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Pancreatic islet cell membranes: Extraction of a possible glucoreceptor

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SUMMARY

Solubilized cell membranes of dog pancreatic islets, in contrast to membranes from acinar cells, include a component which forms complexes with glucose, mannose, and fructose, each of which is a stimulus to insulin secretion. Galactose and 3-*O*-methyl-glucose, which are not secretory stimuli, are not complexed. Thus, this component has the specificity expected of the receptor site through which these cells are stimulated to secrete insulin.

The primary role of the β cells, which make up the bulk of the pancreatic islets of Langerhans, is to secrete insulin. Glucose is the major secretory stimulant *in vivo*, and certain other sugars can also be effective. Evidence that stimulation involves interaction of the sugar with a receptor site on or in the cell membrane includes the following: glucose stimulates insulin secretion even when the glycolytic pathway is blocked[1]; metabolite concentrations in islet cells do not change significantly during the early phase of glucose-induced insulin release[2]; the time course of the electrophysiological response to sugars is very rapid[3,4] Since islet cells are rather unresponsive to glucose in maturity onset diabetes, it has been suggested that this disease may be based on a defect in the glucoreceptor[5]. If there is a glucoreceptor site on or in the β cell membrane it might be possible to extract and characterize it using methods previously used to extract a possible sugar receptor from taste cells[6–8]. This report deals with extraction of a β cell membrane component which forms complexes with sugars which stimulate insulin secretion.

Cell membrane fractions were prepared from islets of Langerhans which were separated from most of the acinar tissue by the method of Gerner *et al.*[9]. Fresh dog

pancreas was cut into pieces 1–3 cm long and placed into mammalian Ringer solution (2 ml per g of tissue) containing 110–140 units per ml of collagenase (Sigma, Type I). The suspending medium was also injected directly into the mesentery in which the tissue is embedded. The suspensions were incubated at 37 °C for 2 h with constant shaking, strained through 20 mesh stainless steel gauze, then incubated for another 30 min. They were then forced through 18 and 20 gauge needles consecutively. After 30 min of settling the supernatant materials were aspirated and discarded. The sediments were washed twice with collagenase-free Ringer solution. The resulting preparations appear to consist largely of islets when examined microscopically (whole pancreas is only 1% islet tissue), and are referred to below as "islets". Control preparations, referred to as "pancreas", were prepared in exactly the same way except that the acinar cells were centrifuged back into the islet-rich sediment instead of being removed during the aspirations.

The cell membrane fractions were prepared by the method of Kiehn and Holland[10]. "Islets" or "pancreas" were suspended in 50 mM Tris + 2.5 mM $MgCl_2$ (pH 7.2; 1 ml per g original tissue), and incubated for 15 min at 37 °C to promote cell swelling, then homogenized in a glass homogenizer with a motor-driven Teflon pestle. Sucrose was dissolved in the homogenates to give a final concentration of 10% (w/v), which were then layered over coarse density gradients consisting of a layer of 30% sucrose + 5 mM $MgCl_2$ above a layer of 50% sucrose + 5 mM $MgCl_2$. After centrifugation (1400 × *g*; 15 min) the 30% sucrose layer and the fluffy layer at its upper surface were collected. This was centrifuged at 9000 × *g* for 10 min, and the supernatant fluid discarded. The pellet was washed with 100 mM Tris + 5 mM $MgCl_2$, then stored at −20 °C until sufficient material accumulated.

Samples of the membrane fraction were solubilized by suspension for 15 min in 100 mM Tris + 5 mM $MgCl_2$ + 0.2% sodium dodecyl sulfate at room temperature. Undissolved material was removed by centrifugation (35 000 × *g*; 20 min). The solubilized membrane fraction was stable for at least 5 days at 3 °C but was inactivated by being frozen and thawed. Each 35–40 g pancreas yielded enough material for approximately 5 data points.

TABLE I

MAGNITUDES OF SPECTRAL CHANGES INDUCED IN SOLUBILIZED DOG MEMBRANES BY SUGARS

Figures are per cent change in absorbance ± S.E.

Sugar (80 mM)	*Islet cell membranes*	*Pancreas cell membranes*	*P* ★
D-Glucose	2.0 ± 0.2 (*N* = 8)	0.4 ± 0.1 (*N* = 6)	< 0.00001
D-Mannose	2.6 ± 0.3 (*N* = 6)	0.7 ± 0.4 (*N* = 6)	< 0.01
D-Fructose	1.6 ± 0.2 (*N* = 6)	0.8 ± 0.1 (*N* = 5)	< 0.025
D-Galactose	0.5 ± 0.3 (*N* = 4)	0.2 ± 0.1 (*N* = 5)	–
3-*O*-Methylglucose	−0.2 ± 0.2 (*N* = 4)	–	–

★ Determined from two-tailed *t*-test.

Interaction with sugars was assayed by measuring an increase in the ultraviolet absorption induced in the solubilized membrane fraction. Difference spectra were recorded from 240 to 350 nm with a Zeiss DMR 21 spectrophotometer thermostatted at 25 °C. Interaction caused peaks in the difference spectrum at 283 and 292 nm. The increase in absorbance at 283 nm, expressed as a percentage of the total absorbance of the solubilized membranes at 272 nm, was used as R, the magnitude of response (see below). This procedure is essentially identical to that used for the interaction of sugars with a putative sweet taste receptor[8].

Effects of sugars (80 mM) on solubilized membrane fractions from islets and from pancreas were measured as described above. The results are shown in Table I. Glucose, mannose, and fructose are all stimuli to insulin secretion in dogs, while galactose and 3-*O*-methylglucose are not[11,12]. It is clear from the data in Table I that the solubilized membranes of islets exhibit this same sugar specificity. Sugars induce little or no spectral change in preparations from whole pancreas. This demonstrates that the effect seen with preparations from islets is not due to a contaminating component of acinar origin.

The simplest interaction of a sugar with a cell component would be the formation of a 1:1 complex with a dissociation constant, K_m. Such an interaction is described by an adsorption isotherm (for a derivation, see Beidler[13]):

$$C/R = C(1/R_m) + K_m/R_m$$

in which R is the magnitude of the response at sugar concentration C, and R_m is the maximal response. When spectral changes in solubilized islet membranes were measured at varying sugar concentrations the data were reasonably well described by the equation, as can be seen in Fig. 1. Values of K_m and R_m for the three stimulatory sugars are in Table II. Since K_m is the sugar concentration at which the response is half-maximal, it is interesting to compare K_m with sugar concentrations which elicit half-maximal responses in living islet cells. In humans, glucose at blood levels of 5–10 mM elicits half-maximal insulin secretion[14], and concentrations around 10 mM are half-maximal stimuli for insulin secretion[15] and for

TABLE II

CONCENTRATION DEPENDENCE OF SUGAR-INDUCED SPECTRAL CHANGES IN SOLUBILIZED MEMBRANES FROM DOG ISLETS

R_m and K_m were calculated from straight lines drawn by the method of least squares when data were plotted as in Fig. 1. N is the number of graphs, each consisting of five data points.

Sugar	$R_m \pm$ S.E. *	$K_m \pm$ S.E. (mM)
D-Glucose (N = 8)	2.9 ± 0.3	35 ± 7
D-Mannose (N = 5)	2.8 ± 0.4	23 ± 7
D-Fructose (N = 5)	1.9 ± 0.3	14 ± 6

* The units of R_m are per cent change in absorbance.

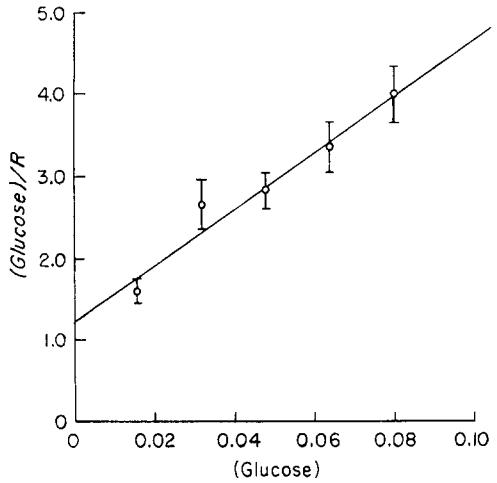

Fig. 1. Concentration dependence of spectral change induced in solubilized dog islet membranes by D-glucose. Data are plotted according to the equation, $C/R = C(1/R_m) + K_m/R_m$. Each point is the mean of 8 values; the vertical bars represent standard errors. The glucose concentration is in the units of mole·l^{-1}

electrical activity[3] when mouse islets are challenged with glucose or mannose. Our preparations give a K_m for glucose which is somewhat higher, but of the same order of magnitude. The strength with which the receptor binds glucose may have been modified by extraction, although there are data suggesting that dog islets respond to higher glucose concentrations than do those of the mouse or human[16].

In summary, it is shown that solubilized membranes from dog islets of Langerhans contain a component which forms complexes with sugars which stimulate insulin secretion, but does not appear to interact with nonstimulatory sugars. The concentration dependence is of the same order of magnitude observed when the sugars are used as stimuli of living islet cells. This component may be the glucoreceptor through which sugars stimulate insulin secretion.

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