Differential Effect of Alpha- and Beta-D-Glucose on Protection Against Alloxan Toxicity in Isolated Islets

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SUMMARY

Using isolated rat islets of Langerhans perifused in vitro, the differential effect of alpha- and beta-D-glucose on the protection against alloxan toxicity was studied in terms of subsequent glucose-induced insulin secretion. Alpha-D-glucose (5,3 and 1.5 mg/ml) produced similar protection to that by a corresponding concentration of equilibrated D-glucose, and was significantly greater than that by beta-D-glucose. There was no apparent protection against alloxan toxicity by a 1 mg/ml medium of either equilibrated D-glucose or beta-D-glucose. However, the addition of 1 mg/ml of alpha-D-glucose to the alloxan solution provided the islets with partial but distinct insulin secretion. These results indicate that stereospecificity plays a role in protection of the islets against alloxan toxicity at the beta cell membrane. The stereospecificity of alpha-D-glucose appears greater for an alloxan site than for an insulin triggering site.

The in vitro perifusion of isolated islets with alloxan (20 mg%) for five minutes abolishes subsequent glucose-induced insulin secretion (1). Alpha-D-glucose injection in rats has been shown to provide the animals with better protection against alloxan-induced hyperglycemia than beta-D-glucose (2,3). Similarly, better protection was observed with alpha-3-O-methyl-glucose injection in rats than with the beta-anomer (4). In isolated islets incubated in vitro with alloxan, significantly greater protection of insulin secretion was reported with alpha-D-glucose (3.0 mg/ml) as compared with beta-D-glucose (5). The insulin secretion per se was also more by alpha-D-glucose than by beta-D-glucose in a 3 mg/ml medium, suggesting that beta cells distinguish alpha and beta anomers of D-glucose for triggering insulin secretion (6). These observations further support the existence of stereospecific glucoreceptor(s) for insulin secretion, which also interreacts with the alloxan site at the beta cell membrane

In addition, alpha-D-glucose not only induced more insulin secretion but also produced more inhibition of glucagon secretion than beta-D-glucose in the perfused rat pancreas (7).

Grill and Cerasi have disclosed that the differential effect of alpha- and beta-D-glucose on insulin secretion is modulated by cyclic AMP generation in isolated islets (8). These facts are all in favor of the stereospecificity for D-glucose in triggering insulin secretion via a glucoreceptor and the adenylate cyclase-cyclic AMP system at the beta cell membrane. The purpose of the present study was to determine the differential effect of alpha- and beta-D-glucose against alloxan toxicity with the use of isolated islet perifusion and to further clarify the stereospecificity of D-glucose at different concentrations in the presence of alloxan.

MATERIALS AND METHODS

Perifusion of Isolated Islets

More than two hundred islets were isolated from the pancreas of one male Sprague-Dawley rat (250-350 gm) using the standard collagenase technique (9). The perifusion system utilized was identical to that described in the studies on alloxan inhibition of insulin secretion (1) except for three chambers perifused simultaneously, each containing 70 to 100 islets in the perifusion chamber (10). Insulin was determined by the radioimmunoassay using crystalline pork insulin (Eli Lilly) as the standard and [125] I labeled pork insulin (New England Nuclear) as the tracer (11). The rate of insulin secretion was expressed as μU/islet/min., and the total glucose-induced insulin secretion was calculated as mU /100 islets/60 min. (1).

Exposure to Alloxan

Alloxan monohydrate (20mg%, Sigma Chemical, St. Louis, Missouri) and either alpha- or beta-D-glucose (both Sigma Chemical) were dissolved in previously warmed and gassed perifusion medium immediately before use for perifusion. The dead space of the tubing connecting the reservoir of medium with the perifusion chamber was about one minute.

RESULTS

The differential effect of anomers of D-glucose was summarized in Table 1. The dose-dependent protection by different concentrations of equilibrated D-glucose (alpha-D-glucose: 36%, beta-D-glucose: 64%) was similar to that previously reported (1). The relative protection by 3 mg/ml, 1.5 mg/ml, and 1.0 mg/ml D-glucose solution were 88.2%, 74.8%, and 15.6% of a 5 mg/ml D-glucose solution, respectively. There was no significant difference in protective effects between

Table 1. Differential effect of alpha- and beta-D-glucose on protection against alloxan toxicity.

Group of Exp.	*Glucose 1 mg/ml Insulin secretion mU/100 islets/45 min. mean ± SEM	Five minute exposure to alloxan (20 mg%) D-glucose	mg/ml	**Glucose 5 mg/ml Insulin secretion mU/100 islets/60 min. mean ± SEM	Percent Protection	P values for effect
I	3.163 ± 0.780	equilibrated	5.0	23.043 ± 1.003 (4)	100%	
	2.766 ± 0.397	alpha-	5.0	22.646 ± 1.098 (4)	98.3	$p < 0.80$
	2.615 ± 0.169	beta-	5.0	18.326 ± 1.979 (4)	79.5	$p < 0.05$
II	3.167 ± 0.618	equilibrated	3.0	20.318 ± 1.157 (4)	88.2	
	3.123 ± 0.409	alpha-	3.0	21.609 ± 0.776 (4)	93.8	$p < 0.30$
	2.138 ± 0.150	beta-	3.0	14.650 ± 1.876 (4)	63.6	$p < 0.02$
III	3.183 ± 0.465	equilibrated	1.5	17.231 ± 1.080 (4)	74.8	
	2.982 ± 0.342	alpha-	1.5	17.388 ± 0.491 (4)	75.5	$p < 0.50$
	3.352 ± 0.336	beta-	1.5	11.751 ± 0.605 (4)	51.0	$p < 0.005$
IV	2.548 ± 0.322	equilibrated	1.0	3.592 ± 0.570 (4)	15.6	
	2.581 ± 0.434	alpha-	1.0	8.014 ± 0.962 (4)	34.8	$p < 0.005$
	2.678 ± 0.431	beta-	1.0	3.510 ± 0.956 (4)	15.2	insignificant

* The total baseline insulin secretion with a 1 mg/ml D-glucose before alloxan exposure was calculated as mU/100 islets/45 min.

** Total glucose (5 mg/ml) -induced insulin secreted in 60 minutes by 100 islets. Numbers in parentheses are the numbers of the experiments.

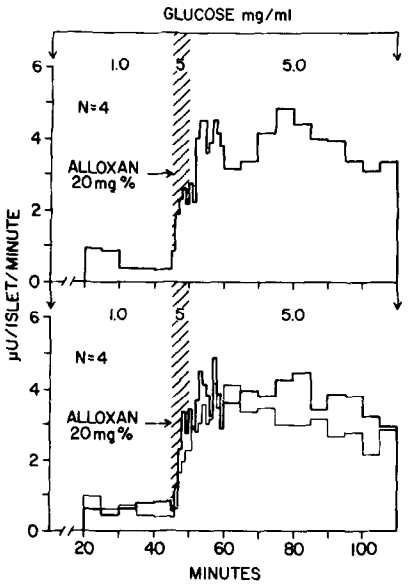

Fig. 1. Three perifusion chambers were perifused simultaneously with a 1 mg/ml solution for 45 minutes; then alloxan solution (20 mg%) was introduced for 5 min, followed by a 5 mg/ml glucose solution for 60 min.
Upper: Alloxan was dissolved in a 5 mg/ml equilibrated D-glucose solution.
Lower: Alloxan was dissolved in a 5 mg/ml alpha-D-glucose (thick line) or beta-D-glucose solution (thin line).

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equilibrated D-glucose and alpha-D-glucose in the range of 5.0 mg/ml and 1.5 mg/ml levels; however, the relative protection by beta-D-glucose was significantly lower than that by equilibrated D-glucose and alpha-D-glucose (Table 1). Fig. 1 shows a glucose-induced insulin secretory profile after exposure to alloxan with a 5 mg/ml D-glucose solution. Stimulated insulin secretion started during exposure to alloxan in all of the D-glucose media, but subsequent first and second phase secretion was more prominent with equilibrated D-glucose and alpha-D-glucose as compared with beta-D-glucose. A similar secretory profile was also observed with a 3 mg/ml and 1.5 mg/ml D-glucose medium (Fig 2). It was also noticed that a slightly higher insulin secretion was obtained by alpha-D-glucose than by equilibrated D-glucose, when 3 mg/ml and 1.5 mg/ml concentrations were used for alloxan solution (Table 1).

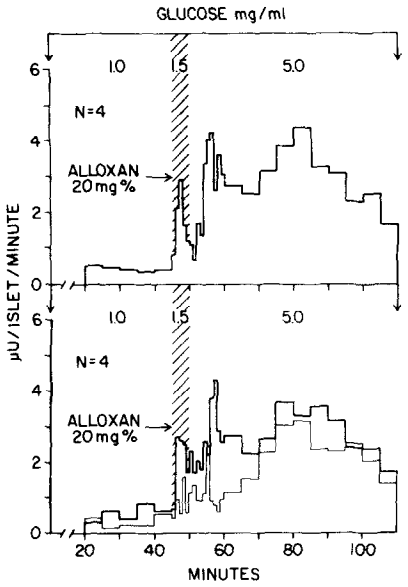

Fig 2. Three perifusion chambers were perifused simultaneously with a 1 mg/ml glucose solution for 45 minutes; then alloxan solution (20 mg%) was introduced for 5 minutes, followed by a 5 mg/ml glucose solution for 60 minutes.
Upper: Alloxan was dissolved in a 1.5 mg/ml equilibrated D-glucose solution.
Lower: Alloxan was dissolved in a 1.5 mg/ml alpha-D-glucose (thick line) or beta-D-glucose solution (thin line).

Fig. 3 depicts an early spike of insulin secretion during exposure to alloxan in a 1 mg/ml D-glucose medium. The secretory pattern was at, or slightly above, the baseline (<1 μU/islet/min.) by equilibrated and beta-D-glucose. However, alpha-D-glucose demonstrated an initial spike of insulin secretion during alloxan exposure as well as a subsequently sustained secretion above the baseline levels (Fig. 3). This selective partial protection by alpha-D-glucose was statistically significant ($p < 0.005$) when compared with equilibrated D-glucose (Table 1).

DISCUSSION

The present study has demonstrated that at all levels of glucose concentration tested there was increased protection against alloxan toxicity with alpha-D-glucose as compared with the beta anomer. This observation supports the presence of stereospecificity for D-glucose and completion with the alloxan

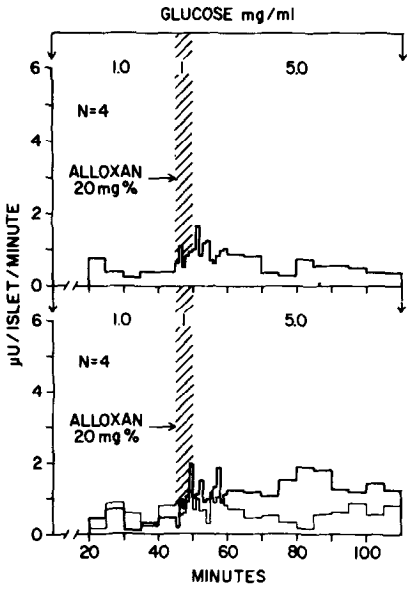

Fig. 3. Three perifusion chambers were perifused simultaneously with a 1 mg/ml glucose solution for 45 minutes; then alloxan solution (20 mg%) was introduced for 5 minutes, followed by a 5 mg/ml glucose solution for 60 minutes.
Upper: Alloxan was dissolved in a 1.0 mg/ml equilibrated D-glucose solution.
Lower: Alloxan was dissolved in a 1.0 mg/ml alpha-D-glucose (thick line) or beta-D-glucose solution (thin line).

site at the beta cell membrane. There was no significant difference in the protective effect between equilibrated and alpha-D-glucose in the range of 5.0 mg/ml and 1.5 mg/ml; and, this would further imply that beta-D-glucose as a major portion of equilitrated D-glucose (alpha-D-glucose: 36%, beta-D-glucose: 64%) potentiates the protective effect against alloxan toxicity in the presence of alpha-D-glucose as a minor portion (6).

At least three minute perifusion was necessary for alloxan (20 mg/%) to exert its constant inhibitory effect on subsequent insulin secretion (10). Three minute incubation was also shown to elevate islet tissue cAMP levels in response to a 3 mg/ml medium of alpha- or beta-D-glucose (8), implying that both the alloxan effect and the activation of the adenylate cyclase-cAMP system take place in this short period.

In the differential insulin secretion by alpha- and beta-D-glucose so far reported, there was little difference between the two anomers in a 3 mg/ml medium (13); but, in a 1.5 mg/ml medium a considerable difference was shown with apparent first and second phase secretion by alpha-D-glucose and absence of phases with the beta-anomer (12,13). Matschinsky et. al. also reported that alpha-D-glucose (0.54 and 1.8 mg/ml) induced more insulin secretion and more inhibition of glucagon secretion than the beta-anomer, which was added to the 10 mM mixture of amino acids (7).

Thus, the present study showed less protection by stimulatory levels (5,3 and 1.5 mg/ml) of beta-D-glucose than its insulin triggering effect in the reported literature. This probably implies more stereospecificity for alpha-D-glucose in the protective effect against alloxan toxicity than in the insulin triggering effect, including the adenylate cyclase-cAMP system. This difference between the protective effect and the insulin triggering effect is again in favor of the adenylate cyclase-cAMP system as a partial modulator for glucose-induced insulin secretion (14).

The in vitro protection against alloxan toxicity has been extended to include mannose and 3-O-methyl-glucose (1). Phlorizin, up to 1 mM, was also shown to protect the islets against alloxan effect while partially reducing the subsequent glucose-induced insulin secretion (10). Since 1 mM phlorizin does not interfere with glucose metabolism, but does reduce glucose transport to 50% in the isolated islets (15), glucose transport appears to be involved in alloxan toxicity. The results of the protective effect of cytochalasin B against the alloxan effect further suggest that the primary site alloxan toxicity was not the hexose carrier system per se, but was mediated on the beta-cell membrane in proximity to the hexose transport site (16).

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