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Diabetes and Hypertension: Role of Electrolytes and Na⁺-K⁺-ATPase

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Abstract: Diabetes mellitus and hypertension frequently coexist. A variety of aberrations have been reported in the metabolism of body chief electrolytes (such as Na⁺, K⁺, Ca⁺⁺ and Mg⁺⁺) and their transport systems in intra and extracellular environment in various metabolic disorders. The study was aimed to examine the similar abnormality in diabetes with hypertension. Thirty hypertensive diabetic patients were selected verses same number of age and sex matched controls with no known history of hyperglycemia and hypertension. A significant difference was observed in blood pressures of both the groups. Intra-erythrocyte sodium level was observed elevated significantly (P<0.01) where as intra-erythrocyte potassium was observed lower significantly (P<0.05) in hypertensive diabetic patients. The serum sodium, calcium, magnesium as well as membrane Na-K-ATPase activity were observed reduced to a significant level (P<0.01) where as serum potassium level was also found reduced significantly (P<0.05) in hypertensive diabetic patients as compare to controls. These findings suggest that the constellation of disturbed cellular ions and their transport mechanisms may critically contribute to pathophysiology of these syndromes and may help to explain their frequent clinical coexistence.

Key words: Diabetes, hypertension, electrolytes, Na-K-ATPase

INTRODUCTION

Hypertension is about twice as prevalent in patients with diabetes as in those without disease. In patients with type II diabetes, elevated blood pressure usually occurs in association with obesity and insulin resistance. Endothelial dysfunction and pressor effects of hyperinsulinemia may contribute directly to raised arterial pressures; however, hypertension is generally considered a renal phenomenon (Elliot *et al.*, 2001). In contrast with diabetes type I, hypertension in type II diabetes mellitus develops even without renal involvement (Huang *et al.*, 1998).

Patients with Diabetes mellitus are at high risk of cardiovascular events. The risk of vascular events is further heightened by coexistent hypertension. (Turner *et al.*, 1998).

The hypertension that frequently accompanies Diabetes mellitus is characterized by abnormalities of sodium metabolism at all physiologic levels, whole-body, renal and cellular. The most consistently described abnormality is an expansion of exchangeable sodium which seems to be closely associated with increased proximal renal tubular sodium reabsorption and suppression of membrane Na-K-ATPase in circulating cells (Weder, 1994).

Abnormal sodium metabolism has a critical role in the etiology of essential hypertension. This has lead to the

hypothesis that can increase in the circulating concentration of Na-K-ATPase inhibitor, which is responsible for the cause of essential hypertension (Blaustein, 1977). Insulin resistance with hyperinsulinemia is characteristic of the metabolic syndrome, and this condition has been associated with high cardiovascular risk, morbidity and mortality (DeFronzo and Ferranini, 1991). Each of these conditions has a cellular resistance to insulin action and also a common underlying abnormality of cellular ion content, viz., higher basal cytosolic free calcium, and/or reciprocally lowers cytosolic free magnesium levels (Barbagallo *et al.*, 2000).

The rise in plasma insulin levels may elevate blood pressure levels by a variety of mechanisms, including increased sympathetic activity and sodium retention (Landsberg, 1994). Obesity-induced changes in the renal medulla, resulting in activation of the renin-angiotensin system, may also contribute to sodium retention and hypertension (Hall, 1994).

Hyperinsulinemia due to exogenous insulin administration may also play a role, because insulin stimulates sodium reabsorption (Elliot *et al.*, 2001). This technique can be associated with urinary losses of sodium and bicarbonate, leading to volume depletion and metabolic acidosis (Hricik *et al.*, 2000). Previous studies have identified ionic aspects of insulin resistance in hypertension, in which cellular responses to insulin were influenced by the basal intracellular ionic environment-the

lower the cytosolic free magnesium, the less magnesium increased following insulin stimulation (Barbagallo *et al.*, 2001).

Potassium depletion is a common feature of hypertension and type II diabetes. Treatment of hypertension at least partially restores potassium levels towards normal and fasting steady state potassium levels are closely linked to calcium and magnesium homeostasis. Altogether these findings in previous studies emphasize the similar and coordinate nature of ionic defects in diabetes and hypertension and suggest that their interpretation requires an understanding of their interaction (Resnick *et al.*, 2001). Glucose related excess calcium is a fundamental lesion in diabetes that contributes the elevated blood pressure and cardiac mass in this disease (Barbagallo *et al.*, 1996).

The aim of the present study was to investigate the changes in membrane Na⁺-K⁺-ATPase, serum and red cell electrolytes in hypertensive diabetic patients.

MATERIALS AND METHODS

Study population: Thirty hypertensive diabetic patients with out intercurrent illness or sever diabetic complications were studied on the occasion of a regular follow-up after informed consent was obtained. Their mean age was 38±2.5 years. None were taking any medication known to influence Na⁺-K⁺-ATPase, such as drugs like calcium blockers, thyroxin, glucocorticoid, mineralocorticoid or digitalis. Thirty age matched healthy normotensive subjects with no known history of hyperglycemia were selected as controls.

Sample collection: Fasting blood samples were collected from control and diabetic subjects in lithium heparin coated tubes followed by measuring their blood pressure readings using sphygmomanometer. A portion of blood was collected to obtain serum.

Intra-erythrocyte sodium and potassium estimation: Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room temperature by suspension in the magnesium chloride solution (112 mmol L⁻¹), centrifugation at 450 x g at 4°C for 5 minutes and aspiration of the supernatant as described earlier (Meyer and Starkey, 1977). Final supernatant was retained for the estimation of intra-erythrocyte sodium and potassium concentration. Neither electrolyte was detectable in the final wash. Washed erythrocytes were then lysed and used for the estimation of intra-erythrocyte sodium and potassium.

Erythrocyte membrane preparation: The red cell pack extracted by centrifugation at 4°C were resuspended and diluted in 25 volumes of Tris buffer at pH 7.4. The hemolyzed cells were then centrifuged at 12,000 rpm at 4°C and the membrane pellet was suspended in 30 ml of 0.11 mol L⁻¹ Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein ml⁻¹ of Tris buffer. The membrane suspension was stored at -80°C until the assay was performed.

Erythrocyte Na⁺-K⁺-ATPase activity measurement (Racciah *et al.*, 1996): ATPase activity was measured in a final volume of 1 ml as follows: Membrane (400 ug) were preincubated for 10 minutes at 37°C in a mixture containing 92 mmol L⁻¹ Tris-HCl (pH=7.4), 100 mmol L⁻¹ NaCl, 20 mmol L⁻¹ KCl, 5 mmol L⁻¹ MgSO₄ .H₂O and 1 mmol L⁻¹ EDTA. Assays were performed with and without 1mmol L⁻¹ Ouabain, a specific inhibitor of Na-K-ATPase. After incubation with 4 mmol L⁻¹ ATP (Vanadate free, Sigma) at 37°C for 10 minutes, the reaction was stopped by adding ice-cold trichloroacetic acid to a final concentration of 5%. After centrifugation at 4°C, 5500 g for 10 minutes. The amount of inorganic phosphate in the supernatant was determined (Dryer and Tammes, 1957). Na⁺-K⁺-ATPase activity was calculated as the difference between inorganic phosphate released during the 10-minute incubation with and without Ouabain. Activity was corrected to a nanomolar concentration of inorganic phosphate released milligram⁻¹ protein h⁻¹. The concentration of protein was estimated by Biuret method.

All assays were performed in duplicate, and blanks for substrate, membrane and incubation time were included to compensate for endogenous phosphate and non-enzyme related breakdown of ATP. Under these experimental conditions, the coefficient of variation was 7.5%.

Serum electrolyte estimation: Serum sodium, potassium and calcium were estimated by flame photometer (Corning 410). Serum magnesium was estimated by the method described earlier (Hallry and Sky peck, 1964).

Statistical analysis: Results are presented as mean±SD. Statistical significance and difference from control and test values evaluated by Student's t-test.

RESULTS

An intra-erythrocyte sodium concentration was elevated significantly (P<0.01) in hypertensive diabetic patients as compared to normotensive control subjects where as intra-erythrocyte potassium level was

Table 1: Intra-erythrocyte sodium, potassium and membrane Na-K-ATPase activity in control and hypertensive diabetic patients

	Control	Patients
Intra-erythrocyte sodium (mmol L ⁻¹)	11.55±4.83	17.99±3.35*
Intra-erythrocyte potassium (mmol L ⁻¹)	106.61±21.41	97.55±9.52**
Membrane Na-K-ATPase activity (nmol mg ⁻¹ protein h ⁻¹)	443.37±282	58.86±24.62*

Table 2: Blood pressure values and serum electrolytes in control and hypertensive diabetic patients

	Control	Patients
Serum sodium (mmol L ⁻¹)	138.1±13.79	92.67±13.06*
Serum potassium (mmol L ⁻¹)	5.03±1.51	4.59±1.71**
Serum calcium (mmol L ⁻¹)	2.11±0.41	1.57±0.49*
Serum magnesium (mmol L ⁻¹)	1.25±0.68	0.72±0.25*
Systolic blood pressure (mm Hg)	123.77±8.13	143.87±13.55*
Diastolic blood pressure (mm Hg)	79.36±6.79	88.9±7.02*

Values are mean±SD. *P<0.01, **P<0.05

significantly lower (P<0.05) in patients as that of in control subjects (Table 1). The Na-K-ATPase activity was estimated reduced significantly (p<0.01) in diabetic patients with hypertension as compared to control subjects (Table 1).

The concentrations of serum sodium, calcium and magnesium were observed lower significantly (P<0.01) in hypertensive diabetic subjects as compared to control subjects, where as the serum potassium level was also found reduced significantly (P<0.05) in diseased persons as that of control subjects (Table 2).

There was a significant difference (P<0.01) was observed in the levels of systolic as well as diastolic blood pressures between hypertensive diabetic patients when compared to normotensive non-diabetic control individuals (Table 2).

DISCUSSION

Several studies on the mechanisms and progression of hypertension in Diabetes mellitus have been done in human and animal subjects, but these studies some times are contradictory; although the role of sodium, potassium, calcium and magnesium in the blood pressure regulation particularly during Diabetes mellitus is well established (Bloomgarden, 2001).

The increased intra-erythrocytes sodium with decreased intra-erythrocyte potassium, serum sodium, calcium and magnesium in diabetic subjects is a consequence of decreased Na⁺-K⁺-ATPase activity due to the increased systolic and diastolic blood pressures as observed during the presented study.

Na⁺-K⁺-ATPase is a ubiquitous enzyme that ensures that the transmembrane gradients of sodium and potassium concentrations are maintained. Alterations of this transport enzyme are thought to be linked to several complications of Diabetes mellitus, hypertension for example (Totan and Greaby, 2002).

In present study, the intra-erythrocyte concentrations of sodium were found increased with a decrease in intra-erythrocyte potassium level in hypertensive diabetic patients as compare to normal individuals (Table 1). Inhibition of Na⁺ K⁺ ATPase activity is the main factor as in most of the cardiovascular diseases, inhibited or reduced ATPase activity has been observed. Various studies reported such a fact; inhibition of Na⁺ K⁺ ATPase activity during cardiovascular problems (Lees, 1991; Walter and Muller, 1985; Hamlyn and Ringel, 1982; Gault *et al.*, 1983; Lijnen, 1990).

The other factor involved in increased intra-erythrocyte sodium and decreased potassium levels during cardiovascular diseases are impaired Na⁺-Ca²⁺ exchange system and enhanced activity of Na⁺-Li⁺ counter transport system. The Na-Li counter transport system is significantly higher in diabetic patients with hypertension than in those with normotension and significantly higher in patients with a positive family history of hypertension. These facts strongly reflect a predisposition of hypertension in Diabetes mellitus type II (Fujita *et al.*, 1994).

Sodium retention occurs as a characteristic alteration in type I as well as type II diabetes; exchangeable body sodium is increased by 10% on average. This abnormality develops in the uncomplicated stage of diabetes and differentiates diabetic from non-diabetic essential hypertensive subjects. Possible sodium retaining mechanisms include increased glomerular filtration of glucose leading to enhanced proximal tubular sodium-glucose cotransport, hyperinsulinemia, which activates several tubular sodium transporters, an extravascular shift of fluid with sodium, and, once it occurs, renal failure. This pathogenetic role of sodium in diabetes-associated hypertension is supported by positive correlations between systolic or mean blood pressure and exchangeable sodium of the body (Weidmann and Ferrari, 1991). The chronic excessive sodium intake leads to the elaboration of a substance that inhibits the Na⁺ K⁺ ATPase activity with subsequent rise in intracellular sodium concentration. Angiotensin converting enzyme inhibitors (ACEI) are used to treat hypertension as they increase the secretion of Aldosterone thus enhancing the activity of Na⁺ K⁺ ATPase (Sansom and O'Neil, 1981; Muto *et al.*, 1987). ACEI are preferred over diuretics and β blockers in the treatment of hypertension, as hypertensive patients treated with β blockers or diuretics had higher mortality rates (Roman and Cuvaevs, 1998).

The present study revealed a decreased serum calcium and magnesium levels in association with increased blood pressure (Table 2). The decreased levels of serum magnesium were also reported in hypertensives as compared to normotensives. Magnesium appears to be

a special kind of calcium antagonist in vascular smooth muscles. At vascular membrane it can lower peripheral and cerebral vascular resistance (Altura *et al.*, 1987). Decreased level of magnesium also results in the inhibition of Na-K-ATPase enzyme because magnesium is a co-factor for this enzyme.

From the results obtained during present study, it can be hypothesized that, hypertension and peripheral insulin-resistance may be different clinical expressions of a common abnormal ionic environment, characterized at least in part by suppressed free magnesium levels (Resnick *et al.*, 1990). In various previous studies it has been shown that the total calcium concentration is increased in the red cells from essential hypertension and, to a lesser extent, in the red cells from renal hypertension. The use of calcium-potassium ratios in red cells may thus be useful in assessing cellular calcium content in hypertension (Zidek *et al.*, 1986).

In conclusion, the results reported here suggest that Na⁺-K⁺-ATPase dysfunction and alterations in intra-erythrocyte and serum electrolytes induced by diabetes is implicated in the complications of Diabetes mellitus most commonly in the pathogenesis of hypertension and vascular diseases in humans. The observations are also focused on the possibility that apart from direct angiotensin II induced blood pressure elevation, the basic cellular abnormality in Diabetes mellitus is an inability to maintain a normal transmembrane electrolyte gradient.

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