Effect of Losartan on the Na^+/Ca^{2+} Exchanger in Left Ventricle of the Insulin Resistant and Hypertensive hHTg Rat

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Abstract. As the Na^+/Ca^{2+} exchanger plays an important role in the regulation of myocyte contractility, it has been suggested that alterations in this system might be involved in the development of insulin resistance and/or diabetes-induced myocardial alterations. Moreover, gene expression and function of the Na^+/Ca^{2+} exchanger in states of combined hypertension and insulin resistance is of a special interest. Thus, we used hereditary hypertriglyceridemic (hHTg) rat (a model of genetically induced insulin resistance and hypertension) to study the effect of losartan, the blocker of type 1 angiotensin receptors, on the Na^+/Ca^{2+} exchanger in the rat heart. We found that gene expression, but not activity of the Na^+/Ca^{2+} exchanger was decreased in the left ventricle of hHTg rats when compared to their normotensive mates. No changes were observed in the right ventricle. In addition, losartan decreased mRNA levels of the Na^+/Ca^{2+} exchanger in the left, but not in the right ventricle of normotensive rats. In hHTg rats, losartan had no effect on the gene expression of this transporter. Our results point to different modulatory pathways of Na^+/Ca^{2+} exchanger in normotensive and hHTg rats.

Key words: Na^+/Ca^{2+} exchanger — Losartan — hHTg rats — Insulin resistance — Hypertension

Introduction

Cardiac complications remain the leading cause of increased mortality and morbidity in diabetics. Cardiac output, rate of relaxation, onset of relaxation, and velocity of muscle shortening have all been shown to be depressed in hearts of rats with experimentally induced diabetes (Kashihara et al. 2000). Also, the incidence of hypertension is higher in patients with diabetes (Lea and Nicholas 2002). Mechanisms

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of the blood pressure up-regulation seem to involve the stimulatory action of insulin on the activity of the sympathetic nervous system, stimulation of the cation transport with consequent changes in vascular reactivity, growth promoting effects on arteriolar smooth muscle cells and increased Na^+ reabsorption (for review Klimes and Sebokova 1997).

Calcium is one of the driving forces for the excitation-contraction coupling of the cardiac muscle (for review see Bootman et al. 2001; Krizanova 1996). A small amount of extracellular calcium enters the myocyte through the L-type calcium channels and during depolarization it triggers release of additional calcium into the cytosol from the sarcoplasmic reticulum (Fabiato 1983). The Na^+/Ca^{2+} exchanger serves as the principal extrusion mechanism in the myocyte and hence plays a pivotal role in the myocyte relaxation (Bridge et al. 1990; Yao et al. 1998). Given the important role of the Na^+/Ca^{2+} exchanger in regulating myocyte contractility, it has been suggested that alterations in this exchanger may play a role in the diabetes-induced myocardial alterations (Bridge et al. 1990; Philipson and Nicoll 2000).

Many clinical and experimental studies suggest that the renin-angiotensin system (RAS) plays an important role in the pathogenesis of hypertension (for review see Krizanova 1998; Fischer et al. 2002). Angiotensin II represents one of the mechanisms that have been proposed to trigger cardiomyocyte growth and left ventricular hypertrophy. In terms of the latter it is interesting to note that treatment of Zucker rats (obese insulin resistant rats which are also hypertensive) or of spontaneously hypertensive rats (SHR; genetically hypertensive animals with moderate insulin resistance) with inhibitors of the angiotensin converting enzyme corrected not only the raised blood pressure, but also stimulated their insulin action (Henriksen and Jacob 1995). Inhibition of the RAS at different levels, e.g. feeding losartan – an antagonist of the angiotensin AT1 receptors – to normal rats with the fructose-induced insulin resistance syndrome prevented both, the blood pressure elevation and the hyperinsulinemia (Navarro-Cid et al. 1995). Similar results were obtained in the SHR animals when treated with valsartan (Chow et al. 1995).

The present study was designed to gain further insights into the mutual influence of the hypertension and insulin resistance on the heart. An interesting animal model for studies of the relationships between blood pressure and metabolic abnormalities, i.e. the hereditary hypertriglyceridemic (hHTg) rats (Klimes et al. 1995) was used in these studies. This strain has been produced in the Vrana's group (Vrana and Kazdova 1990) by selective inbreeding from Wistar rats according to the rise of plasma triglycerides induced by a high sucrose diet. Though hHTg rats display hypertriglyceridemia, insulin resistance, impaired glucose tolerance and raised blood pressure even without nutritional stimuli, the high sucrose feeding further aggravates these symptoms (Klimes et al. 1995). Thus, we studied the gene expression and activity of the Na^+/Ca^{2+} exchanger in hearts of normotensive (NTg) and hHTg rats in control conditions and in rats treated by the AT1 receptors blocker – losartan.

Materials and Methods

Animals

Adult male hereditary hypertriglyceridemic (hHTg) rats (Klimes et al. 1995) were obtained from the hHTg colony at the Institute of Experimental Endocrinology (Bratislava, Slovakia). Age-matched healthy Wistar animals (NTg) were purchased from Charles River (Germany). Animals were housed two *per* cage in a temperature controlled room (22 ± 2℃) on a 12 hour-light/12 hour-dark cycle (lights off at 18.00 hrs) and fed standard lab chow (ST 1) commercially available from VELAZ (Prague, Czech Republic). Rats had always free access to food and water. Food and water consumption were monitored daily throughout the course of the study and the body weight was taken once a week. Rats were sacrificed by cervical dislocation, hearts were withdrawn, separate left and right ventricles were immediately frozen in liquid nitrogen and stored at −70℃ until analysis.

Pharmacological treatment

The animals were treated for 8 days with losartan, which was admixed into the drinking water in the amount to receive a dose of 10 mg/kg of body weight *per* day. The water consumption was monitored daily and the drug concentration in the water was adjusted in two days intervals in dependence on the average water consumption of animals in the given cage. This approach created following groups of animals: a) control NTg and hHTg, b) NTg and hHTg rats pretreated with losartan.

RNA isolation and relative quantification of mRNA levels by RT-PCR

Isolation of RNA was performed according to the procedure of Chomczynski and Sacchi (1987), using guanidine isothiocyanate and phenol-chloroform extraction. Reverse transcription was carried out using Ready-to-Go You-Prime First Strand beads and $pd(N)_6$ primer (Amersham Bioscience). Specific PCR for the Na^+/Ca^{2+} exchanger was performed as described earlier by Zacikova et al. (1999) and compared relatively to the housekeeper glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Results were expressed as optical density (od) *per* mm^2.

Calcium transport into the membrane proteoliposomes from rat hearts

Calcium transport was measured as described by Zacikova et al. (1999). Briefly, the reconstitution was done with an azolectin-protein ratio of 40 : 1. The mixture was solubilized in 1% 3-[(3-Cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS). After solubilization, CHAPS was removed on a Sephadex G-50 column (Pharmacia, Sweden). Multilamellar vesicles were eluted with 20 mmol/l Tris-HCl, 160 mmol/l NaCl, pH 7.4. The proteoliposomes were sonicated for 2×30 s to obtain unilamellar particles. Proteoliposomes were then incubated with 40 mmol/l $^{45}Ca^{2+}$, 20 mmol/l Tris-HCl, pH 7.4, 160 mmol/l KCl and/or NaCl solution for 10 min. Afterwards, free calcium was removed on CM-Sepharose (Pharmacia, Sweden). Radioactivity was measured after addition of Bray's scintillation cocktail using a Beta counter (Rackbeta, LKB).

Statistical analysis

Results are presented as mean ± S.E.M. Each value represents an average of five animals. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Values of $p < 0.05$ were considered to be significant. For multiple comparisons, an adjusted t-test with p values corrected by the Bonferroni method was used (Instat, GraphPad Software, USA).

Results

Comparison of heart/body weight (HBW) index in hHTg and NTg rats

In hHTg rats, the HBW index was significantly higher compared to their normotensive mates (0.312 ± 0.002 a.u. *vs.* 0.288 ± 0.005 a.u.; Figure 1). Losartan treatment for 8 days significantly decreased HBW index in hHTG rats when compared to the untreated group (0.293 ± 0.006 a.u. *vs.* 0.312 ± 0.002 a.u., $p < 0.05$). On the other hand, the HBW index did not change in normotensive NTg rats after losartan treatment when compared to controls (0.292 ± 0.007 a.u. *vs.* 0.28 ± 0.005 a.u.).

Gene expression and activity of the Na^+/Ca^{2+} exchanger in NTg and hHTg rats

In rats without losartan treatment, mRNA levels of the Na^+/Ca^{2+} exchanger in the left ventricle of hHTg rats were significantly reduced when compared with mRNA levels of the Na^+/Ca^{2+} exchanger of NTg rats (548 ± 21 *vs.* 760 ± 52 od/mm^2; Figure 2, top graph). Significantly lower amount of the Na^+/Ca^{2+} exchanger mRNA

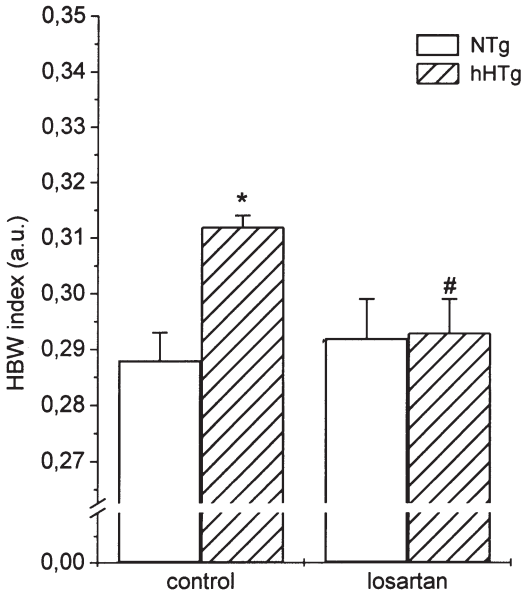

Figure 1. HBW index of the control and losartan treated NTg (empty columns) and hHTg (striped columns) rats. Results are displayed as mean ± S.E.M. and each column represents an average of five animals. Statistical significance * represents $p < 0.05$ relatively to NTg control, # represents $p < 0.05$ relatively to hHTg control.

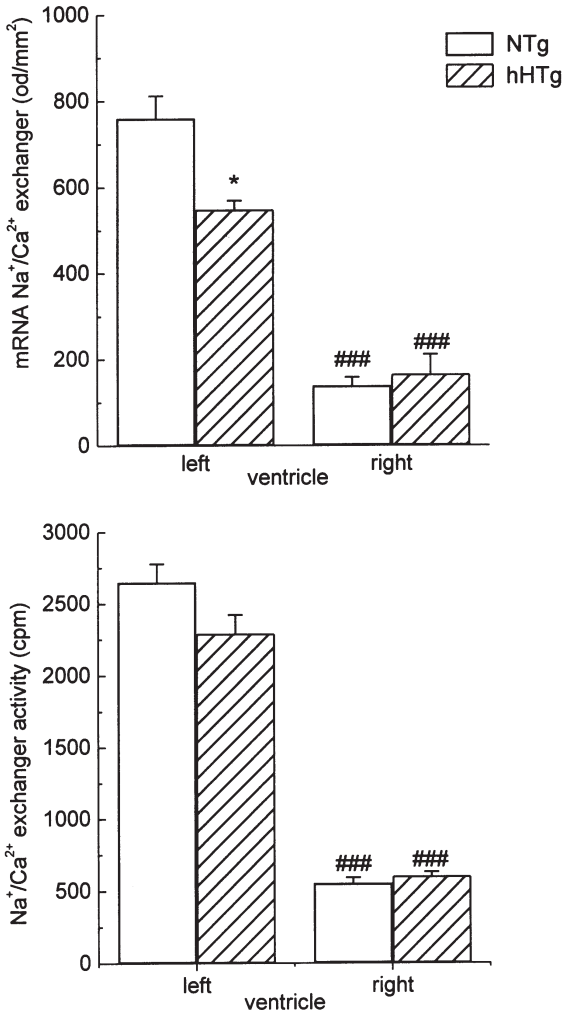

Figure 2. Na^+/Ca^{2+} exchanger's mRNA (top graph) and activity (bottom graph) in the left and right ventricle of NTg (empty columns) and hHTg (striped columns) rats. Results are displayed as mean ± S.E.M. and each column represents and average of five animals. Statistical significance * represents $p < 0.05$ relatively to NTg control, ### represents $p < 0.001$ relatively to corresponding NTg and/or hHTg control.

was observed in the right ventricle of either NTg (138 ± 20 od/mm^2) or hHTg (165 ± 16.5 od/mm^2) rats. Activity of the Na^+/Ca^{2+} exchanger (Figure 2, bottom graph) was higher in the left ventricles of both, the NTg and hHTg rats when compared to the corresponding right ventricle. Nevertheless, no significant difference

was observed in the Na^+/Ca^{2+} exchanger's activity in the left ventricle of NTg and hHTg rats (2650 ± 126 cpm *vs.* 2289 ± 131 cpm).

Effect of losartan on the Na^+/Ca^{2+} exchanger

In NTg rats, losartan treatment resulted in significant decrease in Na^+/Ca^{2+} exchanger mRNA level (426 ± 72 *vs.* 760 ± 52 od/mm^2; Figure 3, top graph) in the left ventricle. Nevertheless, no significant change was observed in mRNA levels of Na^+/Ca^{2+} exchanger of hHTg rats treated with losartan as compared to the un-

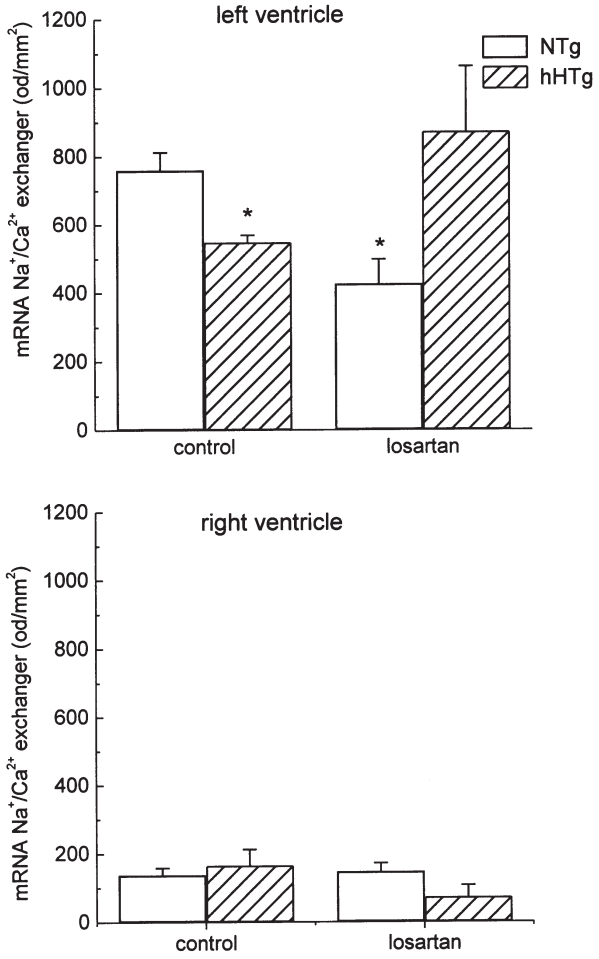

Figure 3. The effect of losartan treatment on the Na^+/Ca^{2+} exchanger's mRNA levels in the left (top graph) and right (bottom graph) ventricle of NTg (empty columns) and hHTg (striped columns) rats. Results are displayed as mean ± S.E.M. and each column represents an average of five animals. Statistical significance * represents $p < 0.05$ relatively to NTg control.

treated group of hHTg rats (548 ± 21 *vs.* 872 ± 190 od/mm^2; Figure 3, top graph). In the right ventricle, no significant change in Na^+/Ca^{2+} exchanger mRNA was found either in NTg, or in hHTg rats which were treated with losartan (Figure 3, bottom graph).

Discussion

Our study has shown that the HBW index was significantly higher in hHTg than in NTg rats. Similar data on cardiac hypertrophy in hHTg rats have been obtained very recently in another study by Simko et al. (2002). The hHTg rats display hypertriglyceridemia, insulin resistance, impaired glucose tolerance and raised blood pressure (Klimes et al. 1995). Thus, cardiac hypertrophy (as indicated by an increased HBW index) might be a result of either hypertension, or hyperinsulinemia. Increased HBW index in hypertensive and diabetic rats was also observed by Kashihara et al. (2000). These authors compared hearts of the control, diabetic, hypertensive and diabetic/hypertensive rats and observed the highest HBW index in hypertensive hearts, followed by diabetic/hypertensive, diabetic and control hearts. Nevertheless, their model of diabetes and hypertension is based on genetically-induced hypertension and streptozotocin-induced diabetes, while in our model both phenotypes of interest (i.e. hypertension and insulin resistance) have a hereditary background.

We observed significant differences in the mRNA levels, but not in the activity of the Na^+/Ca^{2+} exchanger in the left ventricles of NTg and hHTg rats. In the right ventricles, both mRNA level and activity of the Na^+/Ca^{2+} exchanger were not significantly different. In accordance with our previous studies (Zacikova et al. 1999) we found a significantly higher amount of Na^+/Ca^{2+} exchanger mRNA in the left ventricle of both NTg and hHTg rats.

The Na^+/Ca^{2+} exchanger is a trans-sarcolemmal protein that plays an important role in controlling levels of intracellular calcium (Blaustein and Lederer 1999). Na^+/Ca^{2+} exchanger has been found to be up-regulated in the failing human heart at both mRNA and protein level (Reinecke et al. 1996; Flesch et al. 1996; Pogwizd et al. 1999). Also, in spontaneously hypertensive rats (SHR), activity of the Na^+/Ca^{2+} exchanger was demonstrated to be significantly higher compared to normotensive Wistar-Kyoto rats (David-Dufilho et al. 1986). On the other hand, in hearts of streptozotocin-induced diabetic rats, lower levels of the Na^+/Ca^{2+} exchanger were observed (Makino et al. 1987). Nevertheless, the physiological and/or pathophysiological significance of the decreased Na^+/Ca^{2+} exchanger in hearts with altered handling of insulin remains elusive.

Treatment with losartan, an AT1 receptor blocker, significantly suppresses the gene expression of the cardiac Na^+/Ca^{2+} exchanger in NTg, but not in hHTg rats. Several explanations can be conceived to clarify this result. First, the amount of angiotensin II might be much bigger in hHTg rats when compared to NTg rats. Indeed, in our previous studies we have observed a significantly higher gene expression for the intracardiac renin in hHTg rats as compared to their normotensive

mates (Jurkovicova et al. 2001). Also, the amount of type 1 angiotensin receptors might differ between the hHTg and NTg rats.

Angiotensin II, a product of the renin-angiotensin pathway, is known to affect the Na^+/Ca^{2+} exchanger at the level of gene expression (Krizanova et al. 1997), or activity (Ballard and Schaffer 1996; Fukuta et al. 1998). Different sensitivity of the gene expression of the Na^+/Ca^{2+} exchanger to losartan might indicate towards an impaired communication of the renin-angiotensin system and Ca-transport systems, at least of the Na^+/Ca^{2+} exchanger. However, further studies are required to clarify the physiological consequences of our observations.

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