Effect of diabetes on the rat hepatic glucose-6-phosphatase system in endoplasmic reticulum subfractions

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Diabetes-induced alterations in the activities of the components of the glucose-6-phosphatase system (i.e., the enzyme, the glucose-6-P translocase (T_1), and the phosphate translocase (T_2) were examined in smooth and rough subfractions of hepatic endoplasmic reticulum from streptozotocin-injected rats. A significant effect of diabetes on the maximal velocity of glucose-6-P hydrolysis by the enzyme was present in both endoplasmic reticulum subfractions (3.1-fold increase in rough endoplasmic reticulum; 3.8-fold increase in smooth endoplasmic reticulum). Based on latency values, diabetes did not result in a proportional increase in capacity of T_1 or T_2. In contrast to the control condition, the relationship between transport capacity and hydrolytic capacity was not significantly different in the two subfractions from diabetic animals. Elucidation of the effects of diabetes on the components of the glucose-6-phosphatase system associated with smooth and rough endoplasmic reticulum membranes enhances our understanding of the hepatic contribution to diabetic hyperglycemia.

Keywords: Glucose-6-phosphatase; streptozotocin-induced diabetes; kinetic properties; endoplasmic reticulum

Introduction

Hyperglycemia is maintained in the diabetic animal through hepatic glucose overproduction[1] and peripheral under-utilization.[2] The terminal reaction in the pathways leading to glucose formation by the liver is catalyzed by the multicomponent glucose-6-phosphatase system. In the model proposed by Arion et al.,[3] glucose-6-P hydrolysis in glucogenic cells involves the coupled functions of three integral components of the endoplasmic reticulum: (i) a glucose-6-P specific transporter, denoted T_1, that mediates penetration of the hexose phosphate into the lumen; (ii) a relatively nonspecific enzyme, glucose-6-phosphate phosphohydrolase (EC 3.1.3.9), located with its active site facing the luminal surface of the membrane; and (iii) a phosphate transporter, denoted T_2, that mediates efflux of P_i as well as penetration of inorganic pyrophosphate (PP_i). The coupling of T_2 and intrinsic enzyme constitutes the functional system for PP_i hydrolysis by intact liver microsomes.

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Elucidation of the mechanisms involved in the control of glucose-6-P hydrolysis is essential for our understanding of the increased hepatic glucose output in diabetes. The purpose of the present study was to expand our knowledge of the effects of diabetes on the glucose-6-phosphatase system[4] by quantifying the contributions made by the enzyme and translocases associated with the two subfractions of the endoplasmic reticulum. (Pertinent definitions of the terms used in this study are provided in references 5 and 6.)

Materials and methods

Animals and preparations

Male Spraque-Dawley rats were obtained from Sasco-King (Madison, WI) and housed individually under a 12-hour light/dark cycle. Animals were randomly selected to be injected with streptozotocin (75 mg/kg body weight) via the tail vein, as described previously.[4,7] After injection, rats were given food and water ad libitum until killed on the seventh day. The diabetic condition was assessed initially by urine testing with glucostix and verified by glucose testing of trunk blood samples taken at the time of sacrifice.

Following sacrifice, livers were excised and liver homogenates were prepared as previously described.[7] Smooth and rough microsomes were isolated by adjusting previously described proportions[8] to a total of

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3.5 ml in a Beckman TL100 ultracentrifuge.[9] All membrane preparations were stored at −80°C until assayed. Membrane preparations were treated for assay as described previously.[5,10]

Analytical Procedures

The protein content of membrane preparations was assessed by a modification of the Lowry procedure[11] using bovine serum albumin as a reference standard. RNA concentrations were determined by the method of Munro and Fleck[12] to ascertain degree of subfractionation of endoplasmic reticulum. Blood glucose concentration was assayed enzymically by a glucose oxidase method.[13]

Hexose phosphate phosphohydrolase activities were assayed at 30°C by determining the inorganic phosphate (P_i) formed during 10-minute incubations, as described by Bickerstaff and Burchell.[14] Assay media, pH 6.5, contained 50 mM Tris-cacodylate buffer, substrate concentrations between 1 and 10 mM, and enzyme preparation in a final volume of 0.1 ml.

Pyrophosphate hydrolysis was assayed at 30°C in media, pH 6, containing 50 mM Tris-cacodylate buffer, PP_i concentrations between 1 and 10 mM, and enzyme preparation in a final volume of 0.1 ml. The assay was terminated after 10 minutes by addition of 0.9 ml acid solution to the 0.1 ml reaction mixture [final concentration in 1.0 ml equal to 0.8 M perchloric acid, 1% (w/v) sodium dodecyl sulfate, 0.5% (w/v) ammonium molybdate and 0.025% (w/v) ascorbate]. Color development in standards, "zero-time" controls, and assay tubes proceeded for exactly 20 minutes, when formation of phosphomolybdate complex was assessed spectrophotometrically by absorbance at 820 nm.

Phosphohydrolase activities of intact membranes were calculated as previously described.[7] Values for the maximal velocities and Michaelis constants for glucose-6-P hydrolysis by the system and enzyme were estimated from intercepts and slopes of Lineweaver-Burk plots.[15] One unit of activity is defined as the amount of enzyme that catalyzed the hydrolysis of 1 μmol of substrate per minute.

Results

Table 1 gives the means (± SD) for body weights, blood glucose, liver protein, and the protein and RNA content of smooth and rough microsomes for the animals used in this study. The sums of the smooth and rough membranes recovered from liver homogenates were 40 ± 5% and 35 ± 4% of the total endoplasmic reticulum membranes in livers of controls and diabetic rats, respectively; and the distribution between subfractions was the same for both groups. For both control and diabetic rats, 48% of the recovered activity was present in smooth microsomes.

The influences of diabetes on the glucose-6-phosphatase enzyme and system in endoplasmic reticulum subfractions are summarized in *Table 2*. When glucose-6-phosphatase enzyme activity alone was assessed, no significant differences between endoplasmic reticulum subfractions were present. A significant effect of diabetes on the enzyme in both subfractions was apparent, however, in that dramatic increases in maximal velocity of glucose-6-P hydrolysis were observed. The corresponding increase in latency indicated that transport capacity (T_1) did not parallel the response of the enzyme.

A significant difference between rough and smooth microsomes was noted for the glucose-6-phosphatase system in control rats. The maximal velocity of glucose-6-P hydrolysis by the system was higher in rough microsomes. The diabetes-induced increase in the maximal velocity in smooth microsomes negated the difference between the two subfractions from diabetic animals.

A similar pattern was observed for pyrophosphate hydrolysis; there were no differences in maximal velocity between the enzyme associated with the two endoplasmic reticulum subfractions in the control condition, and both showed a dramatic increase in response to diabetes. The significantly higher latency in smooth microsomes compared to rough indicated that the capacity of T_2 was substantially less in that subfraction.

In control animals, a significant difference between rough and smooth microsomes was present in regard

Table 1 Average body weights, blood glucose, liver weights, liver protein, and protein and RNA contents of smooth and rough microsomes from control and diabetic rats.*

	Control	Diabetic
Number of animals	3	3
Body weights (g)	245 ± 9	190 ± 10
Blood glucose (mM)	9.6 ± 1.3	21.3 ± 0.6
Liver weights (g)	10.5 ± 0.3	8.9 ± 0.6
Liver protein (mg/g wet liver)	147 ± 9	162 ± 10
Smooth microsomal protein (mg/g wet liver)	7.48 ± 0.64	6.62 ± 0.51
Rough microsomal protein (mg/g wet liver)	7.84 ± 0.43	8.25 ± 0.64
Smooth microsomal RNA (ng-P/mg protein)	36 ± 3	39 ± 1
Rough microsomal RNA (ng-P/mg protein)	223 ± 18	195 ± 24

* Means ± SD.

Table 2 Influence of diabetes on the kinetic constants for hydrolysis of glucose-6-P and pyrophosphate by the system and enzyme in smooth and rough subfractions of rat hepatic endoplasmic reticulum**

Microsomes: Component	Latency* (%)		Michaelis constants (*mM*)		Maximal velocities (*units/mg protein*)	
	Rough	Smooth	Rough	Smooth	Rough	Smooth
Glucose-6-phosphatase						
Enzyme						
Control			0.25 ± 0.10	0.31 ± 0.22	0.39 ± 0.07	0.37 ± 0.09
Diabetic			0.51 ± 0.21	0.28 ± 0.01	1.2 ± 0.28$^{\Phi}$	1.4 ± 0.40$^{\Phi}$
System (T_1 plus enzyme)						
Control	40 ± 4	56 ± 4^{+}	0.88 ± 0.22	1.1 ± 0.19	0.34 ± 0.06	0.25 ± 0.06^{+}
Diabetic	78 ± 6$^{\Phi}$	74 ± 6$^{\Phi}$	2.8 ± 1.7	1.7 ± 0.16$^{\Phi}$	0.82 ± 0.34	0.73 ± 0.13$^{\Phi}$
Pyrophosphatase						
Enzyme						
Control			0.70 ± 0.08	0.83 ± 0.05^{+}	0.44 ± 0.07	0.42 ± 0.08
Diabetic			0.80 ± 0.16	0.99 ± 0.22	1.8 ± 0.28$^{\Phi}$	2.0 ± 0.83$^{\Phi}$
System (T_2 plus enzyme)						
Control	40 ± 12	74 ± 3^{+}	0.96 ± 0.20	0.87 ± 0.13	0.31 ± 0.07	0.10 ± 0.01^{+}
Diabetic	66 ± 5$^{\Phi}$	82 ± 4	1.6 ± 0.40	1.6 ± 0.67	0.90 ± 0.28$^{\Phi}$	0.45 ± 0.20$^{\Phi +}$

* Latency is the percentage of activity in fully disrupted micrsomes that is not expressed in intact preparations. Latency is calculated as 100 × (activity in fully disrupted membranes − activity in intact membranes)/activity in fully disrupted membranes. Latency provides a measure of the extent to which substrate transport restricts the rate of the coupled hydrolytic process.[5,7] ** Values are means ±SD. $^{\Phi}$ Denotes significant difference ($p < 0.05$) between value of parameter in control and diabetic rats. $^{+}$ The difference between mean values of the parameter in smooth and rough microsomes obtained from the same group of rats is significant at the 0.05 confidence level as determined by paired t-test.

to the maximal velocity of PP_i hydrolysis by the intact system. This difference between subfractions was maintained in microsomal preparations from diabetic rats.

Discussion

Overall, the increases in maximal velocity of glucose-6-P and PP_i hydrolysis by the enzyme were similar to those reported by others.[16] In contrast to the results of Garfield and Cardell,[17] however, a rise in phosphohydrolase maximal velocity was indicated clearly in both endoplasmic reticulum subfractions (3.1-fold increase in rough microsomes versus a 3.8-fold increase in smooth microsomes). Although caution in interpretation must be exercised due to the limited recovery of glucose-6-phosphatase activity, the absence of significant differences between recoveries or distributions of recovery in endoplasmic reticulum subfractions from control and diabetic rats does not support the previously proposed dominant contribution by smooth microsomes to the enhanced phosphohydrolase activity in diabetes.[17]

While the impact of diabetes was primarily on the enzyme component of the system, as previously reported,[4] some interesting effects on T_1 and T_2 were inferred from the system activity and latency values. Alterations in glucose-6-phosphatase system activity may represent changes in enzyme capacity, transport capacity, or both. In the control condition, there were no differences in the enzyme from rough compared to smooth microsomes. Therefore, the distinctions in the system for the two subfractions in the control state must be due to inherent differences in transport capacity for the two subfractions. Accordingly, latency values indicate the degree to which hydrolysis is limited by capacity, for transport is greater in smooth endoplasmic reticulum than in rough endoplasmic reticulum.

The effects of diabetes included obscuring the differences between endoplasmic reticulum subfractions in regard to the relationship between capacity for transport and hydrolysis of substrate.

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References

1. Felig, P. (1975). The liver in glucose homeostasis in normal man and in diabetes. In *Diabetes: Its Physiological and Biochemical Basis*, pp. 93–123, University Park Press, Baltimore
2. Forbath, N. and Hetenyi, G. (1966). Glucose dynamics in normal subjects and diabetic patients before and after a glucose load. *Diabetes* **15,** 778–789
3. Arion, W.J., Lange, A.J., Walls, H.E. and Ballas, L.M. (1980). Evidence of the participation of independent translocation for phosphate and glucose-6-phosphate in the microsomal glucose-6-phosphatase system. *J. Biol. Chem.* **255,** 10396–10406
4. Arion, W.J., Lange, A.J. and Ballas, L.M. (1976). Quantitative aspects of relationship between glucose-6-phosphate transport and hydrolysis for liver microsomal glucose-6-phosphatase system. *J. Biol. Chem.* **251,** 6784–6790
5. Arion, W.J., Schulz, L.O. and Walls, H.E. (1987). Phenobarbital-induced alterations in the activities of the transport and hydrolytic components of the glucose-6-phosphatase system in smooth and rough subfractions of the rat hepatic endoplasmic reticulum. *Arch. Biochem. Biophys.* **252,** 467–477
6. Schulz, L.O. (1988). Suppression of the hepatic glucose-6-phosphatase system in diabetic rats by vanadate. *Ann. Nutr. Met.* **32,** 289–296
7. Arion, W.J., Schulz, L.O., Lange, A.J., Telford, J.N. and Walls, H.E. (1983). The characteristics of liver glucose-6-

phosphatase in the envelope of isolated nuclei and microsomes are identical. *J. Biol. Chem.* **258,** 12661–12669
8 Goldsmith, P.K. and Stetten, M.R. (1979). Different developmental changes in latency for two functions of a single membrane bound enzyme. Glucose-6-phosphatase activities as a function of age. *Biochim. Biophys. Acta* **583,** 133–147
9 Schulz, L.O. (1989). Dietary fructose and the glucose-6-phosphatase system in rat hepatic endoplasmic reticulum subfractions. *Nut. Rep. Intl.* **40,** 993–99
10 Schulz, L.O. (1988). Glycogen influences recovery of hepatic glucose-6-phosphatase activity in microsomal subfractions. *Nut. Rep. Intl.* **38,** 1185–1191
11 Markwell, M.K., Haas, S.M., Bieber, L.L. and Tolbert, N.E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87,** 206–210
12 Munro, H.N. and Fleck, A. (1966). The determination of nucleic acids. *Methods Biochem. Anal.* **14,** 113–176
13 Sigma Technical Bulletin No. 510. (1978). The enzymatic, colorimetric determination of glucose in whole blood, plasma or serum. Sigma Chemical Co., St. Louis
14 Bickerstaff, G.F. and Burchell, B. (1980). Studies on the purification of glucose-6-phosphatase from rabbit liver microsomal fraction. *B. Biochem. Soc. Trans.* **8,** 389–390
15 Lineweaver, H. and Burk, D. (1936). The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56,** 658–666
16 Burchell, A. and Cain, D.I. (1985). Rat hepatic microsomal glucose-6-phosphatase protein levels are increased in streptozotocin-induced diabetes. *Diabetologia* **28,** 852–856
17 Garfield, S.A. and Cardell, R.R. (1979). Hepatic glucose-6-phosphatase activities and correlated ultrastructural alterations in hepatocytes of diabetic rats. *Diabetes* **28,** 664–679