ANOMERIC SPECIFICITY FOR THE RAPID TRANSIENT EFFLUX OF PHOSPHATE IONS FROM PANCREATIC ISLETS DURING SECRETORY STIMULATION WITH GLUCOSE

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When pancreatic islets prelabeled with [^{32}P]-orthophosphate are stimulated with glucose solutions differing only in their anomeric composition, the α anomer induces a greater efflux of radiophosphate than the β anomer. This anomeric specificity suggests that the altered anionic flux may be initiated by the glucose molecule itself or by disposition of glucose which does not entail initial phosphorylation.

INTRODUCTION: The sequence of events whereby the glucose stimulus is coupled to the secretion of insulin in pancreatic islets has not been fully clarified. We have recently shown that a transient efflux of [^{32}P]-orthophosphate from [^{32}P]-prelabeled islets occurs as an early and highly specific response to glucose or other sugars which act as insulin secretagogues (1).

As further evidence for the specificity of this phenomenon, we now report that the α anomer of glucose is a more effective stimulus for the enhanced release of [^{32}P]-orthophosphate than the β anomer. The same anomeric preference has been demonstrated for glucose-mediated insulin release *in vitro* (2, 3), and for the protection of β cells from damage by alloxan *in vivo* (4).

MATERIALS AND METHODS: Details of isolation, labeling, and perifusion of rat islets of Langerhans have been described previously (1). For each experiment 60-100 islets were isolated from two female albino rat pancreases by the method of Lacy and Kostianovsky (6) using collagenase (Worthington Biochemical Corporation, Lots CLS 257, 44M240). Following washing in Krebs-

Ringer bicarbonate (KRB) modified so that each 100 ml contained 500 mg bovine serum albumin, 0.05 mmoles Na_2HPO_4, 0.24 mmoles calcium, 10,000 U penicillin, 10 mg streptomycin sulfate, and 100 mg glucose, the islets were placed in 1 ml of the above medium, supplemented with 150 μC [^{32}P]-orthophosphate, and incubated for 90 minutes at 37°C in an atmosphere of 95% O_2 - 5% CO_2. Thereafter, the labeled islets were washed of adherent radioactivity with basic medium consisting of KRB modified so that each 100 ml contained 20-500 mg bovine serum albumin, 0.1 mmoles Na_2HPO_4, 0.24 mmoles calcium, the same antibiotics as above, and 50 mg glucose, which had been prepared 5 hours before use. The islets were then divided into two equal groups, of 30-50 islets each, and placed via a siliconized Pasteur pipette onto 5.0-μm millipore filters (Gelman) mounted in plastic perifusion chambers (1 ml capacity, Millipore Corp., Bedford, Mass.). The dual chambers were perifused simultaneously at 37°C at a rate of 0.9 ml/min with continuously gassed basic medium using a Gilson peristaltic pump. The effluent perifusates were collected during two 10 min intervals, then one 5 minute interval, and subsequently at one minute periods for the remaining 20 minutes of the experiment. Aliquots (0.2 ml) of the eluate from each collection period were counted in a Nuclear-Chicago Mark II liquid scintillation counter.

The concentration of α-D-glucose in the perifusate was determined as follows: A Beckman glucose analyzer, employing the β-D-glucose specific glucose oxidase, was calibrated against analytical standards consisting of basic medium supplemented with glucose which had been kept at room temperature for at least 5 hours prior to use so that mutarotational equilibrium (approximately 36% α and 64% β-D-glucose (7)) could be attained. Within 30 seconds of collection, the β-D-glucose content of each eluate was determined by injecting a 0.02 ml aliquot into the analyzer and multiplying the reading by 0.64. Following 1 hour at 20° and at least 24 hours at 4°C the perifusates were again analyzed for total glucose content, the amount of α-D-glucose being the difference between the total and the initial β-D-glucose value.

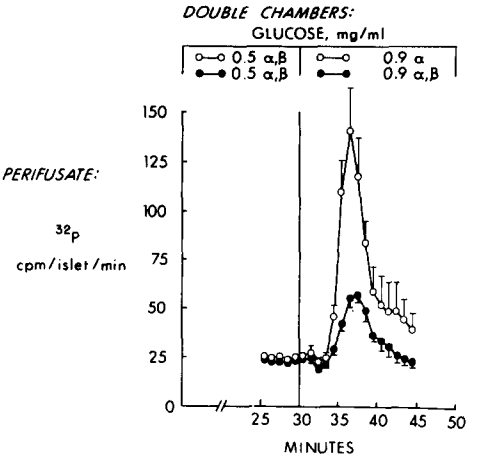

Fig. 1 Effect of glucose anomers on the efflux of [^{32}P]-orthophosphate from prelabeled islets. Dual chambers were perifused with modified KRB containing 0.5 mg/ml of D-glucose which had achieved mutarotational equilibrium (α,β). After 30 minutes, perifusion media were changed to modified KRB containing 0.9 mg/ml α, β-D-glucose in the control chamber and 0.9 mg/ml of freshly prepared α-D-glucose in the experimental chamber. The illustration depicts Mean ± SEM values cpm [^{32}P]-orthophosphate/islet/minute recovered in the effluents in 3 separate experiments.

RESULTS AND DISCUSSION: Results from three separate 2-channel experiments have been pooled and summarized in Fig. 1.

INSERT FIGURE 1 HERE

Basal rates of ^{32}P efflux from each chamber were achieved within 25 minutes, when the two chambers were perifused with the basic medium containing equilibrated glucose at a concentration of 0.5 mg/ml. After 30 minutes of perifusion, the medium for each channel was changed by transferring the intakes for the pump to fresh solutions (Fig. 1). One consisted of basic medium containing 0.9 mg/ml of glucose that had attained mutarotational equilibrium; the other consisted of a 0.9 mg/ml glucose solution which had been prepared 15 seconds prior to use by dissolving powdered α-D-glucose (Mallinckrodt, Lot ABN) in gassed glucose-free basic medium. The glucose concentration of 0.9 mg/ml was selected because prior experience (1) indicated that it was near the threshold for effecting heightened [^{32}P]-orthophosphate efflux from prelabeled islets.

In confirmation of our earlier studies (1), perifusion with equilibrated 0.9 mg/ml glucose in the control chambers produced a transitory increased rate of release of radioactivity into the perifusate which peaked at approximately threefold above basal values by the seventh minute (Fig. 1). Contrariwise, stimulation with 0.9 mg/ml glucose enriched in the α anomer produced a pulse of [^{32}P]-orthophosphate release which was greater than the concurrent rates of efflux in the control chambers at the sixth ($p<.05$), seventh ($p<.05$) and eighth ($p<.05$) minute after the change of media, and a peak which was approximately sevenfold greater than basal values (Fig. 1). Immediate analyses of the effluents from the α-D-glucose channel disclosed α-D-glucose concentrations of 0.61 ± 0.02, 0.59 ± 0.03 and 0.54 ± 0.04 mg/ml at these three time points whereas the concentration of α-D-glucose remained constant at 0.33 ± 0.01 mg/ml in the effluent from the control chamber perifused with the equilibrated mixture. The declining α-D-glucose concentrations in the effluents from the experimental chamber reflected the rapid rates of mutarotation at 37° and the 2.8 ml of dead space in our perifusion system.

Since the mutarotational half-life of glucose-6-phosphate is approximately 1.5 seconds under physiologic conditions (5), any manifestation of glucose disposition distal to this point in glucose handling, either via the glycolytic or phosphogluconate pathways, would presumably be indifferent to the anomeric composition of the stimulating glucose (3). This evidence makes it less likely that the pulse of heightened phosphate efflux is purely the result of increases in metabolic processes for which the stimulatory glucose served as substrate. Moreover, although the stereospecificity of islet glucokinase has not been tested, the known low K_m of islet hexokinase (8) and the lack of anomeric preference with rat brain hexokinase (9) and rabbit liver glucokinase (10) renders it unlikely that the initial phosphorylation of glucose would be an anomer sensitive step. Thus, the anomeric specificity which we have observed for the rapid transient flux of phosphate in response to secretory stimulation with glucose suggests that this new

index of islet activation (1) is "triggered" either by a) a pathway of glucose utilization which is anomer sensitive but does not entail initial phosphorylation; or b) an event prior to glucose phosphorylation such as the interaction of unmetabolized anomer with a receptor capable of distinguishing between anomeric forms. The parallelism between the anomeric specificities of the phosphate efflux, insulin release (2, 3) and protection from alloxan damage (4) favors the latter and suggests a common mechanism for the three effects which is unrelated to the fuel function of the stimulus.

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