The Polyol Pathway for Glucose Metabolism in Tissues from Normal, Diabetic, and Ketotic Chinese Hamsters

G.N. Holcomb, L.A. Klemm and W.E. Dulin

Department of Medicinal Chemistry, Ferris State College, Big Rapids, Michigan, USA, and Diabetes and Atherosclerosis Research, The Upjohn Company, Kalamazoo, Michigan 49001, USA

Summary. Increased activity of the polyol pathway for glucose metabolism and increased intracellular levels of glucose, polyol, and fructose have been implicated in the onset of chronic complications of diabetes in certain tissues where the transport of glucose is not insulin-dependent. Levels of all three carbohydrates were found to be significantly higher in sciatic nerves and lenses from diabetic and ketotic hamsters than in tissues from nondiabetic control animals ($p < 0.01$). Glucose, polyol, and fructose levels were not significantly different in spinal cords or gastrocnemius muscles from diabetic and ketotic hamsters than in tissues from nondiabetic hamsters. There was a linear relationship between blood glucose levels and tissue levels of glucose ($r = 0.885$), polyol ($r = 0.797$), fructose ($r = 0.886$) and the total tissue concentration of all three substances ($r = 0.916$) in hamster

sciatic nerves. Similarly, a linear correlation between blood glucose levels and levels of glucose ($r = 0.934$), polyol ($r = 0.784$), fructose ($r = 0.866$) and the total tissue concentration of the three carbohydrates ($r = 0.915$) in lenses was also established. The results of these studies provide evidence that glucose, polyol, and fructose levels are elevated in certain tissues from animals with spontaneous diabetes. The significance of these observations with respect to the pathogenesis of complications of diabetes remains to be determined.

Key words: Aldose reductase, Chinese hamster, complications of diabetes, fructose, glucose, ketotic, lens, polyol pathway, sciatic nerve, sorbitol, sorbitol dehydrogenase, spontaneous diabetes.

Increased activity of the polyol pathway for glucose metabolism leading to increased intracellular glucose, sorbitol, and fructose levels has been implicated in the pathogenesis of certain chronic complications of diabetes [1].

The Polyol Pathway for Glucose Metabolism

The polyol pathway is dormant or of minimal importance under normal physiological conditions since the K_M of hexokinase for glucose is much smaller than is the K_M of aldose reductase for glucose [1]. At normal levels, glucose is metabolized primarily by phosphorylation and very little of it is reduced to sorbitol. At elevated blood glucose levels, however, normal glycolytic pathways become saturated and excess glucose is metabolized by way of the polyol pathway [1]. Significant quantities of sorbitol and fructose are produced under these conditions. Sorbitol penetrates cell membranes poorly [2]; fructose is poorly metabolized in most tissues and it diffuses out of cells very slowly [1]. These factors lead to elevations in intracellular levels of both sorbitol and fructose [1]. The resultant hypertonicity may then contribute to the

onset of morphologic and pathologic changes in certain tissues in which glucose transport is not insulin-dependent [1].

Elevated levels of glucose, sorbitol, and fructose have been reported in lenses [3–5], sciatic nerves [6–8], spinal cords [7, 8], and kidney papillae [9] of alloxan and streptozotocin-diabetic rats. Levels of these carbohydrates are also regulated by ambient glucose concentrations in aortas [10] and in pancreatic islets [11] incubated *in vitro*. It has been suggested that the complications of diabetes in these tissues may result from the elevated levels of these carbohydrates [1].

The studies reported here were undertaken to determine whether glucose, polyol, and fructose levels were elevated in tissues from animals with spontaneous diabetes. The Chinese hamster was selected as an experimental animal because of the similarity of its disease to diabetes mellitus in humans [12] and also because morphologic and pathologic changes have been observed in a number of tissues from diabetic Chinese hamsters. Moreover, segmental demyelination and other changes similar to those observed in human diabetic neuropathy have been reported to occur in chronically diabetic Chinese hamsters [13].

Materials and Methods

Chemicals

Sheep liver sorbitol dehydrogenase (L-iditol: NAD oxidoreductase EC 1.1.1.14) was purchased from

Boehringer. Hexokinase (ATP: D-hexose-6-phosphotransferase EC 2.7.1.1), glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP oxidoreductase EC 1.1.1.49), phosphoglucose isomerase (D-glucose-6-phosphate ketol-isomerase EC 5.3.1.9), NAD, NADP, ATP, triethanolamine, and sorbitol were obtained from Sigma. D-Glucose and magnesium chloride were supplied by J.T. Baker. D-Fructose was from Pfaffstiehl Laboratories.

Animals

The nondiabetic hamsters used in these studies had fasting blood glucose levels of 80–112 mg% and had never exhibited glucosuria. They were from inbred lines (7–16 generations) in which glucosuria had never been detected. The average age of the animals at the time of sacrifice was 13.4 months. Their weights ranged from 24 to 32 g.

Diabetic hamsters had fasting blood glucose levels of 159–300 (average 216) mg%. They had demonstrated glucosuria (4+ Clinistix® urine glucose), but not ketosis. The average age of these animals was 13.3 months and they had been diabetic for an average of 10.1 months. Their weights ranged from 26 to 33 g. Each diabetic hamster was matched with a nondiabetic animal of the same age and sex.

The ketotic hamsters had fasting blood glucose levels of 160 to 383 (average 280) mg%. They had demonstrated glucosuria (4+ Clinistix®) and ketonuria (3+ or 4+ Ketostix®). The average age was 14.2 months. They had been diabetic for an average of 11.0 months and ketotic for an average of 7.0 months. Their weights ranged from 25 to 36 g. Each ketotic hamster was matched with a nondiabetic animal of the same age and sex.

Blood glucose was measured by Auto-Analyzer® by a modification of the method of Hoffman [14].

Tissue Samples

Animals were sacrificed by decapitation and tissues removed, weighed, frozen, and stored at -15°C . Tissues were homogenized in 1 ml of 6% perchloric acid in glass homogenizer tubes at 0°C according to the method of Morrison *et al.* [11]. The extracts were centrifuged, neutralized with 2 N KOH and cooled to 0°C for 30 min and centrifuged again. The supernatant fractions were frozen for subsequent assays.

Tissue Polyol Assays

Polyol levels were measured by the fluorometric method of Clements, Morrison, and Winegrad [10]. The assay mixture contained 50 mM glycine sodium glycinate buffer (pH 9.6), 0.1 mM NAD and sample at a final volume of 2 ml. Sorbitol dehydrogenase (1.25 units) was added in a volume of 0.02 ml and the increase in NADH fluorescence at 456 nm was measured using an Aminco Fluoro-Microphotometer equipped with a 360 nm narrow passage primary filter and

appropriate secondary filters (Corning numbers 7–60, 5–61, and 4–70). Duplicate determinations were performed on all samples.

Assays of Glucose and Fructose in Tissue

Glucose and fructose levels were measured by the method of Klotzsch and Bergmeyer [15]. The assay mixture contained 50 mM triethanolamine (pH 7.6), 0.1 M magnesium chloride, 0.017 M ATP, 0.012 M NADP, hexokinase (0.01 ml, 1 mg/ml) and samples at a volume of 3 ml. Glucose-6-phosphate dehydrogenase (0.01 ml, 1 mg/ml) was added and the increase in fluorescence of NADPH at 456 nm was measured to determine glucose concentrations. This phase of the reaction was usually complete within 10 min. Phosphoglucose isomerase (0.01 ml, 1 mg/ml) was then added and the increase in fluorescence was measured to determine fructose concentration. Duplicate determinations were performed on all samples.

Statistics

Regression analyses were done according to the method of Draper and Smith [16].

Results

Tissue glucose concentrations were significantly higher in lenses from ketotic Chinese hamsters (3.82 $\mu\text{moles/g}$) than in lenses from their matched controls (0.23 $\mu\text{mole/g}$) (Table 1). Polyol levels were higher in lenses from ketotic animals (3.04 $\mu\text{moles/g}$) than in tissues from normal animals (0.16 $\mu\text{mole/g}$). Fructose levels were also higher in lenses from ketotic hamsters (7.09 $\mu\text{moles/g}$) than in those from the controls (0.42 $\mu\text{mole/g}$).

Lens glucose levels were 1.91 $\mu\text{moles/g}$ in tissues from diabetic hamsters and 0.15 $\mu\text{mole/g}$ in tissues from their matched controls (Table 1). Polyol levels were also higher in lenses from diabetic animals (2.85 vs 0.12 $\mu\text{mole/g}$). Lens fructose levels were 8.08 $\mu\text{moles/g}$ in tissues from diabetic animals and 0.26 $\mu\text{mole/g}$ in tissues from nondiabetic hamsters.

Glucose, polyol, and fructose levels were also significantly higher in sciatic nerves from ketotic hamsters than in nerves from the control animals. Glucose concentrations were 9.64 $\mu\text{moles/g}$ in nerves from ketotic animals and 2.91 $\mu\text{moles/g}$ in tissues from nondiabetic animals (Table 2). Polyol levels were significantly higher in nerves from ketotic hamsters (0.41 vs 0.22 $\mu\text{mole/g}$). Sciatic nerve fructose levels were also higher in tissues from ketotic animals (3.90 vs 2.13 $\mu\text{moles/g}$).

Glucose levels were 3.91 $\mu\text{moles/g}$ in sciatic nerves from diabetic hamsters and 1.91 $\mu\text{moles/g}$ in nerves from the matched controls (Table 2). Polyol levels were also higher in sciatic nerves from diabetic animals (0.39 vs 0.15 $\mu\text{mole/g}$). Fructose concentrations were

also higher in nerves from diabetic hamsters (2.49 vs 1.35 $\mu\text{moles/g}$).

Polyol levels were not significantly elevated in spinal cords from ketotic hamsters (0.10 ± 0.03 vs 0.05 ± 0.02 $\mu\text{mole/g}$). Similarly, there was no difference between polyol levels in gastrocnemius muscle from ketotic and nondiabetic hamsters (0.11 ± 0.01 vs 0.10 ± 0.01 $\mu\text{mole/g}$).

Correlation studies revealed a linear relationship between blood glucose levels and lens concentrations of glucose ($r = 0.934$, $p < 0.01$), polyol ($r = 0.784$, $p < 0.01$), fructose ($r = 0.866$, $p < 0.01$), and the total concentration of all three substances ($r = 0.915$, $p < 0.01$) (Fig. 1). Similarly, there was a linear correlation between blood glucose levels and sciatic nerve concentrations of glucose ($r = 0.885$, $p < 0.01$), polyol

Table 1. Carbohydrate levels in lenses from normal, diabetic, and ketotic Chinese hamsters

Type of animal	Number of animals	Blood glucose ($\mu\text{mole/ml} \pm \text{SEM}$)	Lens content ($\mu\text{mole/g wet weight} \pm \text{SEM}$)		
			Glucose	Polyol	Fructose
Normal	10	5.12 ± 0.21	0.15 ± 0.04	0.12 ± 0.02	0.26 ± 0.04
Diabetic	8	11.92 ± 1.20^a	1.91 ± 0.48^a	2.85 ± 0.63^a	8.08 ± 1.25^a
Normal	8	4.82 ± 0.12	0.23 ± 0.06	0.16 ± 0.02	0.42 ± 0.07
Ketotic	7	16.89 ± 1.36^a	3.82 ± 0.51^a	3.04 ± 0.16^a	7.09 ± 1.05^a

^a $p < 0.001$ compared with normals

Table 2. Carbohydrate levels in sciatic nerves from normal, diabetic, and ketotic Chinese hamsters

Type of animal	Number of animals	Blood glucose ($\mu\text{mole/ml} \pm \text{SEM}$)	Nerve content ($\mu\text{mole/g wet weight} \pm \text{SEM}$)		
			Glucose	Polyol	Fructose
Normal	10	5.12 ± 0.21	1.91 ± 0.16	0.15 ± 0.02	1.35 ± 0.16
Diabetic	8	11.92 ± 1.20^a	3.91 ± 0.26^a	0.39 ± 0.07^a	2.49 ± 0.38^b
Normal	8	4.82 ± 0.12	2.91 ± 0.21	0.22 ± 0.03	2.13 ± 0.15
Ketotic	7	16.89 ± 1.36^a	9.64 ± 0.97^a	0.41 ± 0.04^a	3.90 ± 0.23^a

^a $p < 0.001$ compared with normals

^b $p < 0.01$ compared with normals

Fig. 1. Relationship between blood glucose levels and the total concentration of glucose, polyol, and fructose in Chinese hamster lenses

Fig. 2. Relationship between blood glucose levels and the total concentration of glucose, polyol, and fructose in Chinese hamster sciatic nerves

($r = 0.797$, $p < 0.01$), and fructose ($r = 0.866$, $p < 0.01$), and also between blood glucose levels and the total concentration of all three carbohydrates in nerves ($r = 0.916$, $p < 0.01$) (Fig. 2).

Discussion

Elevated glucose, polyol, and fructose levels have been detected in a number of tissues from alloxan-diabetic rats and rabbits and galactosemic rats [3–9]. The results of the studies reported here show that levels of these substance are elevated in certain tissues from animals with spontaneous diabetes.

The levels of glucose, polyol, and fructose found in lenses and sciatic nerves from diabetic and ketotic Chinese hamsters were not as high as levels which have been found in lenses [3–5] and sciatic nerves [6–8] from alloxan-diabetic, streptozotocin-diabetic, and galactose fed rats. This may be attributable to the fact that blood glucose levels were not as high in the diabetic and ketotic hamsters as in the alloxan-diabetic and streptozotocin-diabetic rats studied by other investigators [3–8]. This idea is supported by the finding that there is a linear correlation between blood glucose levels and tissue levels of glucose, polyol, and fructose in lenses and sciatic nerves from Chinese hamsters.

Polyol levels were not significantly elevated in either spinal cords or gastrocnemius muscle from ketotic Chinese hamsters. Others have reported small but significant increases in polyol levels in spinal cords from alloxan-diabetic and streptozotocin-diabetic rats [7, 8].

The data reported here are consistent with the hypothesis that elevated blood glucose levels lead to increased activity of the polyol pathway resulting in elevated polyol and fructose levels in certain tissues in which glucose transport is not insulin-dependent [1]. The intracellular accumulation of carbohydrates and the resulting increase in osmotic pressure can cause tissue swelling and interfere with the cellular transport of amino acids and other substances [17]. The results of *in vitro* experiments with rabbit lenses indicate that a 16 milliosmolar change in tonicity is sufficient to induce water uptake by lenses and to interfere with the transport of α -aminoisobutyric acid [18]. Since the accumulation of 1 μ mole of solute per g of tissue water is equivalent to 1 milliosmole per liter in osmotic pressure, the carbohydrate levels which were observed in lenses from diabetic and ketotic Chinese hamsters are probably of osmotic importance.

Similarly, the levels of glucose, polyol, and fructose which were detected in sciatic nerves from diabetic and ketotic Chinese hamsters are probably of osmotic importance. The elevations in nerve carbohydrate levels are further emphasized by the fact that the polyol pathway is apparently localized within Schwann cells in peripheral nerves [19] and Schwann cells

comprise only a small proportion of the nerve samples which were assayed.

Schlaepfer has observed a variety of structural alterations which occur in peripheral nerves from diabetic and ketotic Chinese hamsters [13]. Some of these changes are similar to changes observed in diabetic neuropathy in humans [13]. It is not possible at this time, however, to relate the abnormalities in tissue glucose metabolism to the morphologic and pathologic changes observed in tissues in Chinese hamsters. Further studies will be required to relate the biochemical abnormalities to the chronic complications of diabetes in the Chinese hamster.

Acknowledgment. We thank Dr. George Gerritsen for his advice and cooperation, Dr. John Schultz for statistical analysis, and Mrs. Beatrice Wyse for technical assistance.

References

- Gabbay, K.H.: The sorbitol pathway and the complications of diabetes. *New Engl. J. Med.* **288**, 831–836 (1973)
- Wick, A.N., Drury, D.R., Del Monte, V., Holbrook, A., Almen, M.C., Blackwell, M.E.: Action of insulin on the permeability of cells to sorbitol. *Amer. J. Physiol.* **166**, 421–423 (1951)
- van Heyningen, R.: Formation of polyols by the lens of the rat with "sugar" cataract. *Nature (Lond.)* **184**, 194–195 (1959)
- Gabbay, K.H., Kinoshita, J.H.: Mechanism of development and possible prevention of sugar cataracts. *Israel J. med. Sci.* **8**, 1557–1561 (1972)
- Varma, S.D., Kinoshita, J.H.: Sorbitol pathway in diabetic and galactosemic rat lens. *Biochim. biophys. Acta* **338**, 632–640 (1974)
- Stewart, M.A., Sherman, W.R., Kurien, M.M., Moonsammy, G.I., Wisgerhof, M.: Polyol accumulations in nervous tissue of rats with experimental diabetes and galactosemia. *J. Neurochem.* **14**, 1057–1066 (1967)
- Gabbay, K.H., Merola, L.O., Field, R.A.: Sorbitol pathway: Presence in nerve and cord with substrate accumulation in diabetes. *Science* **151**, 209–210 (1966)
- Ward, J.D., Baker, R.W.R., Davis, B.H.: Effect of blood sugar control on the accumulation of sorbitol and fructose in nervous tissue. *Diabetes* **21**, 1173–1178 (1972)
- Gabbay, K.H., O'Sullivan, J.B.: The sorbitol pathway in diabetes and galactosemia: Enzyme and substrate localization and changes in kidney. *Diabetes* **17**, 300 (1968)
- Clements, R.S., Morrison, A.D., Winegrad, A.I.: Polyol pathway in aorta: Regulation by hormones. *Science* **166**, 1007–1008 (1969)
- Morrison, A.D., Winegrad, A.I., Fink, C.J., Lacy, P.E.: Sorbitol synthesis in isolated rat pancreatic islets. *Biochem. Biophys. Res. Comm.* **38**, 491–495 (1970)
- Gerritsen, G.C., Dulin, W.E.: Characterization of diabetes in the Chinese hamster. *Diabetologia* **3**, 74–84 (1967)
- Schlaepfer, W.W., Gerritsen, G.C., Dulin, W.E.: Segmental demyelination in the distal peripheral nerves of chronically diabetic Chinese hamsters. *Diabetologia* **10**, 541–548

14. Hoffman, W.S.: A rapid photoelectric method for the determination of glucose in blood and urine. *J. biol. Chem.* **120**, 51—55 (1937)
15. Klotzsch, H., Bergmeyer, H.: In: *Methods of enzymatic analysis*. (Bergmeyer, H., ed.), pp. 156—159. New York: Academic Press 1965
16. Draper, N.R., Smith, H.: *Bird's applied regression analysis*. New York: John Wiley and Sons 1967
17. Kinoshita, J.H., Merola, L.O., Dikmak, E.: Osmotic changes in experimental galactose cataracts. *Exp. Eye Res.* **1**, 405—410 (1962)
18. Kinoshita, J.H., Merola, L.O., Hayman, S.: Osmotic effects on the amino acid concentrating mechanism in the rabbit lens. *J. biol. Chem.* **240**, 310—315 (1965)
19. Gabbay, K.H., O'Sullivan, J.B.: The sorbitol pathway: Enzyme localization and content in normal and diabetic nerve and cord. *Diabetes* **17**, 239—243 (1968)

Dr. George N. Holcomb
Dept. of Medicinal Chemistry
Ferris State College
Big Rapids, Mich. 49307
USA