EFFECTS OF GLUCOSE ON THE CONTENT OF ATP AND GLYCOGEN AND THE RATE OF GLUCOSE PHOSPHORYLATION OF ISOLATED PANCREATIC ISLETS MAINTAINED IN TISSUE CULTURE

Arne Andersson, Erik Borglund and Sven Brolin

Department of Histology, University of Uppsala, Uppsala, Sweden

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SUMMARY

Isolated mouse pancreatic islets cultured for 7 days in various glucose concentrations had higher ATP concentrations than freshly collagenase isolated ones. A pronounced increase of the glycogen content was observed in islets cultured in a high glucose medium. Such islets also revealed an enhanced rate of glucose phosphorylation, when assayed at a high glucose concentration, indicating an increased high K_m hexokinase activity. The low K_m hexokinase was unaffected by the glucose concentration of the culture medium. These findings further support the idea that the glucose metabolism of the pancreatic B-cell may show adaptive changes to a prolonged period of high extracellular glucose concentration.

INTRODUCTION

Recent developments in techniques for tissue culture of isolated pancreatic islets (1,2) provide new possibilities for studies of the long-term effects of glucose on the pancreatic B-cells. This should allow an approach to a number of hitherto unsolved problems concerning the role of glucose in both the development of degenerative lesions of the endocrine pancreas and the normal functional regulation of the B-cell. With the aid of a newly developed culture technique (2) we have demonstrated enhanced rates of both oxygen consumption and glucose oxidation of isolated mouse pancreatic islets maintained in tissue culture for 6 days in a high glucose medium (2,3). These data suggested that prolonged exposure to elevated glucose concentrations may induce adaptive metabolic alterations of the islet B-cells. To elucidate further the nature of these changes we have now extended the studies to include measurements of the ATP and glycogen concentrations and the glucose phosphorylation rates of islets cultured for 7 days at various glucose concentrations.

MATERIALS AND METHODS

Tissue preparation: Isolated islets were obtained by a modified collagenase method (4) from male NMRI-mice aged 10 weeks, which had been starved over night before the experiments. In studies of non-cultured islets the isolated specimens were washed twice in Hanks solution and then immediately frozen at -80°C in chilled isopentane before freeze-drying. When islets were prepared for tissue culture they were transferred to plastic Petri dishes containing tissue culture medium (TCM 199) supplemented with 10% calf serum, penicillin 100 U/ml and streptomycin 0.1 mg/ml. The glucose concentration of the culture medium was adjusted to 3.3, 6.1 or 28 mM and the gas phase consisted of 5% CO_2 in humified air (2). After 7 days culture the islets were harvested by treatment for 15 min with a Ca^{++} and Mg^{++} free 0.25% (w/v) trypsin solution (1:300), washed twice in Hanks solution, before freezing and freeze-drying. For estimations of the ATP-content of the B-cells in vivo, pancreatic pieces were frozen rapidly and sectioned in a cryostat (-20°C) and then freeze-dried. Samples from the central parts of the pancreatic islets were dissected by free hand under a stereomicroscope. After the freeze-drying all the different islet samples were stored at -25°C in vacuum or in purified argon gas before weighing on a quartz fibre balance (5) prior to the different assays.

Assay of ATP: The content of ATP in the B-cells was measured utilizing the firefly luciferin-luciferase reaction as described in detail elsewhere (6).

Assay of glycogen: The principles designed by Passonneau et al. (7) for extraction and specific degradation of glycogen were followed. The NADPH finally formed was measured photokinetically (8). The sensitivity was 10 pmoles glucosyl units. Since blanks and glycogen standards extracted from rabbit liver were taken through the whole assay procedure, no corrections for recovery were needed.

Assay of glucose phosphorylation: Glucose phosphorylating enzyme activity

(ATP-D-glucose 6-phosphotransferase) was assayed in the islets as previously described from this laboratory (9) by coupling the phosphorylation of glucose to the glucose-6-phosphate dehydrogenase and 6-phospho-gluconate dehydrogenase reactions. Estimations were made at glucose concentrations of 0.5 mM and 100 mM glucose.

RESULTS AND DISCUSSION

ATP content: The contents of ATP in the different islet samples are shown in Table 1. The highest concentrations were observed for the "in vivo" preparations and they agree well with those previously reported (6,10). The isolation procedure markedly decreased the ATP content of the islets, perhaps due to ischemia during the collagenase digestion. The ATP contents of the cultured islets after the harvesting and washing procedures were below those found in freeze-dried islet sections. In islets cultured at a physiologic (6.1 mM) or a high (28 mM) glucose concentration the ATP content was equal, while that of the low-glucose (3.3 mM) cultured islets was somewhat decreased.

The importance of maintaining a sufficient concentration of ATP in the B-cells during the synthesis and release of insulin has been emphasized (12, 13). Both these B-cell functions, however, apparently continue even with the lower steady state level of ATP found in the cultured islets (3), suggesting that the artificial conditions during culture were not injurious to the islets. Furthermore, the ATP content found in the freeze-dried sections reflects almost exclusively the situation in the centrally located B-cells, whereas the in vitro preparations consist of a mixed islet cell population. Other studies in this laboratory have shown that the total sum of adenine nucleotides in cultured islets is close to that found in vivo (13), indicating a well preserved metabolism of the cultured islets.

Glycogen content: The glycogen concentrations of the different islet samples are shown in Table 1. The freshly isolated islets and those cultured at the lower glucose concentrations contained approximately the same amount of gly-

Table 1: ATP and glycogen concentrations and rates of glucose phosphorylation in cultured pancreatic islets.

ISLET PREPARATION	ATP CONTENT	GLYCOGEN CONTENT	GLUCOSE PHOSPHORYLATION		
			0.5 mM	100 mM	100 mM–0.5 mM
	(mMoles/kg dry weight)		(mMoles/kg dry weight per hour)		
"In vivo"	12.3 ± 0.7 (5)				
Islets cultured in 3.3 mM glucose	6.5 ± 0.4 (9)	3.0 ± 0.6 (8)	104 ± 6 (10)	178 ± 17 (10)	75 ± 16 (10)
Islets cultured in 6.1 mM glucose	8.5 ± 0.6 (13)	1.9 ± 0.2 (10)	88 ± 7 (17)	169 ± 9 (17)	81 ± 10 (17)
Islets cultured in 28 mM glucose	8.2 ± 0.7 (13)	32.7 ± 4.8 (14)	97 ± 9 (17)	227 ± 15 (17)	131 ± 11 (17)
Non-cultured islets	5.2 ± 0.7 (6)	2.7 ± 0.5 (9)			

Islets which were freshly isolated or cultured for seven days as well as sections of rapidly frozen pieces of pancreas ("in vivo" preparations) were used. Glycogen concentrations are given as mMoles glycosyl units/kg dry weight and glucose phosphorylation rates as mMoles glucose phosphorylated/kg dry weight per hour at 0.5 mM and 100 mM glucose. The calculated differences in phosphorylation rates at these two concentrations are also given. Results are given as mean values ± S.E.M.. Number of observations are given within parentheses; each observation represents the mean of 3-6 determinations on each islet preparation.

cogen, which conforms to previous reports on the existence of glycogen in normally functioning B-cells (10,14). Islets cultured at a high glucose concentration showed a large increase of their glycogen stores, which were about ten times higher than in those cultured at the lower glucose concentrations ($p < 0.001$). It should be noted in this context that the assay used actually measured the sum of glycogen and glucose-6-phosphate. The concentration of the latter, however, was too low to contribute significantly to the recorded differences in glycogen contents of the different islet samples.

Deposition of glycogen in the islet cells has been reported in animals subjected to different types of hyperglycemia (14,15). In the present study the increase of the glycogen content of the high-glucose cultured islets was observed already on the first day of culture, and further investigations have shown that these glycogen deposits are consumed, if the islets are incubated in a glucose free medium (Riesenfeld, T., Andersson, A., and Borglund, E.: unpublished data). Whether these elevated levels of glycogen of islets subjected to prolonged periods of glucose stimulation reflect adaptive changes at the enzyme level of the glycogen synthesis (16) or rather can be explained by a continuous high access to glucose-6-phosphate remains to be settled. The present experimental model would be suitable for studies of this and other problems related to the mechanism of glycogen deposition in the islet cells.

Glucose phosphorylating enzyme activities: The method used here for the determination of two different glucose phosphorylating activities by measurement at a low (0.5 mM) and a high (100 mM) glucose concentration was first described for liver homogenates by Viñuela et al. (17), and has since been widely used for studies of the various liver hexokinases. It has recently been utilized also for mouse pancreatic islets (10,18). Using a radioactive method for measurements of ATP-D-glucose 6-phosphotransferase Ashcroft et al. (19) detected two phosphotransferase activities in mouse islet homogenates, one with a low K_m for glucose and another with a high K_m. The present observa-

tions on the glucose phosphorylation rates (Table 1) of cultured pancreatic islets confirm these observations in that all groups of islets had a significant additive phosphorylating activity ($p < 0.001$), when the assay was performed at 100 mM glucose. The glucose phosphorylating rates at 0.5 mM glucose in the different groups of cultured islets were similar. However, islets cultured in 28 mM glucose had a higher phosphorylating activity than those cultured in 3.3 and 6.1 mM glucose, when the assay was performed at the higher glucose concentration. The difference between the phosphorylating rates at 100 mM and 0.5 mM glucose was calculated in each experiment in order to estimate the high K_m phosphorylating activity. These results suggest that the high glucose cultured islets have a significantly increased high K_m hexokinase activity ($p < 0.01$) and further support the view that there are both low and high K_m hexokinases in the mouse pancreatic islets.

It appears from the present observations that long-term elevations of the extracellular glucose concentrations may induce glucose phosphorylating enzyme activity in the B-cells. In previous reports a regulatory influence of a high K_m hexokinase on the rate of glucose phosphorylation and insulin release in the B-cells has been suggested (20). We have recently demonstrated a marked alteration of the dose response curve relating glucose oxidation to extracellular glucose concentration in islets cultured for 6 days in a medium with a high glucose concentration (3). It is an intriguing possibility that altered enzyme activities may also be the cause of the increased glucose sensitivity of the insulin release, which follows prolonged periods of glucose stimulation (21,22).

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