Regulatory Role of Nitric Oxide on the Cardiac Na, K-ATPase in Hypertension

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Summary
The present study was focused on regulatory role of nitric oxide on functional properties of the cardiac Na, K-ATPase in three various animal models of hypertension: spontaneously hypertensive male rats (SHR) with increased activity of nitric oxide synthase (NOS) by 60 % (Sh1), SHR with decreased activity of NOS by 40 % (Sh2) and rats with hypertension induced by L-NAME (40 mg/kg/day) with depressed activity of NOS by 72 % (LN). Studying the utilization of energy substrate we observed higher Na, K-ATPase activity in the whole concentration range of ATP in Sh1 and decreased activity in Sh2 and LN. Evaluation of kinetic parameters revealed an increase of Vmax value by 37 % in Sh1 and decrease by 30 % in Sh2 and 17 % in LN. The Km value remained unchanged in Sh2 and LN, but was lower by 38 % in Sh1 indicating increased affinity of the ATP binding site, as compared to controls. During the activation with Na+ we observed increased Vmax by 64 % and increased KNa by 106 % in Sh1. In Sh2 we found decreased Vmax by 40 % and increased Km by 38 %. In LN, the enzyme showed unchanged Vmax with increased Km by 50 %. The above data indicate a positive role of increased activity of NOS in improved utilization of ATP as well as enhanced binding of Na+ by the cardiac Na, K-ATPase.

Key words
Sodium pump • Hypertension • Nitric oxide • Heart

Introduction
One of the complications accompanying hypertension is the increase of intracellular concentration of sodium ions in the cardiac tissue (Jelicks and Gupta 1994). Intracellular Na+ homeostasis is based on a balance between the influx and efflux of Na+. The influx is mediated by various pathways including Na+ leakage through different Na+ channels, Na+-K+-2Cl− cotransport, Na+/H+ exchanger and Na+-HCO3− cotransport. It was suggested that in hypertension the enhanced Na+ leakage is mainly responsible for increase of the intracellular Na+ concentration (Friedman 1979, Jones 1982, Zicha and Kuneš 1999). For the efflux of excessive Na+ ions out from the cells is responsible the Na, K-ATPase called also as the sodium pump. Na, K-ATPase (EC 3.6.3.9) is an important integral membrane protein that transports three Na+ ions out of the cell and two K+ ions into the cell using the energy derived from hydrolysis of one molecule of ATP. This enzyme was also hypothesized to be involved in systemic vascular hypertension through its effects on smooth muscle reactivity and myocardial contractility (Herrera et al. 1988). Various recent studies showed that development of functional alterations of the Na, K-ATPase in response to hypertension induced complications in the cardiovascular system is influenced by nitric oxide (NO) (Vrbjar et al. 1999a,b, Vrbjar and Pecháňová 2002, dos Santos et al. 2003, Xu et al. 2003, Zhou et al. 2002). Therefore, in the present study besides estimating the production of nitric oxide (NO) we focused our attention on two main issues concerning the response of the Na, K-ATPase, i.e. Na+-handling and
ATP utilization in hypertensive rats with various intensity of NO synthase activities.

Methods

Three various animal models of hypertension were studied. The first group was represented by spontaneously hypertensive male rats (SHR) at the age of nine months with increased activity of NOS (Sh1). The second group of SHR (16 weeks old) revealed a decreased activity of NOS (Sh2). In the third group (LN), the hypertension was induced by 4 weeks lasting administration of an inhibitor of NO synthase, L-NAME, in the dose of 40mg/kg/day during the period from 13 to 16 weeks of age. The effect of hypertension in three above mentioned models was evaluated in percentage change of investigated parameters as compared to respective age-matched control groups. Number of animals was in each group the same (n=9). The rats were placed in plastic cages and housed in a room with controlled temperature (22–24 °C), a 12-h light–dark cycle and were fed with a regular pellet diet. All procedures used in this study were approved by the Veterinary Council of the Slovakia (Decree 289, part 139, July 9th 2003). The systolic blood pressure (SBP) was measured by the non-invasive method of tail cuff plethysmography.

Cardiac sarcolemmal fraction was prepared from hearts by the hypotonic shock - sodium iodide treatment method (Vrbjar et al. 1984). Protein concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

The kinetics of Na, K-ATPase was estimated by measuring the splitting of ATP by 50 µg of sarcolemmal proteins at 37° C. The incubation medium consisted of (mmol/l): 100 imidazole (pH 7.4), 4 MgCl₂, 10 KCl, 100 NaCl. The utilization of substrate was measured in the range of 0.08-4.00 mmol/l of ATP. The enzyme kinetics for sodium activation was determined by the same approach, but the concentration of NaCl varied in the range of 2-100 mmol/l and the amount of ATP was constant (4 mmol/l).

The calcium- and calmodulin-dependent NO synthase activity was determined in crude homogenates of fresh cardiac tissue by measuring the formation of L-[³H]citrulline from L-[³H]arginine (Amersham, UK) according to the method of Bredt and Snyder (1990) with a modification as described by Bernátová et al. (1996). NO synthase activity was expressed as picokatal per gram of protein [pkat·(g protein)⁻¹].

The kinetic parameters were evaluated by direct non-linear regression of the obtained data. Results were expressed as a mean ± SEM. Data were analyzed with the ANOVA and the Bonferroni test to compare individual groups. P<0.05 value was considered significant.
Results

The investigated three hypertensive groups revealed various intensity of NO synthesis in the myocardium in comparison to respective controls. The first SHR group (Sh1) had a significantly higher activity of NO synthase by 60%. The second SHR group (Sh2) was characterized by significant decrease of NO synthase activity by 40%. In the third hypertensive group (LN), the activity of NOS was decreased by 72% (Fig. 1).

When activating the Na, K-ATPase with increasing concentrations of ATP, we observed significant variations in the three hypertensive groups. In the Sh1 group we found significant increase in the enzyme activity throughout the investigated concentration range of substrate. On the other hand in groups Sh2 and LN with depressed activity of NO synthase we observed a decrease in the Na, K-ATPase activity in the presence of all applied concentrations of substrate ATP (Fig. 2).

Evaluation of the above data resulted in significant increase of the $V_{\text{max}}$ value for the Sh1 group by 37%. In the Sh2 group a significant decrease of $V_{\text{max}}$ by 30% and in the LN group by 17% were observed (Fig. 3). The $K_M$ value was significantly lowered in the Sh1 group by 38%. In the groups Sh2 and LN the $K_M$ value remained unchanged, as compared to respective controls (Fig. 3).

Hypertension resulted also in alterations of Na⁺- handling by Na, K-ATPase. In the Sh1 group the enzyme activity was significantly increased especially in the concentration range of NaCl above 8 mmol/l. In the Sh2 and LN groups the Na, K-ATPase was lower at all applied NaCl concentrations (Fig. 4).

Evaluation of the data resulted in significant increase of the $V_{\text{max}}$ value by 64% in the Sh1 group and significant depression of the $V_{\text{max}}$ in Sh2 group by 40%. In the LN group the $V_{\text{max}}$ did not change as compared to controls. The $K_{\text{Na}}$ was significantly increased in all three hypertensive groups. The increase represented 106% in Sh1, 38% in Sh2 and 59% in LN group (Fig. 5).

Discussion

Concerning the activity of NO synthase in SHR inconsistent data were published. The function of NO synthase was found to be diminished (Malinski et al. 1993), unaltered (Minami et al. 1995) and also augmented in SHR (Nava et al. 1995). In the light of these contradictions and previously documented increased [Na⁺], in hearts of SHR (Jelicks and Gupta 1994), we focused our attention on the functional properties of the Na, K-ATPase in hearts from hypertensive rats with different level of NO synthesis in the heart.

Our data suggest that the activity of NO synthase may be a determining factor in regulating the functional properties of the cardiac Na, K-ATPase. In SHR with...
increased activity of NO synthase (Sh1) the enzyme showed improved ATP-binding properties as documented by the decreased $K_M$ value. This finding is supported by observation that pretreatment of hearts with low concentration of NO donor markedly protected the Na, K-ATPase function against ischemia-induced inactivation (Xu et al. 2003). The importance of NO in the regulation of Na, K-ATPase function is also supported by the fact that in vitro application of NO donors stimulated the vascular sodium pump (Gupta et al. 1994). However, it should be emphasized that the stimulatory effect of NO is limited to the amount of NO produced by the calcium and calmodulin-dependent constitutive NO synthase (cNOS). If the amount of NO increases above this limit, the Na, K-ATPase might be inhibited again, as was demonstrated at very high concentrations of NO generated by the inducible NO synthase (Guzman et al. 1995) or by application of very high concentration of NO donor, sodium nitroprusside (Liang and Knox 1999). On the other hand, decreased synthesis of NO (in Sh2 and LN groups) caused an inhibition of the Na, K-ATPase in a place distinct from the ATP-binding site as documented by unaltered values of $K_M$. Thus, the alterations of Na, K-ATPase function during hypertension with decreased synthesis of NO probably contribute to pathophysiological changes arising during the disease in the cardiovascular system. Previous data have shown that lowered activity of NOS in rats treated with L-NAME results in hypertension associated with many alterations in cardiovascular system, particularly in hypertrophy of the left ventricle (Bernátová et al. 2002, Pecháňová et al. 2004), in ultrastructural alterations of the heart (Tribulová et al. 2000), in structural changes of the geometry of the aorta, carotid and coronary artery in experimental animal models (Gerová and Kristek 2001), maladaptive gap junction remodeling that consequently promotes development of fatal arrhythmias (Fialová et al. 2008) as well as enhanced triggering of angiogenesis (Okruhliková et al. 2000) and angiopathy of cardiac capillary system (Okruhliková et al. 2005).

Concerning the mechanism of the protection of the enzyme molecule by nitric oxide four different hypotheses seem to be plausible.

It was documented that increased amount of NO protects the Na, K-ATPase activity against exogenous $\cdot OH$ induced inactivation by scavenging hydroxyl radicals (Zhou et al. 2000), which directly inhibit the Na, K-ATPase by attacking its ATP binding site (Xu et al. 1997).

The second explanation for the observed hypertension-induced inhibition may be the interaction of an inhibitory compound on the enzyme molecule. The role of such a hypothetical inhibitor probably responsible for this process might be ascribed to a digitalis-like substance which was found elevated in SHR causing a decrease of cardiac Na, K-ATPase activity (Chen et al. 1993a,b). It should be mentioned that the digitalis-like substance is freely circulating in blood and is relatively well soluble. Therefore in our experiment, the action of such an inhibitor seems to be limited to its presence in tightly membrane bound form.

The third explanation may include the involvement of increased production of angiotensin II (Ang II) during hypertension with lowered synthesis of NO as it was indicated by protective effect on the cardiovascular system during NO-deficient hypertension (Pecháňová et al. 1997, Pecháňová and Simko 2007). The conception of nitric oxide and Ang II participation in regulation of cardiac Na, K-ATPase is supported by protective effect of daidzein (4',7-dihydroxyisoflavone), a soy phytoestrogen, on the ventricular remodeling in rats with myocardial hypertrophy induced by pressure overload. The daidzein-induced protection was accompanied with significant increase of NO content and activities of cNOS, Na, K-ATPase and Ca-ATPase. Daidzein simultaneously induced a decrease of the AngII content in left ventricle and serum and also iNOS activity (Zhou et al. 2007).

The fourth explanation may regard to influence of endothelin-1 (ET-1) on the function of Na, K-ATPase in conditions of altered NO synthesis. Recently, it has been shown, that NO may inhibit the synthesis and
hemodynamic effects of ET-1, which in turn may stimulate NO production by means of autocrine interactions with ET_{A} receptors (Cardillo et al. 2000). Indeed, increasing circulating and tissue ET-1 immunoreactivity has been observed in patients with coronary artery disease (Lerman et al. 1991) and acute coronary syndromes (Wieczorek et al. 1994). Concerning the influence of ET-1 on the sodium pump activity, controversial data have been published. On one side, it was shown that the vasoconstrictor peptide ET-1 inhibits Na, K-ATPase activity in non-pigmented ciliary epithelial cells (Prasanna et al. 2001) and in the kidney (Hughes et al. 1992, Zeidel et al. 1989). On the other hand, ET-1 stimulates Na, K-ATPase activity in rabbit aorta (Gupta et al. 1991), cerebral capillary endothelium (Kawai et al. 1995) and vascular smooth muscle cells (Redondo et al. 1995) by the PKC-dependent pathway. Thus, the hypothetical involvement of ET-1 in sodium pump regulation is still unclear and for verification of this presumption more detailed studies of the above mentioned pathway in regulation of Na, K-ATPase pathway will be necessary.

Sodium-binding properties of the Na, K-ATPase were also changed as indicated by increased K_{Na} value in all three investigated hypertensive groups. Thus, this change was independent on the activity of NO synthase. However, it should be mentioned that of the Na, K-ATPase activity in the Sh1 group (with increased activity of NOS) was not lowered at any sodium concentration applied and at concentrations exceeding the K_{Na} value the enzyme was additionally stimulated, as compared to controls. So, the decreased sensitivity to Na' suggests that in the Sh1 group the enzyme adapts to altered homeostasis of ions induced by the increased blood pressure and is able to extrude the excessive Na' out from myocardial cells more effectively also at higher [Na'], while the Na, K-ATPase from control animals is unable to increase additionally its activity. From the fact, that this adaptation of the Na'-binding site was observed in the presence of high activity of NO synthase (Sh1) as well as in the case of lowered activity of NO synthase (Sh2 and LN), it may be hypothesized that this adaptation is probably induced by the increased blood pressure itself, independently on the activity of NO synthase. However, it should be emphasized that in groups with decreased NO synthesis the activity of the Na, K-ATPase was lower in the whole range of NaCl, as compared to Sh1 group. In addition, comparison of our findings and the available data published previously, indicates a progressive increase of the K_{Na} value along with the development of hypertension, amounting to 21 % in 7-week-old rats (Godfraind and Noel 1980), 38 % in 16-week-old animals (Vlkovičová et al. 2005), and 106 % in 9-month-old spontaneously hypertensive rats (Vrbjar and Pecháňová 2002). In these alterations regarding the vicinity of the Na'-binding site again the 'OH may be involved as it was shown in the case of Na, K-ATPase subjected to increased concentration of 'OH radicals during in vitro conditions (Shao et al. 1995, Lehotský et al. 1999), resulting in decrease of enzyme affinity to sodium in cardiac tissue (Ravingerová et al. 1994). Similar decrease in affinity to sodium was observed also in other pathophysiological situation like chronic hypobaric hypoxia (Rauchová et al. 2006).

We conclude that hypertension was followed by remodeling of the Na'-binding site of the Na, K-ATPase molecule in the heart as an adaptation to enhanced [Na'], induced by increased blood pressure, independently on the level of NO synthesis. On the other hand, the elevated activity of NO synthase induced an improvement of the ATP-affinity of the enzyme resulting thus in higher efficiency for utilizing the substrate ATP. In a situation when both effects, the hypertension and elevated activity of NO synthase occurred simultaneously, the influence of elevated activity of NO synthase seems to have a predominant role in regulating the function of cardiac Na, K-ATPase.

Conflict of Interest
There is no conflict of interest.

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