GLUCOSE-INDUCED CYCLIC AMP ACCUMULATION IN RAT ISLETS OF LANGERHANS: PREFERENTIAL EFFECT OF THE ALPHA ANOMER

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1. Introduction

Recent work from our group [1,2] and others [3–5] has demonstrated that D-glucose is a powerful stimulator of cyclic 3′, 5′ adenosine monophosphate (cyclic AMP) accumulation in the pancreatic islets of rats and other species. The effect of glucose on cyclic AMP precedes, or is concomitant with, the stimulation of insulin release, and several characteristics of the dose relationship are similar for the insulin and cyclic AMP responses to glucose [2]. These findings suggest that glucose-induced cyclic AMP generation is an important factor in the coupling of the glucose stimulus to insulin release.

It is not known whether the glucose effects on cyclic AMP generation and insulin release are mediated by the metabolism of the hexose in the B-cells, or if the sugar acts on a specific, membrane-bound glucoreceptor which activates the adenylate cyclase. Two groups [6,7] have recently reported that the alpha anomer of D-glucose is more effective in releasing insulin than its beta anomer. The present report demonstrates that the islet cyclic AMP response to D-glucose shows a similar anomeric preference, suggesting that a glucoreceptor mechanism may indeed be operating in the B-cells.

2. Materials and methods

Pancreatic islets were isolated from fed male Sprague-Dawley rats (150–200 g) by a modified collagenase technique [2]. After isolation under a stereomicroscope the islets were preincubated in Krebs-Henseleit bicarbonate buffer (KHB) containing 2 mg/ml of bovine albumin, 0.6 mg/ml glucose (in anomeric equilibrium) and 100 μCi/ml of [^{3}H]-2-adenine for 60 min at 37° under continuous gasing with CO_2 : O_2 (5:95%). At its termination, the islets were washed thoroughly, twice with buffer containing glucose, and twice in the absence of glucose. Groups of 15–25 islets each were selected under the microscope and transferred to incubation vials, containing KHB. The incubations were started by the addition of glucose and the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) in 50 μl so as to give the chosen final concentrations (for IBMX always 0.1 mM). The incubations were terminated by immersing the tubes in boiling water for 3 min. The method of extraction and purification of the islet content of [^{3}H]cyclic AMP, which is based on the technique of Krishna [8], has been described in detail [2].

The respective anomers of glucose were dissolved in ice-cold buffer shortly prior to initiation of the incubations. The concentrations of the anomers were measured immediately, and after equilibrium for 2 hr at room temperature, using a Beckman glucose analyzer which measures specifically beta D-glucose by glucose oxidase, the difference between the two readings giving the initial concentration of alpha glucose.

Crude collagenase was purchased from Worthington Biochemical Corporation, Freehold, N.J., USA; cyclic AMP, and alpha and beta D-glucose from Sigma Chemicals, Saint Louis, Mo., USA; bovine albumin (Fraction V) from Armour Co., Eastbourne, U.K.; IBMX from Aldrich Co., Milwaukee, Wis., U.S.A. and [^{3}H]2-adenine (specific activity 31.7 Ci/mmol) from New England Nuclear, Dreiechenhain, West Germany.

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3. Results

Fig.1 shows that mutarotation occurs quite rapidly in the incubates. Already after 3 min of incubation (the time used in the majority of experiments) 30% of the initial alpha glucose was converted to its beta anomer, and at 12 min equilibrium was approached. These results are very similar to those presented by Niki et al. [6].

The time-course of the [^{3}H]cyclic AMP accumulation in islets in response to 0.6 and 1.5 mg/ml of glucose is presented in fig.2. It may be seen that, at 3 min, alpha glucose had a much stronger stimulatory effect than beta glucose. At longer time intervals, differences in stimulation induced by the rapidly mutarotating anomers diminished progressively (fig.1).

The effect of different concentrations of glucose anomers on the cyclic AMP response was tested in incubations of 3 min (table 1). In absolute terms, [^{3}H] cyclic AMP levels were different only when 1.5 mg/ml

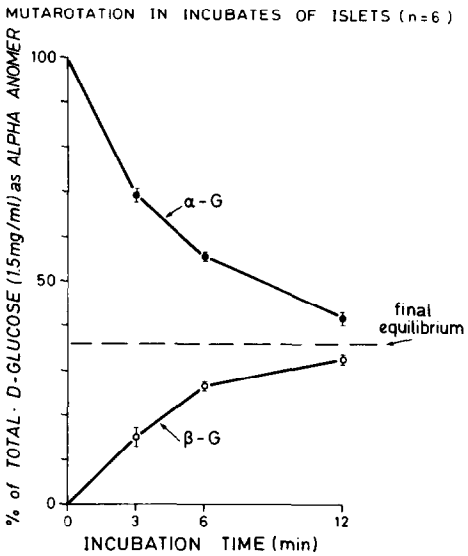

Fig.1. Spontaneous mutarotation in incubates at 37°C. Filled circles denote findings when the starting material was alpha-D-glucose, open circles when beta-D-glucose was used (both 1.5 mg/ml). The glucose concentrations were measured immediately, and after 2 hr at room temperature, with a Beckman glucose analyzer specific for beta-D-glucose. The difference between the two measurements gives the concentration of alpha-D-glucose. Broken line is the proportion of anomers at equilibrium. Mean ± S.E.M. of six experiments.

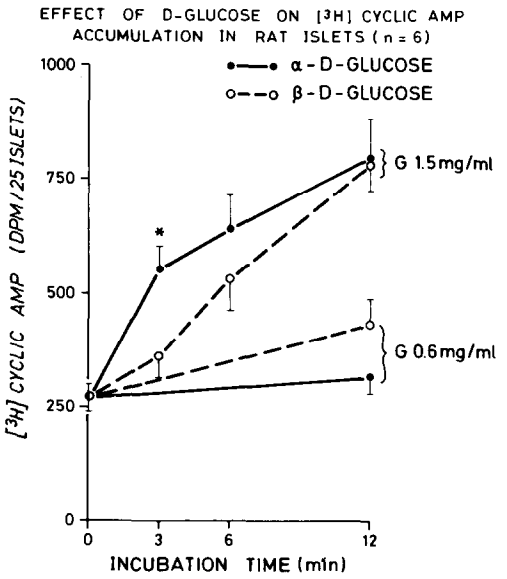

Fig.2. Time-course of [^{3}H]cyclic AMP accumulation in islets incubated with alpha-D-glucose (filled circles) or beta-D-glucose (open circles) at 0.6 and 1.5 mg/ml. * Denotes significant difference ($p < 0.05$) between the two anomers. For details, see text. Mean ± S.E.M. of six experiments. Anomer levels shown in fig.1.

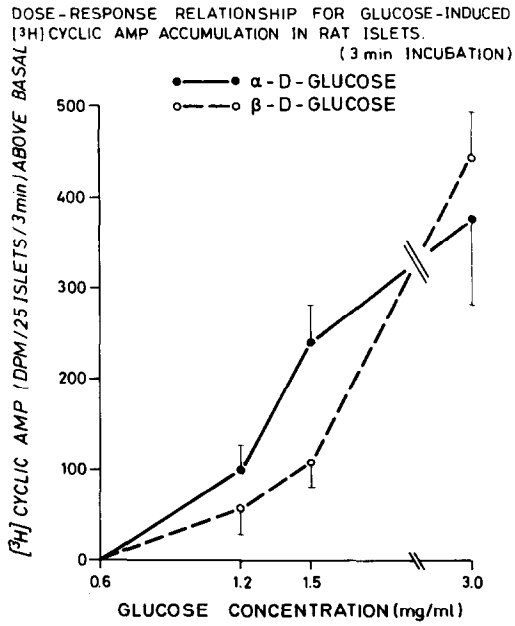

Fig.3. Incremental [^{3}H]cyclic AMP response to different glucose concentrations. Incubations of 3 min with alpha-D-glucose (filled circles) or beta-D-glucose (open circles). For statistical evaluations see table 1. Mean ± S.E.M. of six to twelve experiments.

Table 1
Effect of glucose anomers on [^{3}H]cyclic AMP accumulation (dpm/25 islets) in isolated rat islets, incubated for 3 min with D-glucose and 0.1 mM IBMX

	Glucose concentrations (mg/ml)				Differences		
	I 0.6 (n = 12)	II 1.2 (n = 6)	III 1.5 (n = 12)	IV 3.0 (n = 6)	II - I	III - I	IV - I
α-D-glucose	317 ± 36	460 ± 68	559 ± 52	746 ± 145	99 ± 27 ($P < 0.05$) *	242 ± 45 ($P < 0.005$)	378 ± 96 ($P < 0.02$)
β-D-glucose	331 ± 54	397 ± 100	425 ± 47	842 ± 130	57 ± 24 (N.S.)	111 ± 32 ($P < 0.05$)	445 ± 50 ($P < 0.001$)
P (α vs. β)	N.S.	N.S.	< 0.05	N.S.			

* P values within brackets refer to the significance of differences. For II and IV, the control values at 0.6 mg/ml were 368 ± 60 for α-, and 397 ± 100 for β-glucose.

of glucose was employed. However, if the *stimulatory* effect of glucose is considered (i.e. cyclic AMP accumulation above base-line), a significant response to 1.2 mg/ml was obtained only in the presence of alpha glucose. Furthermore, the response at 1.5 mg/ml was more marked with alpha glucose than with the beta anomer. At a high concentration of glucose (3.0 mg/ml), no difference was noted between the anomers.

The data of table 1 are summarized in fig.3, where dose-response curves for glucose-induced cyclic AMP accumulation are presented. It is seen that the sensitivity of the islets to alpha glucose is greater, but that a similar maximal response seems to be obtained with both anomers.

4. Discussion

Anomeric specificity of the pancreatic B-cell was first suggested by the group of Cahill [9] who demonstrated that alpha-D-glucose protected the islets from the toxic action of alloxan better than the beta anomer. Since then, three different groups, using the isolated perfused rat pancreas [7], static incubations of rat islets [6], and more recently perifused mouse islets [10], have shown that insulin release is preferentially stimulated by alpha-D-glucose. The specificity of the insulin response is not a complete one, however, since some response to beta-D-glucose could always be demonstrated, and at higher glucose concentrations the difference between the two anomers was reduced [7].

The present sets of experiments demonstrate that a similar preference for alpha-D-glucose is found in the cyclic AMP response of the islets, and adds another feature to the parallellism between the insulin and cyclic AMP responses on stimulation by glucose [2].

Our data probably underestimate the difference between the stimulatory effects of alpha and beta glucose on cyclic AMP accumulation, since as shown in fig.1, even during short incubations (3 min) a considerable proportion of glucose is converted into its other anomeric form. Thus, in experiments with beta glucose some of the cyclic AMP response may be due to the presence of 15% of alpha glucose, and in the alpha glucose studies the actual concentration of the anomer was 30% below the expected one. This is also demonstrated by the fact that the differences diminished rapidly with time (fig.2), probably due to mutarotation.

The findings presented in table 1 and fig.3 suggest that the difference in islet cyclic AMP response to alpha and beta glucose is the reflection of differences in the sensitivity of the adenylate cyclase–cyclic AMP system to the respective glucose anomers. Indeed, the maximal response of the islets was not different, whereas the apparent glucose threshold of the stimulation was lower for alpha glucose (1.2 vs. 1.5 mg/ml), as was the calculated 50% maximally effective concentration (around 1.4 vs. 2.3 mg/ml). These quantitative considerations should be accepted with some caution, however, since the dose–response study was not a complete one, and since the absolute amount of alpha glucose present in the 3 min incubates increases with increasing

concentrations of beta glucose, thus obliterating the differences.

We have previously stated that cyclic AMP generation by glucose may be one of the first steps in the transmission of the insulinogenic signal of the hexose [2,11]. The present findings, which in most respects parallel those of Grodsky et al. [7] on insulin response to glucose anomers, support the above statement. It is not known whether glucose itself, through a cell membrane glucoreceptor, stimulates cyclic AMP generation and insulin release, or if the hexose must be metabolized in the islets in order to elicit these effects. The very recent report of the Umeå group [10] demonstrating a more pronounced effect of beta-D-glucose on glucose-6-phosphate accumulation in mice islets compared to the alpha anomer, as well as a greater dilution effect induced by beta glucose on $3H_2O$ production from D-[^{3}H] 5-glucose, indicates that beta-D-glucose utilization in islets is as effective as alpha glucose metabolism, if not more. These results, together with the data presented in this paper, do not contradict, and may even support, the suggestion that a glucose effect on a cell membrane receptor may be the phenomenon initiating insulin release through stimulation of the cyclic AMP accumulation in the islets [11].

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