SORBITOL SYNTHESIS IN ISOLATED RAT PANCREATIC ISLETS

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SUMMARY

Sorbitol and free fructose are present in isolated rat pancreatic islets in concentrations exceeding that found in plasma or acinar tissues. The sorbitol concentration of the islet increases when the tissue is incubated with increasing glucose concentrations. This suggests a mechanism for the production of the "hydropic" changes observed in the pancreatic islet in hyperglycemic states.

INTRODUCTION

In cells containing alditol:NADP oxidoreductase (E.C.1.1.1.21) polyol formation appears to be regulated in part by the intracellular concentration of aldose substrate.[1,2,3,4,5] The presence of this enzyme in cells in which intracellular transport is not rate limiting for glucose or galactose utilization provides the basis of a pathogenetic mechanism that has been implicated in the development of cataracts in diabetes and galactosemia.[1,2] Matschinsky and Ellerman[6] recently reported that the isolated pancreatic islet of the mouse is freely permeable to glucose, and Clements et al[7] have isolated and partially purified alditol:NADP oxidoreductase from rabbit pancreas. The presence of alditol:NADP oxidoreductase activity in the pancreatic islet would thus suggest a mechanism by which persistent hyperglycemia might lead to pathologic changes as a concomitant of increased glucose reduction to sorbitol.

METHODS

Isolated pancreatic islets were prepared from Wistar rats (400 to 500g) by the collagenase digestion method of Lacy and Kostianovsky.[8] Fifty to 100

islets were collected and homogenized in 1.0 ml of perchloric acid (6%) at $0^{\circ}C$ in glass homogenizing vessels. After centrifugation at 5000 x g for 15 minutes, an aliquot of the supernatant was neutralized with KOH (2N), left at $0^{\circ}C$ for 30 minutes, and the $KClO_4$ removed by centrifugation. The sorbitol and fructose contents of the neutralized filtrate were determined by fluorometric enzymatic assays.[4] Samples of acinar tissue from the same rat pancreas were prepared and analyzed in a similar fashion. The protein content of the initial perchloric acid precipitate was determined after treatment with NaOH (1.0 N) for 1 hour as described by Lowry et al.[9] To determine the effect of ambient glucose concentration on the sorbitol concentration in the islet, paired samples of 50 or more islets from the same rat were incubated in 1.0 ml of Krebs-bicarbonate buffer (gas phase 5% CO_2 in 95% O_2) pH 7.4 containing 1.7 and 17.0 mM glucose for 1 hour at $37^{\circ}C$. At the end of the incubation, the vessels were centrifuged at 300 x g for 3 minutes at $2^{\circ}C$, the medium decanted, and 1.0 ml of cold perchloric acid (6%) added. The tissue was then treated as described above. The high intracellular free glucose concentration under these conditions precluded an accurate estimate of free fructose by fluorometric assay, and only values for sorbitol are given.

RESULTS AND DISCUSSION

Sorbitol was present in isolated rat pancreatic islet in concentrations of 1.03 $\pm$ .07 moles per mg protein (Table 1); the concentration of sorbitol in pancreatic acinar tissue was only 1/7th that found in the islets. The water content of the isolated mouse pancreatic islet has been reported to be approximately 75% of the wet weight;[6] if one assumes a similar value for the water content of the isolated rat pancreatic islet the sorbitol content of the rat islet is the order of 250 nmoles per gram wet weight. This is in contrast to rat plasma where the sorbitol concentration is less than 5 nmoles per ml.[5]

The concentration of free fructose in the pancreatic islet was 2.91 $\pm$ 0.39 nmoles per mg of protein, or using a water content of 75% approximately 725 nmoles per gram wet weight. The fructose concentration in the islet is

Table 1

Sorbitol and Fructose Concentrations in Unincubated Rat Pancreatic Islet and Acinar Tissue

Exp. No.	No. of Islets	Sorbitol nmoles per mg protein		Fructose nmoles per mg protein	
		Islet	Acinar	Islet	Acinar
1	50	0.91	0.08	3.56	0.14
2	50	1.00	0.10	4.10	0.23
3	125	0.73	0.08	1.96	0.24
4	125	1.20	0.18	1.15	0.19
5	125	0.97	0.15	2.42	0.32
6	100	1.33	0.19	4.40	0.36
7	100	0.89	0.22	3.02	0.30
8	150	1.18	0.20	2.66	0.23
	mean ± s. e.	1.03 ± 0.07	0.15 ± 0.02	2.91 ± 0.39	0.25 ± 0.03

ten times greater than those found in pancreatic acinar tissue (Table 1), and well in excess of that present in rat plasma. (< 3.0 nmoles per ml). These observations suggest that a significant fraction of the alditol:NADP oxidoreductase activity in the pancreas is present in the islets.

The suggestion that the sorbitol found in the isolated rat pancreatic islet is derived from the reduction of glucose in situ is supported by the data in Table 2. Rat pancreatic islets incubated with glucose (1.7 mM) for 1 hour had a higher sorbitol concentration than that found in unincubated tissue. (Table 1) (The unincubated tissue is exposed to glucose-free medium for periods in excess of 30 minutes in the course of preparing the iso-

Table 2

Effect of Increasing Medium Glucose Concentration on the Sorbitol Concentration in Isolated Pancreatic Islets.

Experiment Number	Number of Islets	Sorbitol nmoles/mg protein		Δ of paired samples nmoles/mg protein
		glucose 1.7 mM	glucose 17 mM	
1	60	12.6	36.7	+ 24.1
2	55	26.4	35.9	+ 9.5
3	75	11.7	19.4	+ 7.7
4	70	17.7	24.0	+ 6.3
5	50	7.2	16.0	+ 8.8
6	60	9.1	12.0	+ 2.9
		Mean Δ $\pm$ s. e. m.		+ 9.9$\pm$3.0
				$p < 0.05$

Paired samples of isolated pancreatic islets from the same rat incubated in 1.0 ml of Krebs-bicarbonate buffer, gas-phase 5% CO_2 in 95% O_2, pH 7.4 containing glucose in concentrations indicated for 1 hour at 37°C.

lated islets). Comparison of the sorbitol concentration of paired pancreatic islet samples from the same rat incubated with 1.7 and 17 mM glucose for 1 hour indicates a significant increase with increasing medium glucose concentration. (Table 2)

"Hydropic" changes were first observed in the pancreatic islets of comatose human diabetics,[10] and have subsequently been observed in numerous forms of experimental and spontaneously occurring hyperglycemic states.[11] The relationship between hyperglycemia and the development of hydropic changes in the beta cell was established by Lukens and his co-workers[12] and culminated in the production of permanent diabetes in a cat by the prolonged intraperi-

toneal injection of glucose. The alterations in fine structure responsible for the hydropic changes in the pancreatic islet are still a matter of dispute. However, Lazarus and Volk have presented data to suggest that "ballooning degeneration" which results from the appearance of membrane bound vesicles in the cytoplasma can be distinguished from glycogen accumulation and is the lesion that progresses to beta cell loss.[11] Although the specific cell type or types in the pancreatic islet in which sorbitol formation from glucose occurs has not been determined, the presence of the polyol sequence in the pancreatic islet suggests the possibility that persistent hyperglycemia could result in progressive islet cell damage by a mechanism similar to that operative in the lens.

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