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FASTING-INDUCED IMPAIRMENT OF GLUCOSE-1,6-BISPHOSPHATE SYNTHESIS IN PANCREATIC ISLETS

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SUMMARY : In pancreatic islets removed from rats fasted for 48 hours, the insulin secretory response to glucose is decreased. Although the activity of phosphoglucomutase is unaffected by fasting, the decrease in glucose-stimulated insulin release coincides with a suppression of the glucose-induced increment in both glucose-1,6-P_2 content and lactate or pyruvate output. These findings are compatible with a regulatory role of glucose-1,6-P_2 in the control of glycolysis in pancreatic islets.

It is now generally believed that the capacity of nutrients to stimulate insulin release from the pancreatic B-cell reflects their capacity to stimulate nutrient catabolism in the islet cells (1, 2). In the process of glucose-induced insulin release, the regulation of glucose metabolism apparently depends on a series of tightly interconnected reactions, in which the hexose serves as a precursor of both glycolytic intermediates and suitable activators of phosphofructokinase (3, 4). In the latter respect, glucose-1,6-bisphosphate possibly in concert with fructose-2,6-bisphosphate may play an essential role. For instance, the higher rate of generation of aldohexose-1,6-bisphosphates in islets exposed to the α-anomers of either D-glucose or D-mannose, as distinct from the corresponding β-anomers, is thought to play a critical role in the anomeric specificity of the insulin secretory response to these hexoses (4-7). Islets removed from fasted rats are poorly sensitive to glucose, whereas the secretory response to other secretagogues is much less or

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not affected (8, 9). This situation was attributed, in part at least, to a decrease in the activity of key glycolytic enzymes (e.g. glucokinase and phosphofructokinase) in the islets removed from fasted, as distinct from fed, rats (10). With this information in mind, we have investigated, in the present study, whether fasting affects the glucose-1,6-bisphosphate content of glucose-stimulated islets.

MATERIALS AND METHODS

Pancreatic islets were isolated by the collagenase technique (11) from the pancreas of albino rats given free access to food or deprived of food for 48 hours. In each experiment, the pancreases removed from 3 or more animals in the same nutritional state were minced and mixed together. Each individual observation refers to a distinct batch of islets. The methods used to measure to islet protein content (12), insulin content (13), insulin release (14), glutamate dehydrogenase activity (15), phosphoglucomutase activity (5), glucose-1,6-P_2 content (4) and output of lactate (16) and pyruvate (17) were all described in detail in prior publications. All results are expressed as the mean ($\pm$ SEM) together with the number of individual observations (n). The statistical significance of differences between control and experimental data was tested by use of Student's t-test.

RESULTS

The islet protein content was not significantly different in islets removed from fed and fasted rats, respectively (Table 1). In a large series of experiments, there was a minor decrease ($-$ 11.3 $\pm$ 5.0 %) in the insulin content of the islets from fasted rats. Over 30 min incubation, the secretory response to glucose (16.7 mM) was severely impaired in fasted rats. Fasting failed to affect the activity of glutamate dehydrogenase in islet homogenates. Likewise, fasting failed to affect the activity of phosphoglucomutase in islet homogenates, whether in the absence or presence of glucose-1,6-P_2 (Table 1).

When islets from fed rats were incubated for 30 min at a low (2.8 mM) or high (16.7 mM) glucose concentration, a threefold increase in both the islet content of glucose-1,6-P_2 and output of lactic acid and a twofold increase in pyruvate acid

Table 1. Islet function in fed and fasted rats

	Fed rats	Fasted rats	P
Islet protein content (ng/islet)	745 ± 88 (6)	721 ± 43 (6)	N.S.
Islet insulin content (μU/islet)	641 ± 21 (135)	566 ± 24 (100)	< 0.02
Insulin output (μU/30 min per islet)			
D-glucose (2.8 mM)	16.6 ± 1.4 (82)	18.5 ± 2.0 (74)	N.S.
D-glucose (16.7 mM)	95.6 ± 3.3 (39)	37.3 ± 2.4 (36)	< 0.001
Glutamate dehydrogenase activity (pmol/min per μg islet protein)			
α-KG (7 mM) + NH_4 (50 mM) + NADH (0.2 mM)	161.4 ± 4.6 (3)	169.6 ± 15.7 (3)	N.S.
Phosphoglucomutase activity (pmol/min per μg islet protein)			
Glucose-1-P (0.2 mM)	4.2 ± 0.4 (3)	4.0 ± 0.4 (3)	N.S.
Glucose-1-P (0.2 mM) + Glucose-1,6-P_2 (25 μM)	75.2 ± 1.3 (3)	70.2 ± 3.8 (3)	N.S.
Glucose-1,6-P_2 islet content (fmol/islet at 30th min of incubation)			
D-glucose (2.8 mM)	16.3 ± 4.5 (12)	13.2 ± 2.6 (12)	N.S.
D-glucose (16.7 mM)	49.1 ± 5.1 (12)	18.0 ± 4.7 (12)	< 0.001
Lactic acid release (pmol/30 min per islet)			
D-glucose (2.8 mM)	34.3 ± 2.1 (11)	49.8 ± 10.1 (9)	N.S.
D-glucose (16.7 mM)	109.9 ± 7.2 (11)	44.3 ± 6.8 (11)	< 0.001
Pyruvic acid release (pmol/30 min per islet)			
D-glucose (2.8 mM)	6.1 ± 0.8 (11)	6.5 ± 0.9 (10)	N.S.
D-glucose (16.7 mM)	14.2 ± 2.0 (12)	5.9 ± 0.7 (12)	< 0.001

Mean values (± SEM) are shown together with the number of individual observations (in parentheses) and significance (P) of differences between fed and fasted rats (N.S. : not significant).

output was observed in response to the rise in glucose content ($P < 0.005$ in all cases). The lactate/pyruvate ratio averaged 6.65 ± 0.97 and 10.04 ± 1.69 (n = 10-11) at low and high glucose concentration, respectively ($P < 0.02$ by paired comparison). At low glucose concentration (2.8 mM), the glucose-1,6-P_2 content and the output of both lactic and pyruvic acid were not significantly different in islets from fed and fasted rats, respectively, there being a trend towards higher lactic acid output in the fasted animals. The islets from fasted rats failed to display any significant increase in either their glucose-

1,6-P_2 content or output of lactic and pyruvic acid when the glucose concentration was raised from 2.8 to 16.7 mM ($P > 0.3$ in all cases). Likewise, in the islets from fasted rats, the lactate/pyruvate ratio was not significantly different at low (8.10 ± 1.42) and high (8.06 ± 0.61) glucose concentration, respectively.

DISCUSSION

The present findings confirm that fasting impairs the insulin secretory response to glucose in rat pancreatic islets, as first reported 16 years ago (8). Secretory and metabolic variables were here purposedly measured after a relatively short period (30 min) of exposure of the islets to glucose (16.7 mM), because the fasting-induced anomaly in islet function tends to fade out during prolonged exposure to high concentrations of glucose (8). Although fasting failed to affect the activity of phosphoglucomutase, the glucose-induced increases in both glucose-1,6-P_2 content and output of lactic and pyruvic acid were severely impaired in islets removed from fasted rats. We have indicated elsewhere our reasons to believe that glucose-1,6-P_2 is formed in intact islets mainly from glucose-6-P (or glucose-1-P) and a suitable phosphate donor (e.g. fructose-1,6-P_2 or 1,3-diphosphoglycerate) in a reaction catalyzed by phosphoglucomutase (5). Therefore, the present finding that fasting suppresses the glucose-induced increment in glucose-1,6-P_2 content, whilst failing to affect the activity of phosphoglucomutase is likely to be due, in part at least, to a decrease in the rate of glucose phosphorylation to glucose-6-P itself attributable to the fasting-induced adaptation of glucokinase (10, 18).

After 48-72 hours of fasting, the activity of glucokinase in rat pancreatic islets is decreased by one to two thirds (10, 18). According to Matschinsky et al. (18), this change in acti-

vity is sufficient to explain the alterations of glucose utilization that occurs in intact islets during starvation. If the activity of glucokinase were to represent the rate-limiting factor in islet glucose metabolism, the glucose-stimulated rate of lactate or pyruvate output would be expected to be decreased by no more than 33-67 % in islets removed from fasted rats. The fasting-induced reduction in lactate and pyruvate output was actually more severe. This suggests that, in islets from fasted rats, a reduced availability of fructose-1,6-P_2 and/or 1,3-diphosphoglycerate, secondary to both the repression of glucokinase and phosphofructokinase (10) and the poor activation of the latter enzyme, may have hampered the synthesis of glucose-1,6-P_2. Such an interpretation is supported by the fact that, in mouse islets exposed to glucose (16.7 mM), fasting decreases the tissue content in fructose-1,6-P_2 and triose-P even when the glucose-6-P content appears unaffected (19, 20). The presents results thus illustrate a situation in which phosphofructokinase is apparently not sufficiently activated to keep pace with the rate of glucose-6-P formation.

In summary, the present findings suggest that the fasting-induced alteration of islet glucose metabolism may be due to impairment of the dual role of glucose as a precursor of both glycolytic intermediates and suitable activator(s) of phosphofructokinase. The present findings are compatible, therefore, with the view that activation of phosphofructokinase is indeed required in order for the rate of conversion of fructose-6-P to fructose-1,6-P_2 to keep pace with the rate of glucose phosphorylation in intact islets (3).

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