01 mM and 0.50 mM, whereas somewhat lower values were found in guinea-pig islets (Sams & Montague, 1972). The two concentrations employed in this study, 0.015 mM and 0.5 mM, were thus chosen to measure the low- and high- K_m enzyme activities separately. Since the concentration of cyclic AMP in the pancreatic islets *in vivo* has been reported to be of the order of 0.5–1.0 µM (Cooper *et al.*, 1973) the low- K_m activity would seem to be of more interest as regards the role of cyclic AMP phosphodiesterase in the control of the islet cyclic AMP concentrations. Further, it was observed that the cyclic AMP phosphodiesterase activity measured at the low cyclic AMP concentration was decreased by 90% by 10 mM-theophylline; the effect obtained at the high cyclic AMP concentration was very small, suggesting that the high- K_m enzyme was not involved.

The present data may be taken to indicate that the adenylate cyclase-cyclic AMP phosphodiesterase system of the B-cells is subject to long-term regulatory influences by glucose. Studies in the rat *in vivo* have shown that both intermittent infusions of glucose (Howell *et al.*, 1973) and the increased dietary intake during pregnancy will activate the islet adenylate cyclase (Green *et al.*, 1973), whereas a diminished activity has been observed after a 48 h period of starvation (Howell *et al.*, 1973). It has been shown that exposure to an elevated glucose concentration

Table 1. Activity of cyclic AMP phosphodiesterase in mouse pancreatic islets cultured for 6 days at a high or a low glucose concentration

Details of the procedure for isolation and culture of the islets and for the enzyme assay are given in the Materials and Methods section. Islets isolated from two mice were divided between one Petri dish with a low-glucose medium and another with a high-glucose medium. From each dish five samples of islets were assayed. The results are means $\pm$ S.E.M. Differences between means were analysed with Student's *t* test.

Glucose concentration in the culture medium (mM)	Number of culture analysed	Hydrolysis cyclic AMP (pmol/h per µg dry wt.)	
		0.015 mM-Cyclic AMP	0.5 mM-Cyclic AMP
3.3	5	10.2 $\pm$ 1.5	59.3 $\pm$ 5.4
16.7	6	19.4 $\pm$ 1.7*	67.8 $\pm$ 6.2†

* $P < 0.005$.

† No significant difference between islets cultured at 3.3 mM- and 16.7 mM-glucose.

in vitro, similar to the one used in the present study, during a 6-day culture period will double the adenylate cyclase activity of mouse pancreatic islets (Howell *et al.*, 1973). The present finding of an activation also of the low- K_m cyclic AMP phosphodiesterase shows that this long-term effect of glucose on the islet cells is also manifest in the system for cyclic AMP degradation. In contrast, no short-term regulatory effects of a high glucose concentration on cyclic AMP phosphodiesterase activity were found in homogenates of freshly isolated islets (Ashcroft *et al.*, 1972; Bowen & Lazarus, 1973; Sams & Montague, 1972).

Taken together all the data suggest that glucose in the pancreatic B-cells may initiate a sequence of events causing an increase in adenylate cyclase activity, which raises the cyclic AMP concentration, and this in turn may act as an inducer for the cyclic AMP phosphodiesterase. The net result would be an increased turnover of cyclic AMP, not necessarily leading to a detectable rise of the cyclic AMP concentration in the cells. A role for cyclic AMP as an inducer of cyclic AMP phosphodiesterase has been suggested from studies in glioma cells in culture, in which a noradrenaline-induced rise in the cyclic AMP concentration leads to an enhanced cyclic AMP phosphodiesterase activity (Schwartz & Passonneau, 1974). It cannot be excluded that a similar mechanism operates in islets exposed to a high extracellular glucose concentration for a prolonged period of time. Such islets are characterized by a high functional activity with an increased turnover rate of insulin (Andersson, 1974; Andersson *et al.*, 1974), which thus occurs concomitantly with an enhanced turnover of intracellular cyclic AMP.

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