DISSOCIATION OF THE INSULIN RELEASING AND THE METABOLIC FUNCTIONS OF HEXOSES IN ISLETS OF LANGERHANS

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SUMMARY: It is demonstrated that the fuel and insulin releasing function of glucose can be dissociated. Glycolysis can be blocked without apparent effect on secretion due to glucose. Conversely, glycolysis can be enhanced by various hexoses without triggering hormone secretion, even though the releasing mechanism is clearly functioning. The enhancement of glycolysis by hexoses is attributed to the need for extra energy to support increased sodium pumping. This is ascribed to a high intracellular sodium load caused by Na^+ dependent transmembraneous sugar transport.

Ordinarily, when glucose stimulates insulin release from pancreatic islets there is a concomitant increase in glycolytic flux and O_2 consumption (1, 2). However, it is not clear that the increased metabolism is a necessary prerequisite for glucose to exert its releasing action. Instead, our current working hypothesis is that glucose acts by stimulating glucoreceptor molecules located in the β-cell membrane (3). The present results lend further support to this hypothesis.

METHODS AND MATERIALS: The study was carried out with rat islets isolated by a version of the collagenase method (4). The islets were perifused *in vitro* according to the method of Tomita and Lacy (5), using commercial micro chambers in which the islets are supported on a millipore filter. Batches of 100 islets were perifused with medium containing 115 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1 mM $MgCl_2$, 2.2 mM $CaCl_2$ and .5% bovine serum albumin. The solutions were gassed continuously with a mixture of 95% oxygen and 5% CO_2. Flow rates were adjusted close to 1 ml/min; fractions were collected at intervals of 1 or 5 min. After a control period of 45 min with 5.5 mM glucose in the medium, secretion was initiated by switching to 27.5 mM glucose. This high level of glucose was maintained for 45 min. Insulin was measured by the method of Hales and Randle (6) using a porcine insulin standard.

Lactate formation was usually measured by incubating batches of 50 islets in 100 µ l of Krebs-Henseleit solution. For low rates of lactate production the number of islets was raised to 100 and the incubation volume reduced to 60 µl. The tubes (5 x 70 mm) were equilibrated with a mixture of 95% O_2 and 5% CO_2, stoppered and incubated at 37°, with shaking at 75 strokes per min. After an initial phase of 45 min, the medium was changed by quantitative removal of the solution with a constriction pipette. A second incubation period of 45 min followed, after adding fresh medium. The samples were acidified with 10 µl of 1 N HCl and heated for 15 min at 75° before analysis.

Lactate was measured in duplicate in aliquots of 25 µl using a fluorometric modification (7) of the enzymatic method described by Noll (8). As little as 10^{-10} moles of lactate can be determined accurately.

All reagents used were analytical grade.

RESULTS AND DISCUSSION: Exposure of islets to glucose leads to concentration dependent increase of lactate formation (Fig. 1). The curve depicting the concentration dependency of the lactate production is sigmoidal with an inflection at about 16.5 mM glucose. Iodoacetate at 0.2 mM concentration blocked lactate formation with either low or high glucose. Approximately 45 min elapsed before the inhibition was complete. (Compare the results of the first and second incubation period).

Glycolysis was also stimulated by exposure of islets to 3-O-methyl glucose and galactose provided the incubation medium contained a basal concentration (5.5 mM) of glucose (Fig. 2). (This low level of glucose by itself causes negligible glycolysis). L-glucose and sucrose were not capable of stimulating glycolysis using these conditions. Lactate formation due to added 3-O-methyl glucose or galactose was substantial; the rates approached those observed when the extra sugar above the 5.5 mM basal level was glucose. The rate of lactate production by islet tissue seems to parallel the rate of glucose consumption as well as of CO_2 production and amounts to approximately half the glucose used (compare (1)). Therefore it seems to be a valid measure of glucose metabolism.

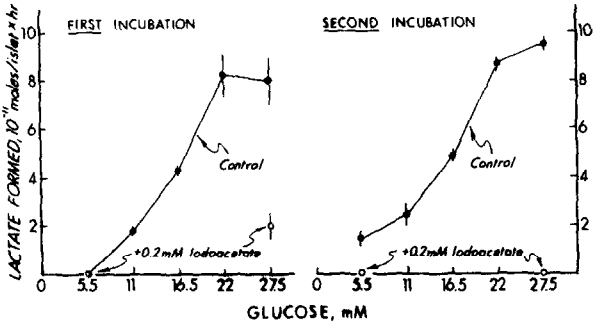

Fig. 1.

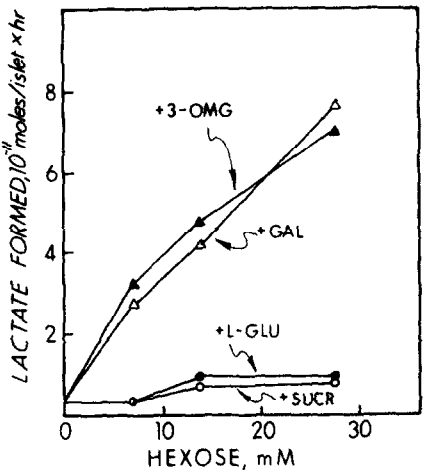

Fig. 2.

Figure 1. Glucose dependency of lactate production by pancreatic islets and the effect of iodoacetate. For experimental details see methodology. The means of at least three experiments ± standard errors are recorded.

Figure 2. Stimulation of glycolysis of islets by non-metabolizable sugars. Batches of 50 islets were incubated at 37° for two consecutive periods of 45 min with the different levels of the sugar, together with 5.5 mM D-glucose. The lactate production was determined separately for the two periods and the average hourly rates were calculated from these two values. Each point represents the mean of two experiments.

The enhancement of lactate formation by glucose on one hand and by galactose and 3-O-methyl glucose on the other seem to have a common cause. We have found that the stimulation of glycolysis by either glucose or 3-O-methyl glucose can be inhibited to the same extent by compounds which inhibit sugar transport (e.g. phlorizin (9) and cytochalasin B (10)), by ouabain and by various conditions of sodium deficiency (Table I). These results suggest that the metabolic effects seen with various hexoses are the consequence of similar ion dependent sugar transport systems. The inhibition of glycolysis in the case of ouabain is presumably due to a decrease in energy requirement (to drive the Na^+ pump) (11); in the case of cytochalasin B it is presumably due to blockade of glucose transport (10). It seems most significant that ouabain (12) and cytochalasin B (13) have been shown to potentiate glucose

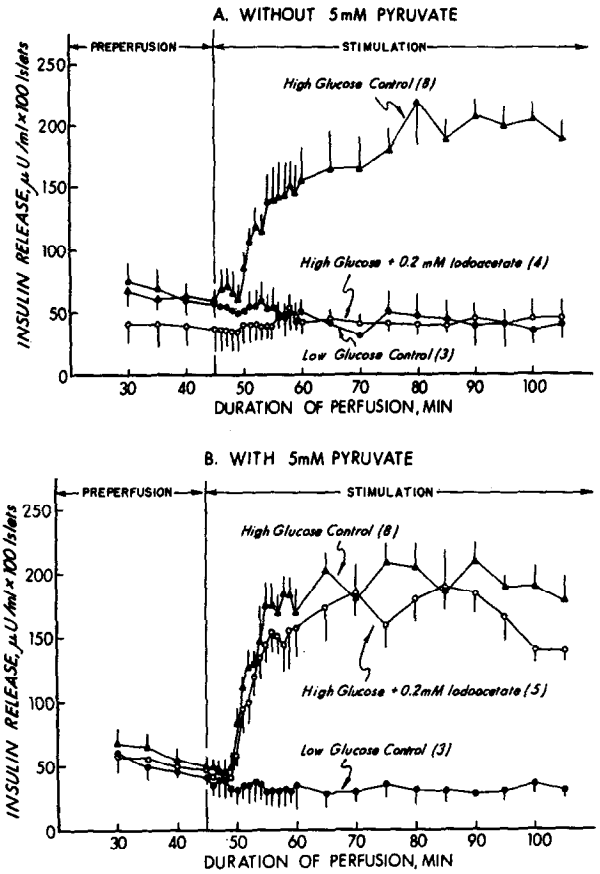

Figure 3A and 3B. Effect of iodoacetate on glucose provoked insulin release in the presence or absence of pyruvate. Batches of 100 islets each were perifused as described in the methods section. The flow rate was 1 ml/min. Pyruvate was absent in A and present in B at 5 mM concentration throughout. Low glucose controls had 5.5 mM glucose throughout. High glucose samples had 5.5 mM glucose in the preperifusion period and 27.5 mM glucose during stimulation, with or without iodoacetate present throughout as indicated. The means of indicated numbers of experiments are given. Standard errors are recorded, except where this would interfere with clarity.

induced insulin release. Inhibition of glucose metabolism by sodium deficiency has been observed previously by Ashcroft et al. (1).

The release of insulin by 27.5 mM glucose was not prevented by blocking glycolytic flux with 0.2 mM iodoacetate, provided 5 mM pyruvate was included in the perfusion medium as an alternate fuel (Figures 3A and B). This level of pyruvate, by itself, when added to 5.5 mM glucose, caused no secretion of the hormone. Furthermore, pyruvate did not interfere with the inhibition of

Table 1: Effects of phlorizin, cytochalasin B, ouabain and Na^+-deficiency on the stimulation of glycolysis by glucose and 3-O-methyl glucose

Incubation Conditions	Glucose, 27.5 mM (16.5 mM in CB studies)	3-OMG, 27.5 mM Glucose, 5.5 mM
	lactate formation, picomoles/islet x hour	
Phlorizin		
none	78.0 ± 7.0 (3)	67.2 ± 5.3 (6)
0.5 mM	-	47.1 (2)
1.5 "	44.5 ± 6.5 (3)	30.8 ± 2.2 (3)
4.5 "	10.7 ± 0.7 (3)	2.2 (2)
Cytochalasin B		
none, 0.007% solvent	56.2 ± 1.4 (10)	50.8 ± 2.3 (3)
10.5 µM	38.4 ± 1.7 (3)	33.9 ± 4.9 (3)
21.0 "	20.4 ± 3.8 (3)	18.6 ± 1.7 (3)
63.0 "	17.7 ± 4.3 (3)	6.2 ± 1.7 (3)
Ouabain		
none	78.0 ± 7.0 (3)	67.2 ± 5.3 (6)
0.2 mM	-	44.5 ± 5.1 (3)
1.0 "	38.2 ± 2.4 (3)	29.6 ± 6.1 (3)
5.0 "	10.4 ± 1.5 (3)	6.6 ± 0.7 (3)
Sodium replacement		
Control a	65.0 ± 4.4 (5)	46.7 ± 3.4 (5)
LiCl	30.3 ± 3.5 (3)	16.4 ± 0.1 (3)
Control b for Li^+	61.0 ± 7.7 (3)	49 (2)
Choline chloride	28.1 ± 4.6 (3)	21.3 ± 0.7 (3)
Control b for choline	55.2 ± 7.0 (3)	43.8 (2)
KCl	30.0 ± 4.7 (3)	26.5 ± 6.7 (3)
Control b for K^+	63.0 ±12.2 (3)	48.1 (2)

For experiments with phlorizin, cytochalasin B (CB) and ouabain, the rate of lactate formation is the average of two incubation periods (see Methods and Fig. 1). The solvent for CB (Imperial Chemical Industries, Macclesfield, England) was dimethyl formamide which at the concentrations used (0.0012 to 0.007%) inhibited lactate formation only slightly (15% or less). When studying Na^+ replacement, $KHCO_3$ was substituted for $NaHCO_3$ in all solutions and NaCl was replaced by either LiCl, choline chloride or KCl. For efficient removal of Na^+, islets were incubated for two 30 min periods in the respective glucose-free Na^+ deficient media; lactate formation was finally determined in a third 45 min period in which 27.5 mM glucose or 3-OMG was added, the latter together with 5.5 mM glucose. Two types of controls were performed in the Na^+ deficiency studies: Type a controls were treated like the experimental samples, except for the presence of Na^+, i.e. no sugar was present for the first two 30 min periods. Type b controls were run after the periods of Na^+ deficiency to determine if the islets were still viable. For this purpose, the Na^+ deficient medium was removed, the islets were washed with 300 µl of medium containing normal NaCl and then incubated with hexoses in 100 µl of medium with normal NaCl. The means of an indicated number of experiments are recorded ± SEM for the number of samples in parentheses.

glycolysis by iodoacetate (not shown). A differential effect of iodoacetate was observed earlier by Loeffler who found that a 0.5 mM concentration of the inhibitor completely blocked glucose oxidation, but decreased insulin release due to glucose by only 30 - 40% (14).

Galactose and 3-O-methyl glucose at 27.5 mM could not accomplish insulin release when perfused together with 5.5 mM glucose. Sucrose and L-glucose were similarly ineffective. On the other hand, galactose and 3-O-methyl glucose do not interfere with glucose provoked insulin secretion. (None of these negative results are shown here).

Since galactose and 3-O-methyl glucose augment glycolysis without causing release, and since a number of agents block glycolysis without interfering with glucose provoked release, it is inescapable that the processes which finally lead to the emiocytosis of the β-granules cannot be triggered or blocked by merely changing the glycolytic flux.

Using an entirely different approach it was concluded earlier that a change of flux through metabolic pathways seemed an unlikely cause of insulin release, since changes in levels of glycolytic metabolites and cofactors either did not occur when secretion was stimulated or were not related to release when they did occur (for references see (3)). In view of these negative results and the present data, which do not support the metabolic theory, as well as other pertinent findings (3), the glucoreceptor hypothesis, that glucose exerts a direct releasing action on the β-cell membrane, becomes increasingly attractive.

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