MEDICINE

Effect of water and saline overload on the pyruvate kinase and glucose-6-phosphatase activities in the liver of streptozotocin diabetic rats

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Abstract. The aim of the present study was to evaluate the effect of water and saline overload on pyruvate kinase (PK) and glucose-6-phosphatase (G-6-Ph) activities in the liver of streptozotocin (STZ) diabetic rats. In streptozotocin diabetic rats under water and salt stress observed inhibition of glycolytic cleavage of glucose and increasing gluconeogenesis. These changes are more pronounced in the liver of diabetic rats undergoing saline load.

Keywords: *water and saline overload, pyruvate kinase, glucose-6-phosphatase, liver, diabetic rats.*

Introduction. Type 1 Diabetes Mellitus (T1DM) is a widespread chronic disease among children and adolescents [8]. This is a complex disease involving a combination of factors, such as genetic susceptibility, immunologic dysregulation and exposure to environmental trigger [18]. Liver plays a vital role in blood glucose homeostasis. Recent studies have provided considerable evidence that hepatic glucose production plays an important role in the development of fasting hyperglycemia in diabetes [14]. Salt plays an important role in the control of blood pressure in obesity and diabetes mellitus [13]. High-sodium intake may increase blood pressure and diabetes is a salt-sensitive condition [4].

Pyruvate kinase (PK, ATP-pyruvate-O-phospho transferase, EC 2.7.1.40), which plays a metabolic role in bacteria, plants, and vertebrates, is found in various organisms. In mammalian tissues, there are 4 types of PK which are as follow: M1 isoenzyme that is found in skeletal muscle, heart and brain; M2 isoenzyme that is present in kidneys, adipose tissue, and lungs; R isoenzyme that exists in erythrocytes, and L isoenzyme found in liver [3].

PK isoenzymes control consumption of metabolic carbon for biosynthesis and use of pyruvate for energy production. The regulatory role of L-PK plays a crucial role in living organisms. Researches on glycation of intracellular proteins show high susceptibility of the enzymes to modification with methylglyoxal (MG) [3].

Despite many research studies on the importance of glycolysis in production of MG as a glycation factor, little data are available about modification of glycolitic enzymes. It has been shown that MG inhibits glycolysis in metastatic cells of Ehrilich tumor and leukemic leukocytes through the formation of glycated glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Furthermore, glycation of GAPDH from rabbit muscle as a result of treatment with MG leads to a significant decrease in the enzyme activity [3].

A recent study in PK activity on cultured fibroblasts isolated from type 1 diabetic patients with and without nephropathy showed a significant decrease in PK activity in diabetic fibroblasts with nephropathy compared to those of subjects without nephropathy and normal people [3].

Glucose 6-phosphatase (EC 3.1.3.9, G6Ph) is an enzyme that hydrolyzes glucose-6-phosphate, resulting in the creation of a phosphate group and free glucose. Glucose is then exported from the cell via glucose transporter membrane proteins. This catalysis completes the final step in gluconeogenesis and glycogenolysis and therefore plays a key role in the homeostatic regulation of blood glucose levels [14].

The aim of the present study was to evaluate the effect of water and saline overload on pyruvate kinase (PK) and glucose-6-phosphatase (G-6-Ph) activities in the liver of streptozotocin (STZ) diabetic rats [5].

Male Wistar rats weighing 180 +/- 50 g were made diabetic by injection with a single intraperitoneally (i.p.) dose of STZ (65 mg/kg b. w.) [1]. After the induction of diabetes, animals were maintained for 5 days with free access to standard rat chow and tap water [4, 12]. After 5 and 12 days was carried out to determine the level of glucose in vivo [16]. Blood was taken from the tail vein evaluate the basal glycemia level with the use of One Touch Ultra (LifeScan, USA) [16]. Water stress was carried out by introducing the animals water at the rate of 5% of body weight. Saline loading diabetic rats was performed by introducing a 0,1% NaCl at a rate of 5% of the body weight of rats. Blood and tissue samples were collected at day 12 post STZ injection (from diabetic group serum glucose level significantly elevated < or = 300 mg%, p < or = 0.05) [1]. The animals were divided into groups : 1) intact rats (the control group); 2) STZ- diabetic rats with overt (basal glycemia >150 mg%) diabetes; 3) animals with overt diabetes undergoing water stress; 4) animals with overt diabetes undergoing saline stress. Determinations of basal levels of glucose, activities of pyruvate kinase (PK) and glucose-6-phosphatase (G-6-Ph) in the liver were by standard methods [2].

Results.

Our results (Tab.) showed changes in activity of the enzymes studied in STZ-diabetic rats: the activity of PK was 42% lower and the activity of G-6-Ph was higher 182% respectively compared with the same indexes of control rats.

Notable are the studies that found the altered activities of the key enzymes of carbohydrate metabolism such as hexokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase [2, 12]. Other studies have shown that [1, 16], liver function, nitric oxide (NO), malondialdehyde and

phosphoenol pyruvate carboxykinase were significantly increased, while superoxide dismutase, reduced glutathione, total protein, lactate dehydrogenase, pyruvate kinase and hexokinase were inhibited after STZ treatment. So, diabetes in rat liver is accompanied by increase phosphorolysis of glycogen and gluconeogenesis; by decrease - glycolysis and characterized by reduction in the activity of antioxidant enzymes [6, 10, 11].

Table. Changes of pyruvate kinase and glucose-6-phosphatase activities on a background of streptozotocin diabetes in rats which had water or saline stress (x±Sx, n=6)

Indexes / Groups	Pyruvate kinase, mkmol / min × g tissue	Glucose-6-phosphatase, mcg (P)/min×g tissue
1. Control group	51,2±2,28	21,9±1,83
2. Overt diabetes	29,8±2,11[a]	61,8±4,15[a]
3. Overt diabetes + water stress	24,5±1,87 [a]	58,5±3,77 [a]
4. Overt diabetes + saline stress	11,9±1,75 [a,b,c]	93,2±4,63 [a,b,c]

1. a, b, c - changes are reliable ($p \leq 0,05$).
2. a - concerning control (intact) rats ; b - concerning rats with overt diabetes;
c - concerning rats with overt diabetes which had water stress.

Activation of gluconeogenesis explains the relative benefit of glucocorticoids, which induce the synthesis of key enzymes of gluconeogenesis. PK activity in the liver is reduced only under conditions of absolute deficiency of insulin, which leads to a decrease in ATP synthesis, in particular through substrate phosphorylation. Histological examination of diabetic liver showed necrosis and degenerative changes of hepatocytes [1].

According to the results obtained in the liver of rats with STZ diabetes, which had water or saline stress, decreased activity of PK and increased activity of G-6-Ph by an average of 65% and 245% respectively compared with the same indexes of control rats. These changes are more pronounced in the group of diabetic rats undergoing saline load. In the liver of animals of this group activity of PK was 52% lower, and the activity of G-6-Ph was 61% higher than in diabetic rats undergoing water stress. There was no significant difference between the performance of diabetic rats without stress and diabetic rats with water loading. It is well known that fluid overload has a higher predictive value of an elevated risk for renal progression than diabetes in late chronic kidney disease [9].

It is known that, the main neurohumoral mechanisms of salt-induced cardiovascular changes in STZ-diabetes are increased sodium and vascular sensitivity to adrenergic stimuli, which act in combination to produce a final result of higher arterial pressure levels [4]. In other studies indicated that defects in the effects of NO, endothelin, and ATP increase blood pressure, especially in a NaCl-sensitive manner. In diabetes, disruption of NO-induced inhibition of transport may contribute to increased blood pressure and renal damage [7]. Other results show that paraoxonase 1 activity is decreased in fructose-fed insulin-resistant rats on a high-salt diet, which may be associated with increased oxidative stress, leading to inflammation [15].

Diabetic nephropathy (DN) is a major cause of end-stage renal disease (ESRD) affecting nearby 20%–30% of diabetic patients worldwide. Therefore, preventing DN as a serious microvascular complication of IDDM to reduce the risk of ESRD is a clinical priority [17].

Mechanisms by which kidney glomerular, interstitial, and vascular functions are injured consist of inflammation, oxidative stress, endothelial dysfunction, and accelerated fibrosis. Endothelium dysfunction that has been described in DM consists of impairment in many aspects of endothelial functions including anti-inflammatory, antiproliferative, and vasodilatation. In vessels, a balance between vasodilatation and vasoconstriction is achieved by normal endothelial function. Vascular inflammation is a result of combining damage in vasomotor response, augmenting cell proliferation, increasing platelet aggregation, and vascular permeability. Furthermore, endothelial dysfunction has been reported as the early sign of atherosclerosis and atherogenesis. The renin–angiotensin–aldosteron system (RAAS) has a main role in the progression of DN. Inhibition of the renin-angiotensin system (RAAS) may be effective in preventing DN through amending all above mentioned complications [17].

Conclusion. In streptozotocin diabetic rats under water and salt stress observed inhibition of glycolytic cleavage of glucose and increasing gluconeogenesis. These changes are more pronounced in the liver of diabetic rats undergoing saline load.

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Влияние перегрузки водой и солевым раствором на активность пируваткиназы и глюкозо-6-фосфатазы в печени крыс со стрептозотоциновым диабетом

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Аннотация. Цель настоящего исследования состояла в том, чтобы оценить эффект водной и солевой нагрузки на активность пируваткиназы и глюкозо-6-фосфатазы в печени крыс со стрептозотоциновым диабетом. В диабетических крыс под действием водного и солевого стресса наблюдается торможение гликолитического расщепления глюкозы и увеличения глюконеогенеза. Эти изменения более выражены в печени диабетических крыс подвергающихся солевой нагрузки.

Ключевые слова: *водная и солевая перегрузки, пируваткиназа, глюкозо-6-фосфатаза, печень, диабетические крысы.*