Short Communications

Glucose Phosphorylation in Mouse Pancreatic Islets

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(Received 1 February 1968)

Glucose, mannose and fructose may stimulate release of insulin by rabbit or rat pancreas *in vitro*, whereas galactose and 2-deoxyglucose are without effect. Glucose stimulation is concentration-dependent, being detected at concentrations above 3.5 mM and half-maximal at a concentration of approximately 10 mM. The glucose effect is abolished by D-mannoheptulose (an inhibitor of ATP-D-glucose 6-phosphotransferases) but not by phlorrhizin (an inhibitor of glucose-transporting systems). These findings have suggested that glucose metabolism in pancreatic β -cells may provide a signal for release of insulin; and that phosphorylation of glucose, catalysed perhaps by an ATP-D-glucose 6-phosphotransferase with a high K_m for glucose (glucokinase, EC 2.7.1.2), may be a rate-determining step in this process (Coore & Randle, 1964; Grodsky *et al.* 1963).

Further support for these suggestions has been obtained in a study of glucose oxidation in mouse pancreatic islets. These were released from acinar tissue by digestion with collagenase (Moskalewski, 1965) and harvested with a wire loop under a dissecting microscope. Batches of ten islets (approx. 50 μ g. of tissue) were incubated in 0.2 ml. of saline medium (Krebs & Henseleit, 1932) containing [U- 14 C]glucose for 2 hr. at 37°. After acidification of the medium, carbon dioxide was collected in Hyamine and assayed for radioactivity by liquid-scintillation spectrometry with toluene scintillator (Synder, 1961). It was found that mouse islets oxidized glucose at a constant rate for at least 3 hr. The curve relating [U- 14 C]glucose-oxidation rate to glucose concentration was either biphasic or sigmoid and not hyperbolic. Thus the rate of oxidation of [U- 14 C]glucose increased slowly as glucose concentration was raised to 1 mg./ml., increased rapidly between 1 mg./ml. and 2 mg./ml. and became constant at approximately 2 mg./ml. Half-maximal rates of glucose oxidation were seen with 7 mM-glucose (1.26 mg./ml.) and the maximum rate of oxidation was approximately 2 μ moles of glucose/g. wet wt. of islets/hr. Phlorrhizin (3 mM) had no effect on the rate of glucose oxidation,

whereas D-mannoheptulose (3 or 6 mg./ml.) almost completely suppressed it at glucose concentrations of 0.55, 1.5 and 2.6 mg./ml. There is thus a positive correlation between the rate of glucose oxidation in mouse islets measured in these experiments and release of insulin, which in mouse islets *in vitro*, as in rabbit or rat pancreas, is stimulated by glucose and inhibited by mannoheptulose but not by phlorrhizin (E. Coll Garcia, personal communication).

ATP-D-glucose 6-phosphotransferase activities have been investigated in mouse islet homogenates. Batches of 100 mouse islets were disintegrated by ultrasonic vibration in 0.5 ml. of assay medium containing (final concns.) 50 mM-triethanolamine chloride, 100 mM-KCl, 10 mM-MgCl₂, 2 mM-N-acetylcysteine and mM-EDTA, pH 7.5. Mouse islet extract (50 μ l.) was added immediately to 100 μ l. of the above assay medium containing ATP (final concn. 0.1 mM or 5 mM) and [6- 3 H]glucose purified by passage through Dowex 1 (Cl⁻ form) (final concns. 0.02–20 mM, 3–150 μ C/ μ mole). After 20 min. incubation at 20° the reaction was arrested by adding 100 μ l. of ethanol; carrier glucose 6-phosphate (10 μ moles) was then added and the incubation mixture applied to a 1 ml. column of Dowex 1 (AG 1-X8; Cl⁻ form) (Bio-Rad Laboratories, Richmond, Calif., U.S.A.) and washed in succession with 50 ml. of 10 mM-glucose and 30 ml. of 2 mM-HCl. Glucose 6-phosphate was then eluted with 6 ml. of 0.1 M-HCl and assayed for radioactivity (after freeze-drying) by liquid-scintillation spectrometry with dioxan scintillator (Butler, 1961). Control incubations were made with addition of ethanol at zero time. It was found that the rate of reaction was constant during the 20 min. period of incubation and proportional to the amount of extract added. The maximum concentration of glucose 6-phosphate at the end of incubation was 2.6 μ M. Islet extracts hydrolysed [6- 3 H]glucose 6-phosphate at this concentration to [6- 3 H]glucose, but the rate was less than 10% of that of glucose phosphorylation. Glucose phosphorylation was also assayed in extracts of mouse liver prepared in the same medium

Fig. 1. Effect of glucose concentration on velocity of the ATP-D-glucose 6-phosphotransferase reaction in extracts of mouse pancreatic islets and mouse liver. The velocity of the ATP-D-glucose 6-phosphotransferase reaction was measured by the rate of formation of [6-³H]glucose 6-phosphate from [6-³H]glucose (see the text). ●, Mouse liver with 5mM-ATP; ▲, mouse pancreatic islets with 5mM-ATP; △, mouse pancreatic islets with 0.1mM-ATP.

by dispersion with a Potter-Elvehjem homogenizer, dilution to a concentration equivalent to that of islets and ultrasonic vibration.

Fig. 1. shows a reciprocal plot of glucose concentration and glucose phosphorylation rate measured in extracts of mouse islets with 5mM-Mg-ATP and 0.1mM-Mg-ATP. The results obtained in extracts of mouse liver are included for comparison. With mouse liver extracts and 5mM-Mg-ATP the results gave evidence for the presence of two ATP-D-glucose 6-phosphotransferases, one with a low K_m for glucose (approximately 0.05mM) and one with a high K_m for glucose (approximately 25mM). These may correspond to hexokinase and glucokinase previously described in rat, rabbit and guinea-pig liver (e.g. see Walker, 1963). With mouse islet extracts and 5mM-Mg-ATP, on the other hand, only one ATP-D-glucose 6-phosphotransferase activity was detected, with a K_m for glucose of 0.03mM. With mouse islet extracts and 0.5mM-glucose, reciprocal plots of MgATP concentrations and phosphorylation rates were curvilinear, the apparent K_m for MgATP being 0.3mM. This suggested the possibility that the glucose K_m in mouse islet extracts might be dependent on MgATP concentration. When the effects of glucose concentration were re-investigated in islet extracts with 0.1mM-Mg-ATP, the K_m for glucose was found to be 5.3mM. Mannoheptulose was found to be a competitive inhibitor (with respect to glucose) of ATP-D-glucose 6-phosphotransferase activity in mouse islet extracts with 5mM-Mg-ATP.

The total ATP-D-glucose 6-phosphotransferase activity of islet extracts (measured at 20° with 5mM-MgATP and 20mM-glucose) was approximately 0.4 unit/g. wet wt. of islets.

Mouse islet extracts thus showed no evidence for the presence of glucokinase under conditions where glucokinase was readily detected in extracts of mouse liver. It appears unlikely therefore that glucokinase is responsible for the increased rates of glucose oxidation and insulin release that are seen with mouse islets when glucose concentrations are increased between 3.3mM and 17mM. The apparent K_m for glucose of mouse islet ATP-D-glucose 6-phosphotransferase activity appeared to be inversely related to MgATP concentration, suggesting the presence of a hexokinase whose affinity for glucose is increased by the binding of MgATP. The concentration of ATP in mammalian cells in which it has been measured is of the order of 2–10mM. If the ATP concentration in mouse islets is of this order the K_m for glucose of mouse islet hexokinase would be approximately 0.03mM, and the participation of other mechanisms would be required to account for increased rates of glucose oxidation and insulin release as glucose concentrations are increased above 3.3mM. One possibility suggested by the present study is an increase in the glucose K_m which might be brought about, for example, by agents that may increase the apparent K_m for MgATP. Alternatively, stimulation of insulin release by glucose may occur by a mechanism that does not primarily involve glucose metabolism but which leads secondarily through the release process to acceleration of glucose oxidation. Hellerstrom (1967) found, for example, that glucose may stimulate oxygen consumption in mouse pancreatic islets.

We thank Professor P. E. Lacy, Dr K. W. Taylor and Dr E. Coll Garcia for details of their methods of preparing mouse islets with collagenase, Mrs I. M. Harris, Mrs J. E. Eaborn and Mrs L. Hansford for skilled technical assistance, and the Medical Research Council and the British Diabetic Association for contributing towards the costs of these studies.

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