J. Sci. I. R. Iran

Vol. 5 No. 4
Autumn 1994

VARIATIONS OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE ACTIVITY IN VARIOUS TISSUES INDUCED BY METABOLIC ALKALOSIS, ACIDOSIS AND DIABETES

B. Haghighi, M. Suzangar and M. Khajeh

Department of Biochemistry, Isfahan University of Medical Sciences, Isfahan, Islamic Republic of Iran

Abstract

The effects of chronic metabolic acidosis, alkalosis and alloxan-induced ketoacidosis on G6PD activity of rat kidney, liver and erythrocytes were studied. Metabolic acidosis significantly increased the activity of kidney enzyme (55%) but decreased the liver (43%) and erythrocyte (38%) enzyme activities. Alkalosis did not make a significant change in the kidney or liver enzyme activity but slightly decreased that of the erythrocyte enzyme. In alloxan-induced diabetic rats, the rise in G6PD activity of kidney (69%) was associated with a decline in the enzyme activities of liver (50%) and erythrocytes (51%). It is suggested that in alloxan-induced diabetes, ketoacidosis is mainly responsible for the change in G6PD activity of various tissues and different organs do not respond similarly toward metabolic acidosis or alkalosis.

Introduction

Glucose 6-phosphate dehydrogenase (G6PD), the first enzyme of hexose monophosphate shunt, generates reducing equivalent in the form of NADPH necessary for biosynthetic reactions and for maintenance of cellular integrity (for review see Ref. 1). The activity of G6PD is regulated under different metabolic and hormonal conditions in several organs [2-5]. In rat kidney, the rate of hexose monophosphate shunt has been affected by chronic metabolic acidosis [6] and other physiological and pathological situations such as growth and diabetes [7-8]. Several other reports on diabetic animals have shown that insulin affects G6PD activity in liver [9], adipose tissue [10] and mammary gland [11]. The possible roles of chronic metabolic acidosis and alkalosis, however, are still uncertain.

The present work was undertaken to investigate the relationship between chronic metabolic acidosis, alkalosis, alloxan-induced keto-acidosis and G6PD activities of several tissues.

Keywords: Acidosis; Glucose 6-phosphate dehydrogenase; Diabetes

Materials and Methods

Chemicals

Glucose 6-phosphate, nicotinamide adenine dinucleotide phosphate, alloxan and β-mercaptoethanol were obtained from Sigma Chemical Co. (U.S.A). All other chemicals were reagent grade.

Animals

Male Wistar rats (200-250 g) were purchased from the Pasteur Institute (Tehran). The animals were fed with standard diet (50% carbohydrates, 20% protein, 5% olive oil, 10% cellulose) and maintained as described previously [12].

Acidosis and Alkalosis

To generate metabolic acidosis, five rats received 0.28 M NH_4Cl solution instead of water for 10 days and five rats were given water as controls [6, 13]. Similarly, a 0.8 M sodium bicarbonate solution was given to a group of animals for six days to produce metabolic alkalosis.

Alloxan-Induced Diabetes

The rats were fasted for 24 h and alloxan solution in saline was injected intraperitonally (200 mg/kg) into a group of five animals to induce diabetes, and saline was injected into the control group [14].

Tissue Preparation

The liver was perfused *in situ* using 0.15 M NaCl to remove erythrocytes. The liver and kidney were removed and separately homogenized in 10 mM Tris-HCl buffer pH 7.6 containing 1 mM EDTA. Each homogenate was then centrifuged at 30,000 g for 30 minutes and the supernatant kept for the enzyme assay. For the erythrocyte enzyme, the blood was collected from the decapitated rats, the erythrocytes separated and washed with 0.15 M NaCl and hemolyzed in a solution containing 10 µM $NADP^+$, 7 mM β-mercaptoethanol and 2.7 mM EDTA pH 7.0 as described before [15]. The hemolyzate was centrifuged at 15000 g for 20 minutes and the supernatant kept for G6PD assay.

Measurement of the Enzyme Activity

The enzyme activity was measured in 50 mM Tris-HCl buffer pH 7.4 containing 0.6 mM $NADP^+$, 2 mM glucose 6-phosphate, 5 mM EDTA and the appropriate amount of the supernatant at 25°C using a Perkin-Elmer spectrophotometer model 551 S. The reaction was monitored by measuring the change in absorbance at 340 nm. Each enzyme unit was taken as the enzyme activity producing 1 µmole NADPH/minute.

Protein Determination

Protein was measured by the method of Lowry *et al.* [16].

Determination of Blood pH and Bicarbonate

Blood pH and bicarbonate were measured using an Az4 blood gas analyzer model 985.

Glucose Measurement

Blood glucose was determined by the standard method [17].

Results

Metabolic acidosis and alkalosis induced by NH_4Cl and $NaHCO_3$ are shown in Table 1. The decline in blood pH of NH_4Cl receiving rats was accompanied by a decrease of about 37% in the concentration of blood HCO_3^-. Conversely, in rats given $NaHCO_3$ both blood pH and HCO_3^- significantly increased. Table 1 also shows that alloxan-induced diabetes, indicated by high plasma glucose concentration (1154 g mg/dl), was associated with a chronic acidosis.

The effects of chronic acidosis, alkalosis and alloxan-induced ketoacidosis on G6PD activities of kidney, liver and erythrocytes are presented in Table 2. Chronic acidosis increased the activity of kidney enzyme (55%) but decreased that of the liver (43%) and erythrocyte (38%) enzymes. Alkalosis, however, did not significantly alter G6PD activity of kidney or liver but slightly decreased the erythrocyte enzyme activity. In alloxan-induced diabetes the data are closely similar to those obtained in metabolic acidotic rats. The rise in G6PD activity of kidney was accompanied by a decline in the enzyme activities of liver (50%) and erythrocyte (51%).

Discussion

The data presented in this article demonstrate the association between chronic metabolic acidosis and diabetic acidosis and the activity of hexose monophosphate pathway in various tissues. The rise observed in kidney G6PD activity of rats with both metabolic and diabetic acidosis might be explained in several ways. The acidosis itself may affect the kinetic behavior of G6PD resulting in the change of the enzyme activity. This was, however, ruled out by Peragon *et al.* [6] who have found no change in the kinetic parameters of kidney G6PD activity in acidotic rats. Schoolwerth *et al.* [18] have reported that in rats with chronic acidosis the increased activity of glutamate dehydrogenase was primarily due to an alteration in the enzyme kinetics. Other workers, however, have suggested that an increase in protein synthesis is mainly responsible for the elevation of kidney glutamate dehydrogenase in the acidotic rats [19, 20]. The concentration of total glutamine in renal cells was also elevated during chronic acidosis where ammonia production is increased [21-22].

In alloxan-injected rats, in which ketoacidosis is

Table 1. Induction of chronic acidosis and alkalosis in rats

Animal Conditions	Blood pH	Blood HCO_3^- mM	Plasma glucose mg/dl
Chronic acidosis:			
Control	7.012 ± 0.01	21.92 ± 0.89	-
NH_4Cl receiving	6.86 ± 0.05*	13.7 ± 1.4*	-
Chronic alkalosis:			
Control	7.032 ± 0.03	20.57 ± 0.7	-
$NaHCO_3$ receiving	7.29 ± 0.02*	41.65 ± 5.9*	-
Ketoacidosis			
Control	7.21 ± 0.02	23.3 ± 0.51	104 ± 2.9
Alloxan-injected	7.02 ± 0.05*	10.8 ± 1.75	1154 ± 168

Rats were given or injected the indicated solutions and the pH, blood bicarbonate and plasma glucose measured as described in Methods. Each point represents mean ± S. E. of five rats.
*, significantly different from control ($P < 0.001$ to 0.05).

Table 2. The effects of metabolic acidosis, alkalosis and alloxan-induced ketoacidosis on G6PD activity

Conditions	Enzyme activity, miu/mg protein		
	Control	Experimental	Change %
Metabolic acidosis:			
Kidney	34.48 ± 5.4	53.54 ± 3.5*	55
Liver	51.5 ± 9.6	29.2 ± 0.8*	43
Erythrocytes	23 ± 1.04	14.2 ± 1.9*	38
Metabolic Alkalosis:			
Kidney	14.05 ± 2.9	13.8 ± 1.5	1.8
Liver	10.45 ± 2.05	12.3 ± 1.3	17.7
Erythrocytes	23.3 ± 1.6	17.3 ± 2.5*	25.8
Alloxan-induced ketoacidosis:			
Kidney	20.5 ± 2.05	34.7 ± 5.95*	69
Liver	14.5 ± 3.1	7.3 ± 0.8*	50
Erythrocytes	26.5 ± 1.9	13 ± 1.6*	51

Metabolic acidosis, alkalosis and alloxan-induced ketoacidosis were generated as described in Methods. Each point represents mean ± S. E. of five rats
*, significantly different from control ($P < 0.01$ to 0.05).

developed [23], the role of insulin may also attribute to the change in G6PD activity. Geisler and Hansen [10] have shown that insulin stimulates G6PD activity in adipose tissue through an increase in protein synthesis. This finding may account for the decrease in liver G6PD in diabetic rats, but such an effect on renal enzyme is unlikely since even at low levels of insulin G6PD activity is elevated. Seyer-Hansen [24] has reported that in diabetic rats renal hypertrophy develops and the rate of protein and nucleic acid synthesis in liver and muscles decreases while in the kidney it is increased [25-26]. Acidosis-induced renal

hypertrophy was also associated with a change in pentose phosphate pathway activity [27]. It is, therefore, suggested that in diabetic rats the ketoacidosis is responsible for the observed renal response.

The decreased activity of liver G6PD in metabolic acidosis might be the result of the change in food intake pattern occurring in NH_4Cl receiving rats which in turn alters hepatic dehydrogenases activities [28].

Studies performed on the effects of pH and anions such as bicarbonate, phosphate and sulfate on G6PD activity have shown that these anions stimulate glucose dehydrogenase but inhibit glucose 6-phosphate oxidation activities of the enzyme [29]. These studies have also shown that the above anions and nucleoside triphosphate occupy the same binding site on G6PD. Unemoto *et al.* [30] have found that a low concentration of chloride ion stimulates the enzyme activity but has inhibitory effects at high concentrations. Therefore, in metabolic acidosis and alkalosis, where the concentrations of chloride and bicarbonate, respectively, are increased, erythrocyte G6PD activity is inhibited probably via changes in the enzyme kinetics. More investigation, however, is needed to prove this.

References

1. Levy, H. R. Glucose 6-phosphate dehydrogenase. *Adv. Enzymol.*, **48**, 97-192, (1979).
2. Haghighi, B., Ani, M. and Zakeri, S. Inhibition of human erythrocyte glucose 6-phosphate dehydrogenase activity by dehydroepiandrosterone and related steroids. *Med. J. I. R. I.*, **3**, 75-78, (1989).
3. Martins, R. N., Stoses, G. B. and Masters, C. L. Regulation of the multiple molecular forms of rat liver glucose 6-phosphate dehydrogenase by insulin and dietary restriction. *Bioch. Biophys. Res. Commun.*, **127**, 136-142, (1985).
4. Fritz, R. S. and Kletzien, R. F. Regulation of glucose-6-phosphate dehydrogenase by diet and thyroid hormone. *Mol. Cell Endocrinol.*, **51**, 13-17, (1987).
5. Haghighi, B. and Ghanbari, S. Involvement of adrenal hormones in hydrazine-induced changes of rat hepatic enzymes. *Iranian J. Med. Sci.*, **16**, 135-142, (1991).
6. Peragon, J., Aranda, F., Garcia-Salguero, L., Corpas, F. J. and Lupianez, J. A. Stimulation of rat-kidney hexose monophosphate dehydrogenase activity by chronic metabolic acidosis. *Biochem. Int.*, **18**, 1041-1050, (1989).
7. Sochor, M., Kunjara, S., Greembaum, A. L. and Mclean, P. Renal hypertrophy in experimental diabetes. Effect of diabetes on the pathways of glucose metabolism: differential response in adult and immature rats. *Biochem. J.*, **234**, 573-577, (1986).
8. Peragon, J., Aranda, F., Garcia-Salguero, L., Corpas, F. J. and Lupianez, J. A. Influence of experimental diabetes on the kinetic behavior of renal cortex hexose monophosphate dehydrogenases. *Int. J. Biochem.*, **21**, (6), 689-92, (1989).
9. Glock, G. E., Mclean, P. and Whitehead, J. K. Pathway of glucose catabolism in rat liver in alloxan diabetes and hyperthyroidism. *Biochem. J.*, **63**, 520-524, (1956).
10. Geisler, R. W. and Hansen, R. J. Effects of insulin on the adaptation of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in rat adipose tissue. *Biochim. Biophys. Acta*, **279**, 139-145, (1972).
11. Leader, D. P. and Barry, J. M. Increase in activity of glucose 6-phosphate dehydrogenase in mouse mammary tissue cultured with insulin. *Biochem. J.*, **113**, 175, (1969).
12. Haghighi, B. and Honargou, S. The effects of hydrazine on phosphatidate phosphohydrolase activity in rat liver. *Biochem. Pharmacol.*, **36**, 1163-1165, (1987).
13. Tong, J. Harrison, G. and Curthoys, N. P. The effect of metabolic acidosis on the synthesis and turn-over of rat renal phosphate-dependent glutaminase. *Biochem. J.*, **233**, 139-144, (1986).
14. Wimhurst, J. M. and Manchester, K. L. A comparison of the effects of diabetes induced with either alloxan or streptozotocin and starvation on the activities in rat liver of the key enzymes of gluconeogenesis. *Ibid.*, **120**, 95-103, (1970).
15. Haghighi, B., Suzangar, M., Yazdani, A. and Mehnat, M. A genetic variant of human erythrocyte glucose 6-phosphate dehydrogenase. *Biochem Biophys. Res. Commun.*, **132**, 1151-1159, (1985).
16. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193**, 265-275, (1957).
17. Dubowski, R. M. An o-Toluidin method for body fluid glucose determination. *Clin. Chem.*, **8**, 215, (1962).
18. Schoolwerth, A. C., Nazor, B. L., La Noue, K. F. Glutamate dehydrogenase activation and ammonia formation by rat kidney mitochondria. *J. Biol. Chem.*, **253**, 6177-6183, (1978).
19. Kunin, A. S., Tannen, R. L. Regulation of glutamate metabolism by renal cortical mitochondria. *Am. J. Physiol.*, **237**, F55-F62, (1979).
20. Seyama, S., Salkl, T. and Katanuma, N. Comparison of properties and inducibility of glutamate dehydrogenases in rat kidney and liver. *J. Biochem.* (Tosyo), **73**, 39-45, (1973).
21. Rodriguez-Nichols, F., Langhrey. E. and Tannen, R. L. Response of renal ammonia production to chronic respiratory acidosis. *Am. J. Physiol.*, **247**, F896-F903, (1984).
22. Tong, J., Harrison, G. and Curthoys, N. P. The effect of metabolic acidosis on the synthesis and turnover of rat renal phosphate-dependent glutaminase. *Biochem. J.*, **233**, 139-144, (1986).
23. Fraser, H. S. and Alleyne, A. O. The effect of malnutrition on the pattern of growth in the rat kidney and the renal response to acidosis. *Br. J. Nutr.*, **31**, 113-124, (1974).
24. Seyer-Hansen, K. Renal hypertrophy in streptozotocin

diabetic rats. *Clin. Sci. Mol. Med.*, **51**, 551-555, (1976).
25. Green, M. and Miller, L. L. Protein catabolism and protein synthesis in perfused liver of normal and alloxan-diabetic rats. *J. Biol. Chem.*, **238**, 3202-3208, (1960).
26. Cortes, P., Levin, N. W., Dumler, F., Rubenstein, A. H., Verghese, C. P. and Venkatachalam, K. K. Uridine triphosphate and RNA synthesis during diabetes-induced renal growth. *Am. J. Physiol.*, **238**, 349-357, (1980).
27. Steer, K. A., Sochor, M. and Mclean, P. Renal hypertrophy in experimental diabetes. Changes in pentose phosphate pathway activity. *Diabetes*, **34**, 485-990, (1985).
28. Tepperman, H. M. and Tepperman, J. On the response of hepatic glucose 6-phosphate dehydrogenase activity to changes of diet composition and food intake pattern. *Adv. Enz. Regul.*, **1**, 121-136, (1963).
29. Anderson, W. B. and Nordie, R. C. Glucose dehydrogenase activity of yeast glucose 6-phosphate dehydrogenase. 1. Selective stimulation by bicarbonate phosphate and sulphate. *Biochemistry*, **7**, 3997-4004, (1968).
30. Unemoto, T., Kanbayashi, N. and Hagashi, M. Kinetic of the salf modification of G6PD purified from a marine vibrio algniolyticus. *J. Biochem.* (Tokyo), **81**, 1435-1445, (1977).