BLOOD PRESSURE LOWERING EFFECTS OF ATENOLOL IN RATS: ROLE OF NA-K-ATPASE, SERUM, RED CELL AND TISSUE ELECTROLYTES

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ABSTRACT
Beta adrenoceptor blocking drugs have been shown to decrease the incidence of ventricular fibrillation and sudden cardiac death in patients with coronary artery disease. Although the blood pressure lowering effect of atenolol is studied through hormonal mechanisms. There is no specific study available regarding the role of electrolyte alterations in blood pressure lowering effects of atenolol. The present work was designed to investigate the role of serum, red cell and tissue electrolytes and Na-K-ATPase in blood pressure lowering effects of atenolol (a beta blocking drug). Rats were divided into two experimental groups. Atenolol (4mg/kg body weight) was administered intraperitoneally to the test group. Control group received same volume of vehicle. Systolic blood pressure was significantly reduced after atenolol administration. An increased membrane Na-K-ATPase activity was observed after atenolol administration. Atenolol treatment decreases sodium and increase potassium in red blood cells. Concentration of sodium, potassium, calcium and magnesium was increased in serum after atenolol treatment. Atenolol treatment decreases sodium and calcium content in heart and kidney tissues whereas an increased content of potassium was observed in these tissues. The results reported in the present study suggests that apart from hormonal mechanism an alteration in electrolytes levels in red cell, serum, heart and kidney tissues and membrane Na-K-ATPase are associated with the blood pressure lowering effect of atenolol. The role of increased activity of Na-K-ATPase in the changes of sodium and potassium in red cell, serum, kidney and heart tissue after atenolol administration is discussed.

INTRODUCTION
Beta blockers are among the drugs most commonly used to treat the patients with essential hypertension and coronary artery disease (Marquez J.M. et al., 1987 and Robert G.G. et al., 1990). Beta adrenergic receptor Mocker therapy also known to be results in decreased mortality after myocardial infarction (Richard G.G. et al., 1990). Beta-adrenoceptor blocking drugs have been shown to decrease the incidence of ventricular fibrillation and sudden cardiac death in patients with coronary artery disease (Peukkurinen K.J et al., 1994; Pasini E. et al., 2000 and Nick F. et al., 1999). The mechanism of antihypertensive action of beta adrenoceptor blocking agent is not well understood. Previous studies showed that the antihypertensive pharmacological action takes place at the site of beta receptor (Richard G.G. et al., 1990; Haye P.C. et al., 1987 and Olofesson B.O. et al., 1989). The antihypertensive mechanism in the pharmacological response of beta blockers are reduction in cardiac output, suppression of renin release, resetting of baroreceptors, direct action on CNS, release of vasodilator prostaglandins and blockade of prejunctional beta receptors. (Barat E. et al., 1989; Buhler F.R. et al., 1972 and Prichard B.N.C. and Owans C.W.I. 1980). Role of electrolytes such as sodium, potassium and calcium in the blood pressure regulation is often described in
various clinical and experimental studies (Leblondel G. and Allain P. 1988; McCarron D.A. 1982 and 1982). A rise of intracellular concentration was suggested to play an important role in the pathogenesis of both genetic and induced form of hypertension (Ousel P.A. and Motulsky H.J. 1984). Hypertensive animals and human demonstrated several distinct alterations in calcium metabolism which include low ionized calcium concentration and increased intracellular calcium concentration (Young E.W. et al., 1988). An stimulated Na-K-pump activity has also been reported in DOCA-salt hypertension (Bin Talib H.K. and Zicha J., 1992). Although the blood pressure lowering effect of atenolol I4-(2-hydroxy-3-isopropylaminopropoxy) phc-nylacetamide] is studied through hormonal mechanisms (Darbar D. et al., 1996 and Insel P.A. and Motulsky H.J, 1984), there is no specific study available regarding the role of electrolytes and Na-K-ATPase activity in the blood pressure lowering effect of atenolol.

From known effects of electrolytes on cell metabolism and function and of hormone on electrolytes homeostasis, a link between electrolyte alterations, Na-KATPase activity and the blood pressure lowering effect of atenolol can be derived. The present study was designed to investigate the role of serum, red cell and tissue electrolytes and Na-K-ATPase activity in blood pressure lowering effect of atenolol, a beta blocking drug.

Using male albino wistar rats we tried to elucidate the following investigations:

i) The relation between changes in serum sodium, potassium, calcium and systolic blood pressure after Atenolol administration.

ii) To investigate the changes in intracellular levels of sodium and potassium in erythrocyte, kidney and heart tissue after Atenolol administration.

iii) To study the changes in Na--K-ATPase activity in red cell membrane after Atenolol administration.

**Materials and Methods**

**Animals and adaptation phase:**

12 weeks old male albino wistar rats (180-250g body weight), bred and grown in our own laboratory were used during the study. Animals were individually caged in a quiet temperature controlled room at 23 ± 4°C and maintained on a 12/12 light dark cycle with lights off at 7:00 a.m. Rats had free access to water and standard rat diet. Experiments on these rats started after two weeks of habituation.

**Drug Administration:**

On the day of the experiment animals were randomly divided to control and test groups (n=10 for each). Test group received atenolol (4mg/kg body weight) intraperitoneally. Control group received same volume of deionized water through the same route.

**Blood pressure measurements:**

Before decapitation blood pressure was measured in terms of mmHg by Harvard indirect rat tail blood pressure system. Rats were placed in restrainer for approximately 10 minutes before the onset of BP determinations. The mean of three-artifact free determination (not differ by 10%) served as an index of Systolic blood pressure (SBP).

**Sample Collection:**

After one hour of injection animals were decapitated and blood was sample from head wound in the lithium heparin coated tubes. A portion of blood was used to collect serum. Heart and kidney were excised, trimmed of connective tissues, rinsed with deionized water to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer until analysis. Preparation of RBC membrane fractions was begun within 30 minutes of blood collection.

**Serum Electrolyte measurements:**

Serum was analyzed for the estimation of sodium, potassium and calcium by flame photometry.
**Intraerythrocyte sodium and potassium estimations:**

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), centrifugation at 450 x g at 4°C for 5 minutes and aspiration of the supernatant as describe earlier (14). Final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration. Neither electrolytes were detectable in the final wash. Washed erythrocytes were then lysed and used for the estimation of intraerythrocyte sodium and potassium (Tabassum M. et al., 1996).

**Erythrocyte membrane preparation:**

The packed red cells extracted by centrifugation at 4°C, 450 x g for 15 minutes were resuspended and diluted in 25 volumes of 0.011 mol/L, Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged for 30 minutes at 12,000 rpm at 4°C and the membrane pellet was resuspended in 30 ml of 0.011 mol/L Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was -4 mg protein /ml of Tris buffer. The membrane suspension was stored at -80°C until the assay was performed.

**Erythrocyte Na-K-ATPase activity measurement:**

ATPase activity was measured in a final volume of 1 ml as follows: Membrane (400µg) were preincubated for 10 minutes at 37°C in a mixture containing 92 mmol/L Tris-HCl (pH=7.4), 100 mmol/L NaCl, 20 mmol/L KCl, 5 mmol/L MgSO4.H2O and 1 mmol/L EDTA. Assays were performed with or without 1mmol/L Ouabain, a specific inhibitor of Na-K-ATPase. After incubation with 4 mmol/L ATP (Vanadate free, Sigma) at 37°C for 10 minutes, the reaction was stopped by adding of ice cold trichloroacetic acid to a final concentration of 5%. After centrifugation at 4°C, 5500g for 10 minutes. The amount of inorganic phosphate in the supernatant was determined (Dryer R.L., 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 protein/hour.

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%.

**Tissue digestion and Electrolyte measurements:**

Frozen tissues (kidney and heart) were digested for 3 hours at room temperature and then at 70°C for another 3 hour in 20 ml deionized water followed by 10 ml of concentration nitric acid and perchloric acid (equal volume). The samples were initially heated very gently. After foaming subsided temperature was increased to produced steady boiling. The excess acids were boiled off to near dryness. The digest then cool to room temperature and analyzed for sodium, potassium and calcium by flame photometry.

**Statistical Analysis:**

Results are presented as mean ± SD. Significance of control and test values was evaluated by student's t-test.

**RESULTS AND DISCUSSION**

Several studies on the mechanism of antihypertensive effects of beta adrenoceptor antagonist have been done in human and animals (Peuhkurinen K.J. et al., 1994; Pasini E. et al., 2000 and Prichard B.NC. and Owans C.W.I., 1980). But these studies sometimes are contradictory and most of the studies available showed the action of these antagonists through various physiological and hormonal mechanism. Although the role of sodium, potassium and calcium in the blood pressure regulation is well established.
However non-of these studies showed the role of electrolytes (sodium, potassium and calcium) and Na-K-ATPase in the blood pressure lowering effect of Atenolol.

The results (tables I, II, III and IV) of this investigation demonstrated that a number of changes in electrolyte contents of red blood cell, serum, heart and kidney tissues were, observed in rats after Atenolol administration

Na-K-ATPase is a ubiquitous enzyme that ensures that the transmembrane gradients of sodium and potassium concentrations are maintained. This enzymes activity is mainly studied in the erythrocyte membranes because these cells are easily accessible. A significant increase of the Na-K-ATPase activity of erythrocyte membrane was observed after atenolol administration with simultaneous decrease in systolic blood pressure (Table 1).

Table 1 showed a significant decrease in systolic blood pressure after atenolol treatment. Atenolol treatment decreased sodium (p<0.05) and increased potassium (p<0.005) in red blood cells (Table 1). These results may be a consequence of increased Na-K-ATPase activity in the erythrocyte membrane as shown in Table I. Our results are in agreement with those published previously (Fagher B. et al., 1993). Serum magnesium is known to be act as a cofactor for the activation of Na-K-ATPase (Ebel H. and Gunther T, 1983; Altura B.M. and Altura B.T., 1985). The increased serum magnesium observed in the present study may also activate the Na-K-ATPase. Activation of Na-K-ATPase decreases sodium influx and potassium efflux across cell membrane and thus an increased intracellular potassium and decreased sodium content. At the same time concentration of sodium in serum increased

**Table I**

Effects of intraperitoneal administration of Atenolol (4 mg/kg) on systolic blood pressure, Na-K-ATPase and red cell sodium and potassium in rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>139 ± 4.0</td>
<td>104 ± 6.0**</td>
</tr>
<tr>
<td>Na-K-ATPase Activity (nmol/mg protein/hr)</td>
<td>212 ± 4.5</td>
<td>260 ± 6.0**</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>5.65 ± 0.8</td>
<td>4.19 ± 0.4**</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>124.9 ± 2.4</td>
<td>136 ± 2.1*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.  
P** < 0.05, P* < 0.005

**Table II**

Effects of intraperitoneal administration of Atenolol (4 mg/kg) on serum electrolytes in rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/L)</td>
<td>138 ± 1.0</td>
<td>153 ± 2.5**</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>5.9 ± 0.4</td>
<td>7.6 ± 0.2**</td>
</tr>
<tr>
<td>Calcium (mEq/L)</td>
<td>2.8 ± 0.2</td>
<td>4.9 ± 0.3**</td>
</tr>
<tr>
<td>Magnesium (mEq/L)</td>
<td>1.8 ± 0.3</td>
<td>2.8 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.  
P** < 0.05, P* < 0.005
(Table II). Potassium levels in erythrocytes and serum are found to be increased after atenolol administration (Tables I, II). These changes in potassium may be due to the fact that beta adrenergic antagonist can decrease renal renin release which causes decrease in angiotensin II (AII), thus aldosterone production is decreased. Beta blocker administration have also been reported to decrease potassium loss (Lind L. et al., 1994; Gullestad L. et al., 1992; Sorensen E.V. et al., 1991 and Levett J.M et al., 1994). The hyperkalemia may also occur by suppression in renin release. It is suggested that renin release is suppressed by small doses of all beta blockers. The fall in blood pressure in response to beta blocker therapy may be related directly to changes in plasma renin activity and thus increased serum potassium as observed in the present study (Table II).

In the present study, it was observed that sodium and calcium content of heart and kidney were decreased whereas potassium content was increased after Atenolol administration as shown in Table III and IV. The decreased sodium in heart and kidney tissue may be due to the activation of Na-K-ATPase. The decrease tissue sodium also decreases the calcium influx through Na-Ca-exchange mechanism and thus caused a decreased intracellular calcium content. The decreased intracellular calcium results in a vasorelaxant action and lowering the blood pressure.

The results reported in the present study suggest that apart from decreased renin release an increased membrane Na-K-ATPase and alterations in the electrolytes particularly decreased sodium and calcium and increased potassium in red cells, heart and kidney tissues are associated with the blood pressure lowering effect of Atenolol.

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REFERENCES


Nick F., John C., Philip Y., James M. and Jan


