Sodium and ATP Affinities of the Cardiac Na^+,K^+-ATPase in Spontaneously Hypertensive Rats

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Abstract. The Na^+,K^+-ATPase is postulated to be involved in systemic vascular hypertension through its effects on smooth muscle reactivity and cardiac contractility. Investigating the kinetic properties of the above enzyme we tried to assess the molecular basis of alterations in transmembrane Na^+-efflux from cardiac cells in spontaneously hypertensive rats (SHR). In the investigated group of SHR the systolic blood pressure and the heart weight were increased by 48% and by 60%, respectively. Upon activating the cardiac Na^+,K^+-ATPase with substrate, its activity was lower in SHR in the whole concentration range of ATP. Evaluation of kinetic parameters revealed a decrease of the maximum velocity (V_{max}) by 28% which was accompanied with lowered affinity of the ATP-binding site as indicated by the increased value of Michaelis-Menten constant (K_m) by 354% in SHR. During activation with Na^+, we observed an inhibition of the enzyme in hearts from SHR at all tested Na^+ concentrations. The value of V_{max} decreased by 37%, and the concentration of Na^+ that gives half maximal reaction velocity (K_{Na}) increased by 98%. This impairment in the affinity of the Na^+-binding site together with decreased affinity to ATP in the molecule of the Na^+,K^+-ATPase are probably responsible for the deteriorated efflux of the excessive Na^+ from the intracellular space in hearts of SHR.

Key words: Na^+,K^+-ATPase — Na^+-binding site — ATP-binding site — Hypertension — Cardiac sarcolemma

Introduction

The Na^+,K^+-ATPase or Na^+ pump (EC 3.6.37) is present in the plasma membrane of practically all eukaryotic cells. This enzyme couples the energy released in the intracellular hydrolysis of ATP to the export of three intracellular Na^+ ions

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and the import of two extracellular K^+ ions. Thus the Na^+,K^+-ATPase maintains the transmembrane ion balance needed to establish and control the membrane potential, i.e., it plays an essential role in cell survival (Skou and Esmann 1992). Na^+ pump inhibition has been described in vessels (Pamnani et al. 1978, 1981a, 1981b) and myocardium (Clough et al. 1983, 1984; Haddy 1990) of animals and patients with low-renin hypertension. Moreover, decreased ouabain-sensitive Na^+,K^+-ATPase activity with increased intracellular Na^+ concentration has been described in erythrocytes, leukocytes, and lymphocytes from hypertensive and normotensive patients with a family history of hypertension (Edmondson et al. 1975, Ambresioni et al. 1981, Heagerty et al. 1982, de Wardener and MacGregor 1983, Cooper et al. 1987). The Na^+ pump is a major cellular transport system that controls Na^+ homeostasis (O'Donnell and Owen 1994) and membrane potential (Hermsmeyer and Erne 1990) – both key factors in the regulation of vascular tone and blood pressure (Marin et al. 1988; Marin 1995; Marin and Redondo 1999), which suggests that the altered Na^+ pump could be a causative factor in essential hypertension (Blaustein et al. 1986). Indeed, alterations of electrogenic ion transport ascribed to the Na^+ pump have been shown in spontaneously hypertensive rats (SHR) (Arvola et al. 1992; Pörsti et al. 1992). Concerning the function and molecular properties of Na^+,K^+-ATPase at conditions of hypertension we focused our attention in the present study to two main questions. One concerned the energy supply by ATP and the second involved the binding of Na^+ to the enzyme molecule.

Materials and Methods

Experimental protocol

One group of male Wistar Kyoto rats ($n = 10$) and one group of male SHR ($n = 10$) were taken for the study. The groups were age-matched and in both groups the systolic blood pressure (SBP) was measured by the noninvasive method of tail cuff plethysmography. In the age of 34 weeks the animals were sacrificed, the hearts were quickly excised, immediately frozen in liquid nitrogen and stored for further biochemical investigations.

Isolation of sarcolemmal membranes

Cardiac sarcolemma was prepared from pooled samples of two hearts by the hypotonic shock-NaI treatment method as described previously (Vrbjar et al. 1984). The protein content was determined by the procedure of Lowry et al. (1951) using bovine serum albumin as a standard.

Kinetics of Na^+, K^+-ATPase

The substrate kinetics of Na^+,K^+-ATPase was estimated measuring the splitting of ATP by 30 μg sarcolemmal proteins at 37 ℃ in the presence of increasing concentrations of ATP in the range of 0.16–8.0 mmol/l in a total volume of 0.5 ml

medium containing 50 mmol/l Imidazole (pH 7.4), 4 mmol/l MgCl$_2$, 10 mmol/l KCl and 100 mmol/l NaCl. After 15 minutes preincubation in the substrate-free medium, the reaction was started by addition of ATP and 15 minutes later was terminated by 1 ml of 12 % solution of trichloroacetic acid. Verification of the time dependence of ATP-hydrolysis showed that the ATP splitting was linear up to 20 min in the whole ATP concentration range applied. The inorganic phosphorus liberated was determined according to Taussky and Shorr (1953). In order to establish the Na$^+$,K$^+$-ATPase activity, the ATP hydrolysis that occurred in the presence of Mg^{2+} only was subtracted. From each sarcolemmal preparation three individual values for Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) were obtained. The Na$^+$,K$^+$-ATPase kinetics for sodium was determined by the same approach, in the presence of increasing concentrations of NaCl in the range 2.0–100.0 mmol/l, using a constant amount of ATP (4 mmol/l).

Data processing

The kinetic parameters were evaluated by direct nonlinear regression of the data obtained. All results were expressed as mean ± S.E.M. The significance of differences between the individual groups was determined with the use of ANOVA, Bonferroni test. A value of $p < 0.05$ was regarded significant.

Results

General haemodynamic parameters

The systolic blood pressure was increased in 34 weeks-old SHR by 48 % as compared to the age matched control group. Heart weight increased by 60 % in the SHR group. On the other hand, the growth of the whole body was slowed down in the SHR group as it reveals from the decrease of body weight by 15 %. The heart weight/body weight ratio was significantly higher by 88 % in the SHR group than in the control group. See Table 1.

Table 1. Haemodynamic characteristics of 34 weeks-old spontaneously hypertensive rats and age matched control Wistar rats

	Controls	SHR
SBP [mm Hg]	129 ± 3	191 ± 2*
Heart weight [mg]	1070 ± 22	1713 ± 49*
Body weight [g]	486 ± 10	413 ± 8*
Hw/Bw ratio × 1000	2.202	4.148

SBP, systolic blood pressure; Hw/Bw, heart weight/body weight ratio. Number of estimations: SHR ($n = 10$), controls ($n = 10$). Data represent means ± S.E.M.; * $p < 0.05$.

Na⁺,K⁺-ATPase study

The activity of sarcolemmal Na⁺,K⁺-ATPase was determined in the presence of constant amounts of the cofactors Na⁺ and K⁺ at eight different concentrations of ATP. The enzyme was significantly less activated by ATP at all tested ATP concentrations in hearts from SHR group as compared to control animals (Fig. 1A). The
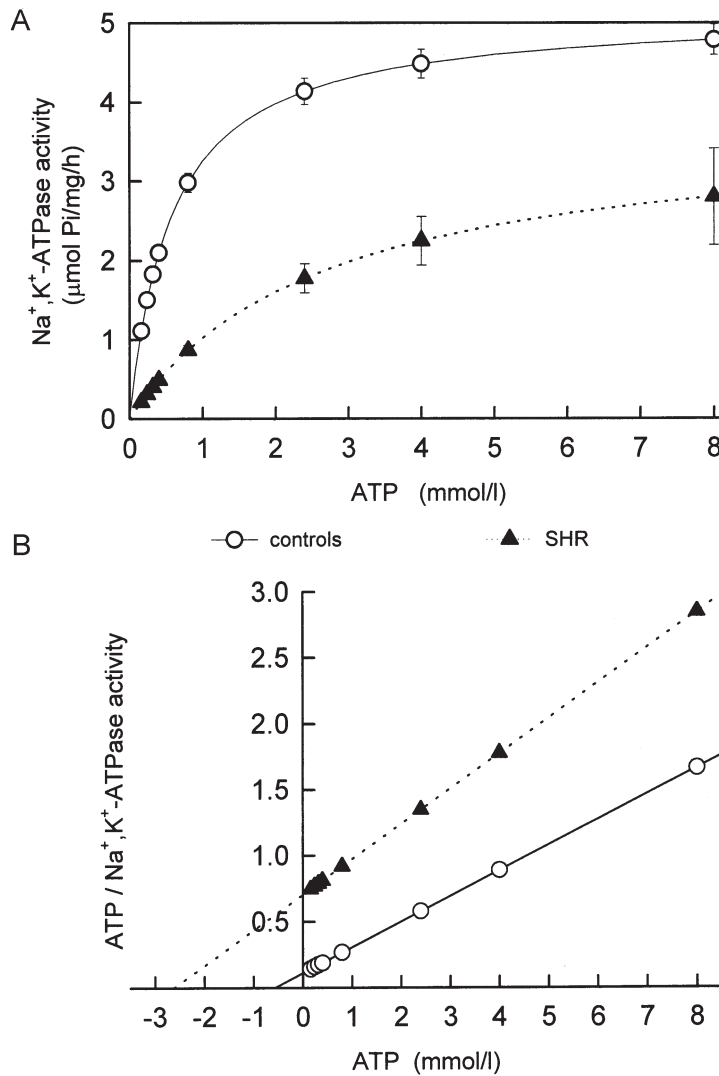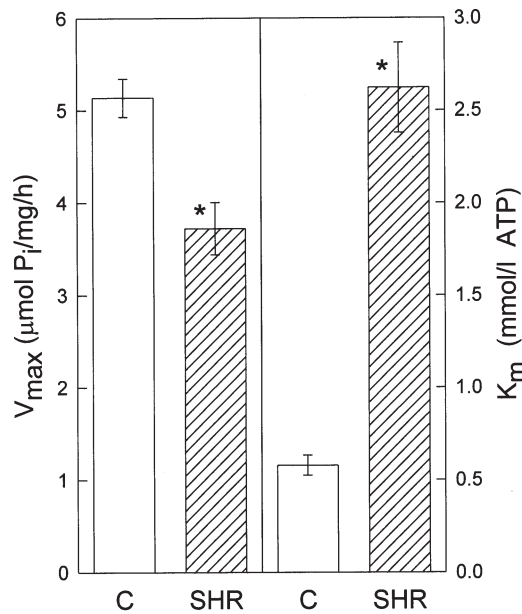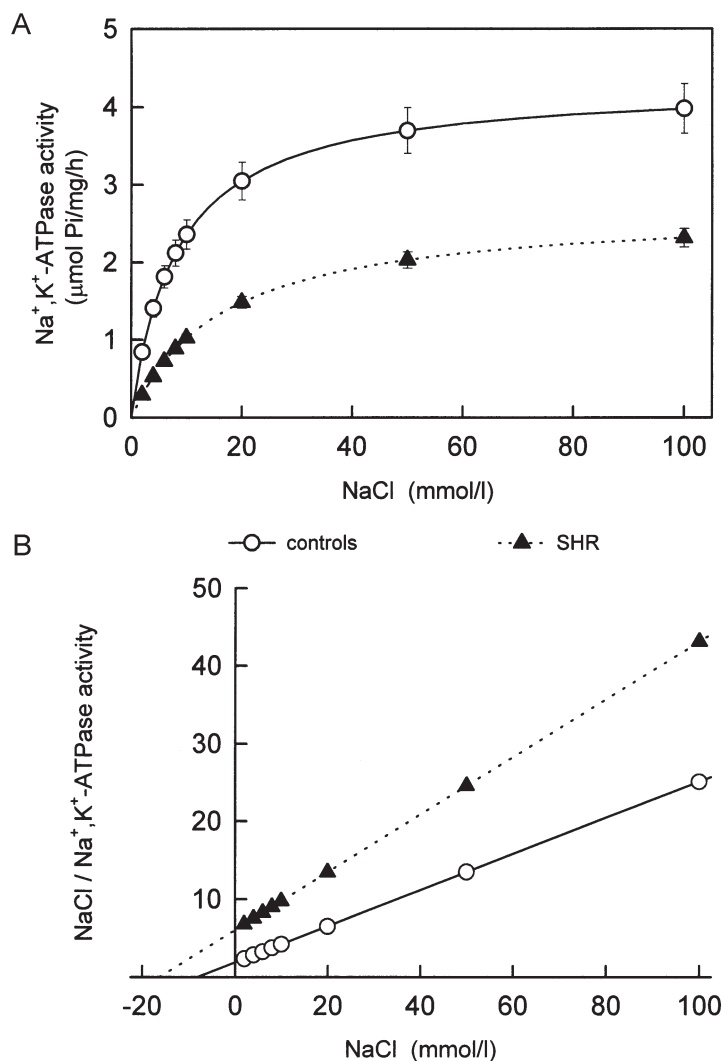

Figure 1. Activity of cardiac sarcolemmal Na⁺,K⁺-ATPase in SHR. **A.** Activation of the enzyme with increasing concentrations of ATP. **B.** "Hanes" plot of the data.

Figure 2. Kinetic parameters of Na$^+$,K$^+$-ATPase activation by substrate ATP in hearts from 34 weeks-old SHR and age matched control Wistar rats (C). Number of estimations: SHR ($n = 15$), C ($n = 15$). Data represent means $\pm$ S.E.M.; * $p < 0.001$.

loss of enzyme activity induced by hypertension was highest at low concentrations of substrate. In the presence of 0.16 and 0.4 mmol/l ATP the inhibition represented 81% and 77%, respectively. With increase of the ATP concentration the effect was gradually reduced and in the presence of 8 mmol/l ATP the inhibition of the Na$^+$,K$^+$-ATPase amounted 41% only, in hypertensive animals. Transformation of the data from representative measurements (Fig. 1A) to Hanes plot (Fig. 1B) points out to variations in kinetic parameters of enzyme from hearts of the SHR group. More precise evaluation of all kinetic measurements by nonlinear regression analysis of the data obtained according to Michaelis-Menten equation revealed significant decrease by 28% of the V_{max} of ATP hydrolysis in the SHR group as compared to controls. The K_m value significantly increased by 354% in the hypertensive group (Fig. 2). Upon activating the Na$^+$,K$^+$-ATPase by its cofactor Na$^+$ we observed again an inhibition of the enzyme in hearts from hypertensive animals at all investigated concentrations of Na$^+$ (Fig. 3A). This inhibition was again concentration-dependent with the highest effect representing 65% in the presence of 2 mmol/l NaCl and the lowest amounting 42% in the presence of 100 mmol/l NaCl. This clearly indicates the alterations in affinity of the Na$^+$-binding site as it is shown also in the Hanes plot of the data (Fig. 3B). Evaluation of the nature of this effect resulted in decreased V_{max} value by 37%. The concentration of Na$^+$ that gives half maximal reaction velocity (K_{Na}) significantly increased by 98% (Fig. 4).

Figure 3. Activity of cardiac sarcolemmal Na^+,K^+-ATPase in SHR. **A.** Activation of the enzyme with increasing concentrations of Na^+. **B.** "Hanes" plot of the data.

Discussion

In the present study the spontaneously hypertensive rats showed significant changes in blood pressure, heart and body weight. These changes are in agreement with those published previously, when 31 weeks-old SHR were investigated (Sallinen et al. 1996). The long-term pressure overload resulted in a profound cardiac hypertro-
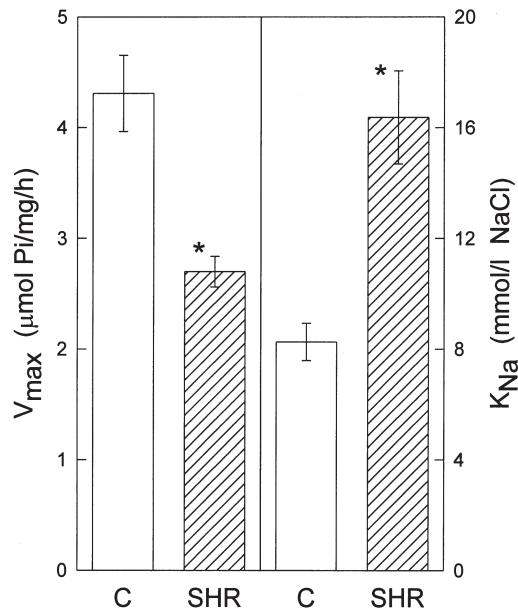
Figure 4. Kinetic parameters of Na$^+$,K$^+$-ATPase activation by Na$^+$ in hearts from 34 weeks-old SHR and age matched control Wistar rats (C). Number of estimations: SHR ($n = 15$), C ($n = 15$). Data represent means $\pm$ S.E.M.; * $p < 0.001$.

phy as shown by 88% increase of the heart weight/body weight ratio in the SHR group.

Previously a significant hypertension-induced inhibition of the Na$^+$,K$^+$-ATP-ase has been documented in blood-vessels (Arvola et al. 1992; Pörsti et al. 1992; Sallinen et al. 1996) and also in myocardium (Clough et al. 1983, 1984; Haddy 1990; Vrbjar et al. 1999a,b). To elucidate further molecular principles of the decreased activity of Na$^+$,K$^+$-ATPase in hypertension we studied its ATP and Na$^+$-binding properties *via* investigating the kinetic properties of the enzyme. Upon activating the enzyme with increasing concentrations of substrate, a significant loss of the activity occurred in the SHR group, through all the concentration range of ATP. This inhibition, resulting in a lowered value of V_{max} is probably caused by decreased affinity of the ATP-binding site in the Na$^+$,K$^+$-ATPase molecule as indicated by the increased value of K_m. This finding implicates that the utilization of energy derived from hydrolysis of ATP, by the enzyme, is less in hypertension as in normotensive conditions. It has to be mentioned that the above inhibition is more apparent at low concentrations of ATP, as the loss of Na$^+$,K$^+$-ATPase activity in the presence of 0.16 and 0.4 mmol/l represented 81% and 77%, respectively. An additional increase of the ATP concentration gradually reduced the effect to only 41% in the presence of 8 mmol/l ATP. This phenomenon might be of physiological importance specially in conditions of increased intracellular ATP requirements under conditions when the heart is working against the increased systemic resistance. Hence, the cardiac Na$^+$,K$^+$-ATPase is unable to hydrolyze enough ATP,

thus the energy supply necessary for the active transport of Na^+ out of the cells is restricted in hypertension. This limitation of the Na^+,K^+-ATPase function may contribute to the elevated intracellular concentration of Na^+ as it is supported by observation that in hearts from hypertensive animals the intracellular concentration of Na^+ is almost doubled as compared to controls (Jelicks and Gupta 1994). The alterations of the ATP-binding site develop probably after longer period of hypertension, because in other experimental model of hypertension, the nitric oxide deficient model, where the hypertension lasted 4 weeks only, no changes in the affinity to ATP were observed (Vrbjar et al. 1999a,b). The other important hypertension-induced alteration in the molecule of Na^+,K^+-ATPase is connected to its Na^+-binding site as it was shown previously by the depressed ability of the enzyme to bind intracellular Na^+ (Charlemagne 1990; Vrbjar et al. 1999a,b). In the present study we confirmed this fact, as revealed from the increased K_{Na} value in hypertensive animals. The concentration of Na^+ necessary for the half maximal activation of the Na^+,K^+-ATPase, which increased from 8 mmol/l NaCl in controls to 16 mmol/l NaCl in hypertension, agrees very well with the intracellular Na^+ levels of 8.4 and 17.3 mmol/l Na^+ reported for rat hearts in the respective physiological and pathophysiological conditions (Jelicks and Gupta 1994). Several mechanisms may account for this decreased affinity to Na^+ during hypertension. One possible explanation involves a potential synthesis of a new enzyme molecule with novel properties of the Na^+-binding site. It is known that the catalytic α-subunit of this enzyme has 3 isoforms which differ in their sensitivity to Na^+ ions. The α_1 subunit has the highest sensitivity to sodium characterized by 12 mmol/l Na^+ for the K_{Na} value and this is the major isoform present in the cardiac tissue. The other two types of subunit, the α_2 and α_3, revealing 22 and 33 mmol/l of Na^+ for the K_{Na} value are present in the heart in a lesser amount (Zahler et al. 1996, 1997). In association with hypertension, changes in Na^+,K^+-ATPase isoforms in cardiac, vascular and skeletal muscle tissues of rats have been reported (Herrera et al. 1988; Sweadner et al. 1994; Zahler et al. 1996). It has to be mentioned that hypertension is often accompanied with cardiac hypertrophy as it was shown also in our study. In contrary to previously mentioned observations, an evidence was found also for posttranslational alterations of the Na^+,K^+-ATPase. In hypertrophied rat hearts, the Na^+,K^+-ATPase revealed kinetic properties typical for α_2 and α_3 isoforms, but the expression of α_2 decreased, and for α_3 the expression was not detectable (Charlemagne 1990). According to these results, it was suggested that other regulatory factors might be involved either at the level of the gene (another yet undetected isoform ?) or at the membrane level (phospholipids, regulatory protein) (Charlemagne 1990). The hypothesis about the involvement of phospholipids in regulating the Na^+,K^+-ATPase activity in hypertension is supported by our previous observation that the concentration of phospholipids and, consequently, the ratio of phospholipids to membrane proteins increased by 100 % and 88 %, respectively (Žiačiková et al. 1999). The above described decrease in activity and also in the affinity of the Na^+-binding site of the Na^+,K^+-ATPase seems to be a specific answer to long lasting pressure overload, as in short-term inhibition

of the NO synthase for four hours, which was accompanied by the increase of the diastolic blood pressure, the enzyme revealed an opposite effect characterized by increased activity. For this limited period of time the Na$^+$,K$^+$-ATPase was able to extrude the excessive Na$^+$ out of myocardial cell more efficiently also at higher intracellular Na$^+$ concentrations (Vrbjar et al. 2000). In chronic models of pressure overload, like in this study, or in nitric oxide deficient hypertension (Vrbjar et al. 1999a,b), the Na$^+$,K$^+$-ATPase is loosing this capability.

We conclude, that in chronic model of hypertension, impairment in the affinity of the Na$^+$-binding site together with the decreased affinity to ATP in the molecule of the Na$^+$,K$^+$-ATPase are probably responsible for the deteriorated efflux of the excessive Na$^+$ from the intracellular space in hearts of SHR. Thus, the Na$^+$,K$^+$-ATPase may contribute to the genesis and maintenance of the hypertension.

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