Effect of losartan on functional and structural properties of conduit atery wall in young spontaneously hypertensive rats

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Abstract— *The effect of losartan on systolic blood pressure (SBP), function and structure of thoracic aorta and mesenteric artery was examined. Four-week-old Wistar rats, spontaneously hypertensive rats (SHR), and SHR treated with losartan (20 mg/kg/day for 5 weeks) were used. SBP was measured by the tail-cuff method weekly. Isometric contraction of aortal rings and neurogenic contractions of mesenteric artery were studied in organ baths. For in vivo investigation after anesthesia, carotid artery was cannulated for SBP measurement and jugular vein for drug administration. Then thoracic aorta was processed according to electron microscopy procedure. In SHR (9-week-old) SBP and relative heart weight were increased, acetylcholine induced relaxation of phenylephrine-precontracted aortal rings was reduced compared to Wistar rats. In losartan treated SHR SBP and relative heart weight were lowered. Impairment of acetylcholine-induced relaxation was prevented and neurogenic responses of mesenteric artery were suppressed. In vivo administration of acetylcholine (0.1µg, 1µg, 10µg) evoked similar decrease of SBP in SHR and Wistar rats. In losartan treated SHR the decrease of SBP was larger than in SHR and Wistar rats. Wall thickness (WT) and cross-sectional area (CSA) of the aorta in SHR were decreased compared to Wistar rats; inner diameter (ID) and WT/ID ratio were not changed. In losartan treated SHR ID was increased and WT/ID decreased while WT and CSA were not changed. Conclusion: Losartan administered to SHR (i) reduced SBP, (ii) prevented impairment of endothelium-dependent relaxation, (iii) improved integrated responses to acetylcholine, and (iv) reduced structural changes in the aortic wall.*

Keywords— Hypertension, Conduit artery, Losartan, Neurogenic contraction, Structure.

I. INTRODUCTION

Systolic blood pressure (SBP) of 4-week-old spontaneously hypertensive rats (SHR) did not differ from that of normotensive Wistar rats. From the 4th to 10th week of age, the SBP of SHR increases faster than that in Wistar rats (1). The rapid phase of increasing blood pressure in SHR coincides approximately with the period of the most intense elevation of sympathetic nervous system activity, progressive increase of plasma noradrenaline and cardiovascular hypertrophy (2). It appears that the rapid increase in blood pressure is the most important risk factor, which triggers the functional and structural alterations that are later observed in cardiovascular diseases. In animals with genetic hypertension, the hypertrophy of blood vessel wall parallels the developmental rise in blood pressure and systemic resistance, which is maximal at about 36-week of age (3). In large conduit arteries nitric oxide is, a major component of acetylcholine-dependent relaxation, in resistance arteries an important role plays hyperpolarizing factor (4). Endothelium-derived hyperpolarizing factor (EDHF) has been described as one of the principal mediators of endothelium-dependent vasorelaxation in small resistance arteries in normotensive animals (5), and may play a crucial role in maintaining peripheral vascular resistance (6). An impairment of endothelial function, as evaluated by the relaxant response to acetylcholine, has been detected in both conduit and resistance arteries of adult animal and human hypertension (7). Our observations indicate that in 4-week-old SHR endothelium-dependent relaxation of aorta to acetylcholine is not impaired (8). There have been found structural changes in resistance vessels at prehypertensive phase in SHR. These changes may belong to factors contributing to the development of hypertension in the SHR (9).

Angiotensin II facilitates sympathetic activity by actions at the central and peripheral nervous systems (10). It was shown that both angiotensin converting enzyme inhibitors as well as angiotensin II blocking drugs reduce blood pressure and sympathetic hyperactivity in SHR (11). Our founding showed that magnitude of noradrenaline-induced contraction of aorta from 4-week-old SHR was smaller than in age-matched Wistar rats (12). This is in good consent with findings of Mizutani et al. (13) which demonstrated that maximum mechanical strength in the 4-week-old stroke-prone spontaneously hypertensive rats were lower than those in the age-matched WKY rats. Previous long lasting treatment of young SHR with losartan showed significant blood pressure reduction and some structural changes in cardiovascular system (14). The effect of angiotensin II (AT_1) receptor inhibition is relatively well documented in the resistant part (especially mesenteric bed) of the vasculature (15,22). Very small attention was addressed to the effect of AT_1 receptors blockade on the function and structure of conduit arteries in SHR, especially in early stage of hypertension development.

The aim of the study was to examine the long-term effect of losartan, nonpeptide AT_1 receptor antagonist, on systolic blood pressure, relative heart weight, functional (endothelium-dependent relaxation and contraction, adrenergic neurotransmission) and structural changes in selected isolated conduit arteries of young Wistar rats and SHRs (4 weeks old) during early developmental of spontaneous hypertension.

II. MATERIALS AND METHODS

Experiments were performed on 4-week-old rats. All procedures and experimental protocols were approved by the Ethical Committee for Experimental Work of the Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, and by an Ethical committee according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, Directive 2010/63/EU of the European Parliament. The animals were housed at a temperature of 22-24 ºC, under a 12 h light: dark cycle and fed a regular pellet diet. They had free access to food and water.

The animals taken for the study consisted from three groups of 20 animals in each: 1) control Wistar rats, 2) spontaneously hypertensive rats (SHR), and 3) SHR treated with losartan daily by gavage in a dose 20 mg/kg/day (dissolved in drinking water). The experiment lasted five weeks. Systolic blood pressure (SBP) was measured indirectly by the plethysmographic method on the tail artery of prewarmed animals each week. At the end of the experiment ten animals from each group was taken for functional in vitro study and ten animals for in vivo and morphological study.

2.1 In vitro study

Ten animals were sacrificed by an overdose of anaesthesia, the chest was opened and the thoracic aorta and mesenteric artery (arteria mesenterica superior) were excised and placed in a Petri dish filled with a modified Krebs solution of the following composition (in mM): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, $CaNa_2EDTA$ 0.03, ascorbic acid 1.1. For the measurement of contractile activity both arteries were cut into rings of 3.5 mm length, fixed on stainless steel hooks and suspended in organ bath filled with modified Krebs solution at 37°C and gassed with 95% O2 +5% CO2. One side of the ring was connected by a thread to a force-displacement transducer (Sanborn FT 10, USA) to measure changes in isometric tension which were recorded with a polygraph TZ 4200 (Labora, Czech Republic). Vascular preparations from both arteries were stretched to 10mN of resting tension, and the stabilization period was 60 min with washing at 15 min intervals.

In mesenteric artery, the electrical field stimulation of perivascular nerves was provided by an electronic stimulator ST-3 (Medicor, Hungary) via two platinum electrodes pointed on each side and parallel to the vessel preparation. The following parameters of stimulation were used: square-wave pulses 0.5 ms duration, 1-32 Hz, supramaximal voltage (approx. 40 V), duration of stimulation 20 s. A period of approx. 10 min was allowed between stimulations.

2.2 In vivo study

Ten animals were anaesthetized with ketamine (0.25 ml/100 g b.w.) and xylazine (0.1 ml/100g b.w.) applied i.p. The right jugular vein was cannulated for administration of the respective drugs. Immediately after preparation, heparin in a dose of 25 i.u. was injected into the jugular vein. The right carotid artery was cannulated and connected to a Statham pressure transducer. Mean arterial pressure was recorded with a Physioscript Schwarzer (Germany) Drugs (acetylcholine, noradrenaline) were administered according protocol described earlier (16). Individual injections of drugs were applied in a 10-15 min intervals (0.1 ml during 10 seconds) after the blood pressure returned to basal level and was stabilized.

2.3 Morphological studies

Immediately after finishing in vivo study the animals were sacrificed by an overdose of anaesthesia, the chest was opened and the cardiovascular system was perfused at a constant pressure of 120 mm Hg for 10 min via a cannula placed in the left ventricle. As a fixative 300 mM glutaraldehyde in 100 mM phosphate buffer was used. After perfusion the hearts were excised and weighed. The descending part of the thoracic aorta was excised, cleaned, divided into three segments (about 1 mm), fixed with the same fixative and embedded in Durcupan ACM (Sigma). Two randomly selected blocks of the artery were cut perpendicularly to the longitudinal axis. The inner diameter (ID) and arterial wall thickness (tunica intima and tunica media) (WT) were measured in light microscopy as it was referred earlier (17). The cross-sectional area of the arterial wall (CSA) was calculated.

2.4 Statistical analysis

Results shown in the text and figures are expressed as mean values ± S.E.M. For statistical evaluation, data were analyzed by ANOVA and Bonferroni test for unpaired variables. Statistical significance was assumed when P< 0.05.

III. RESULTS

3.1 General parameters

The values of systolic blood pressure in SHR and Wistar were similar at the weaning period at 4-week of age. In SHR blood pressure rapidly elevated during follow-up period. At the age of 9th week SBP was significantly higher than in age-matched Wistar controls (Figure 1). Five week-lasting treatment of SHR with losartan slowed down systolic blood pressure increase and at the end of the experiment it was lower in comparison to the untreated SHR but it still remained significantly higher compared to Wistar rats (Figure 1).

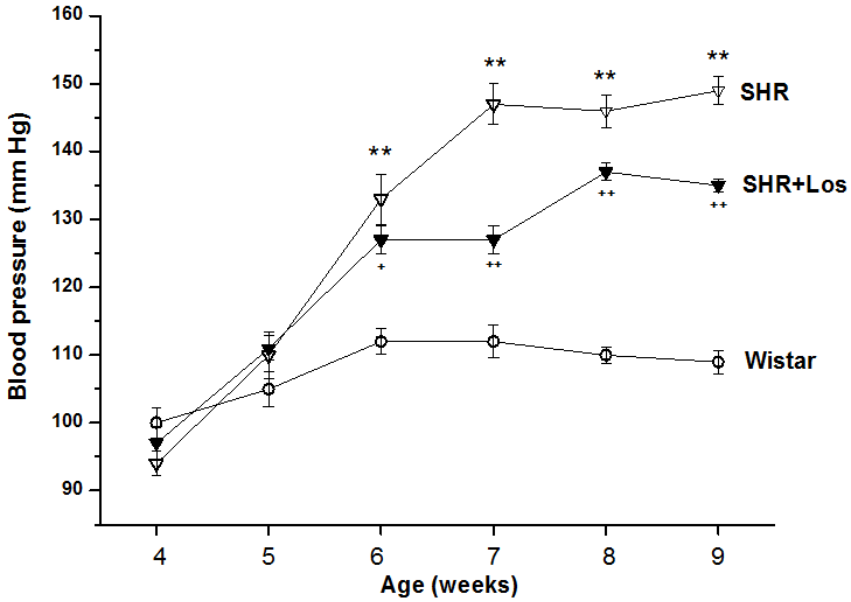

FIGURE 1: THE VALUES OF SYSTOLIC BLOOD PRESSURE IN THE COURSE OF THE EXPERIMENTS. WISTAR RATS (WISTAR), SPONTANEOUSLY HYPERTENSIVE RATS SHR, AND SHR TREATED WITH LOSARTAN (SHR+LOS). VALUES ARE MEANS ± S.E.M. **p<0.01 VS. WISTAR RATS, $^{++}$p<0.01, $^{+}$p<0.05 vs. SHR.

Body weight of the Wistar rats was in the course of the whole experiment (not shown) as well as at the end of the experiment significantly higher than in SHR. Losartan administration resulted in body weight increase in comparison to SHR and no different was observed compared with Wistar rats (Table 1).

TABLE 1
GENERAL PARAMETERS IN THE GROUPS

	HW (mg)	BW (g)	HW/BW
Wistar	1060±0.046	220±4.7	4.7±0.3
SHR	1080±0.023	191±4.2**	5.8±0.2**
SHR+Los	0920±0.024**$^{++}$	218±2.5^{++}	4.3±0.1^{++}

At the end of the experiment the heart weight of Wistar rats and SHR did not significantly differ. Long-term losartan administration of losartan to SHR evoked pronounced decrease of heart weight in comparison to both group Wistar and SHR (Table 1).

The evaluation of trophicity of the heart from heart weight/body weight ratio (relative heart weight) revealed significant difference between Wistar rats and SHR. Due to lower body weight of SHR and unchanged heart weight compared to Wistar

rats the ratio was increased in SHR which manifested hypertrophy of the heart in this group. Losartan administration remarkably prevented hypertrophy of the heart in SHR and the absolute value of the ratio was even lower (not however significant) than in Wistar rats (Table 1).

3.2 In vivo study

Acute administration of increasing concentrations of acetylcholine (0.1 µg, 1 µg, and 10 µg) via jugular vein into cardiovascular system evoked similar decrease of blood pressure in SHR and Wistar rats. In SHR chronically treated with losartan blood pressure was significantly decreased ($p<0.01$) in all three concentrations of acetylcholine used when compared to both SHR and Wistar rats (Figure 2).

Integrated responses of the cardiovascular system of SHR to noradrenaline application in a bolus in concentration of 1 µg resulted in significant ($p<0.01$) blood pressure increase compared to Wistar rats. The application of the same dose of noradrenaline to chronically administered SHR with losartan evoked slightly lower blood pressure increase than in SHR nevertheless the blood pressure was still significantly higher ($p<0.05$) than in control Wistar rats (Figure 2).

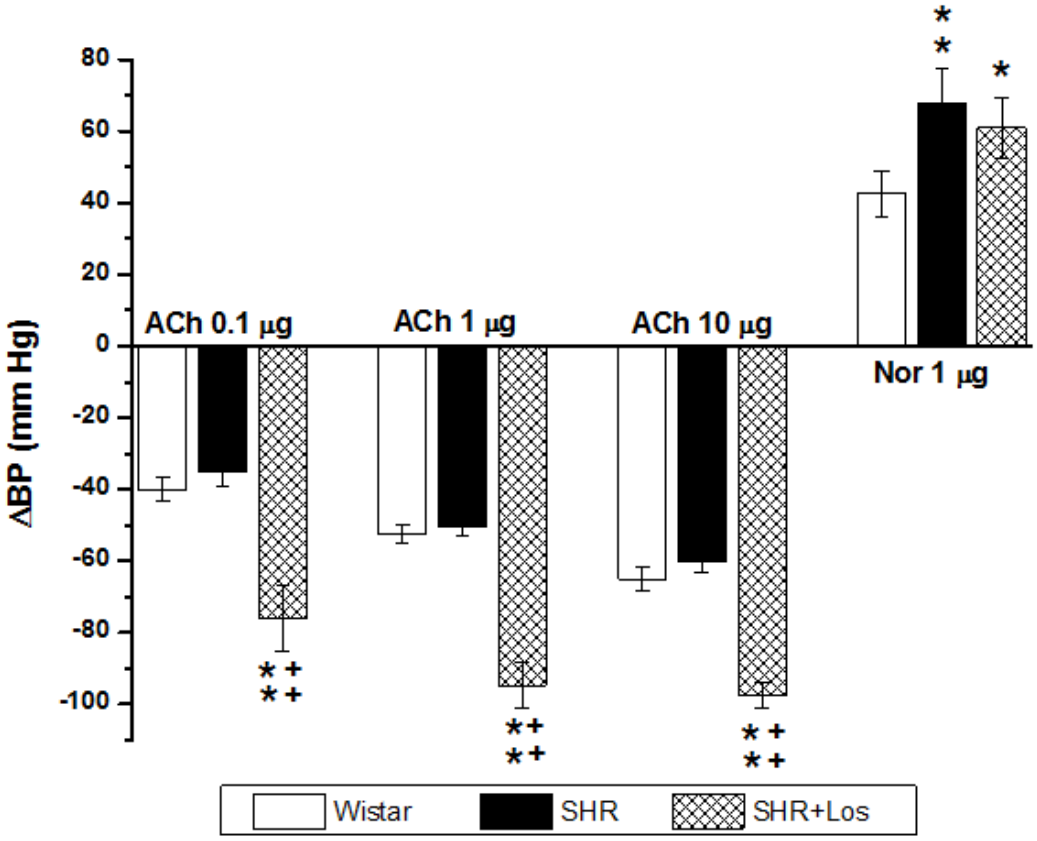

FIGURE 2: CHANGES IN SYSTOLIC BLOOD PRESSURE (ΔBP) INDUCED BY ACETYLCHOLINE (ACh) AT CONCENTRATION 0.1 µg, 1.0 µg, and 10 µg I.V. AND NORADRENALINE (NOR) AT CONCENTRATION 1 µg IN CONTROL WISTAR RATS (WISTAR), SPONTANEOUSLY HYPERTENSIVE RATS (SHR), AND SHR TREATED WITH LOSARTAN (SHR+LOS). VALUES ARE MEANS ± S.E.M. **p<0.01 vs. Wistar rats, $^{++}$p<0.01 vs. SHR.

3.3 In vitro study

In control Wistar rats acetylcholine caused an endothelium-dependent relaxation of precontracted aortic rings. In vascular preparations from SHR relaxation was slightly reduced. Long-term administration of losartan to SHR completely prevented the reduction of acetylcholine-induced relaxation (Figure 3).

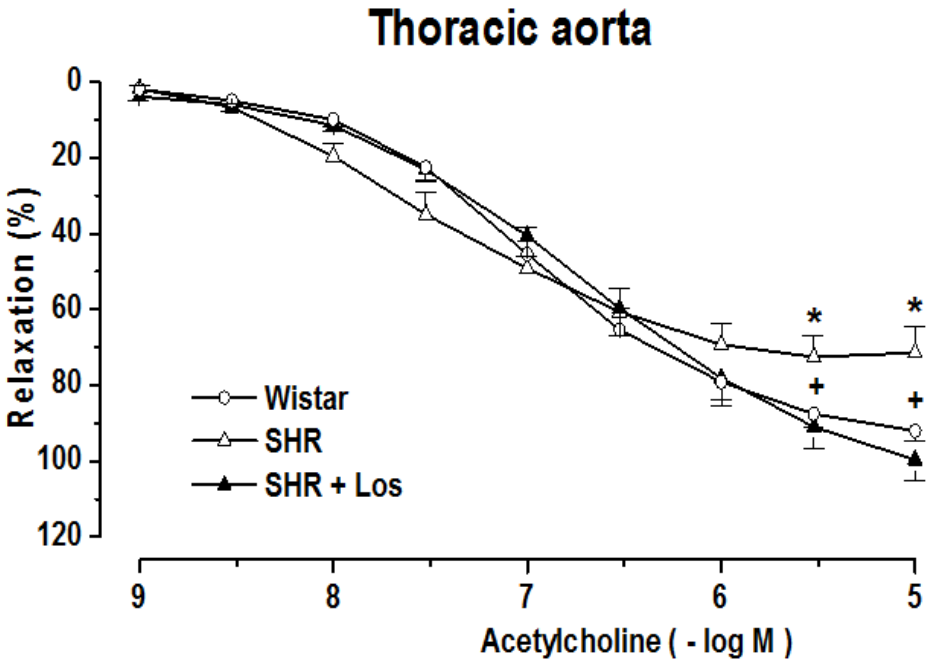

FIGURE 3: CONCENTRATION-RESPONSE CURVES FOR RELAXATION INDUCED BY ACETYLCHOLINE IN CONTROL WISTAR RATS, SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND SHR TREATED WITH LOSARTAN (SHR+LOS). VALUES ARE MEANS ± S.E.M. *p<0.05 VS. WISTAR RATS, $^{+}$p<0.05 vs. SHR.

The concentration-response curves to exogenously administered noradrenaline on thoracic aorta from Wistar rats, SHR and SHR + losartan are shown at Figure 4. The concentration-response curve for noradrenaline was shifted to the left (compared to the Wistar rats) in SHR. In SHR losartan administration normalized sensitivity of aorta to exogenous noradrenaline and reduced the maximum contraction in aorta induced by exogenous noradrenaline (Figure 5).

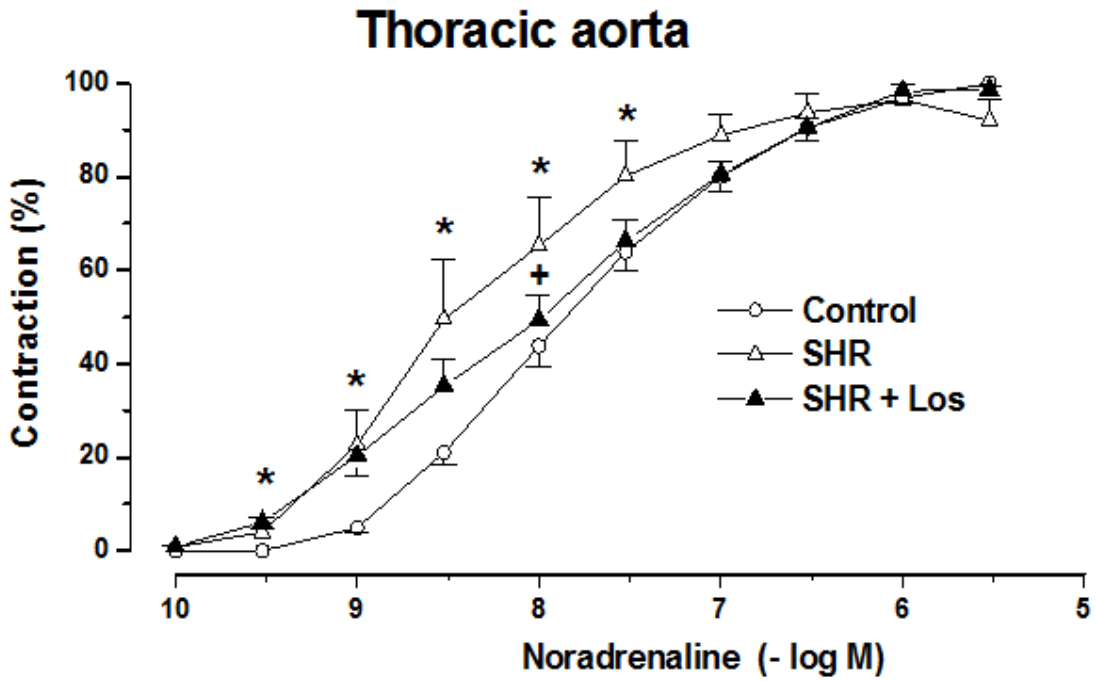

FIGURE 4: CONCENTRATION-RESPONSE CURVES FOR NORADRENALINE-INDUCED CONTRACTION FOR THE THORACIC AORTA IN CONTROL WISTAR RATS, SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND SHR TREATED WITH LOSARTAN (SHR+LOS). VALUES ARE MEANS ± S.E.M. *p<0.05 VS. WISTAR RATS, $^{+}$p<0.05 vs. SHR.

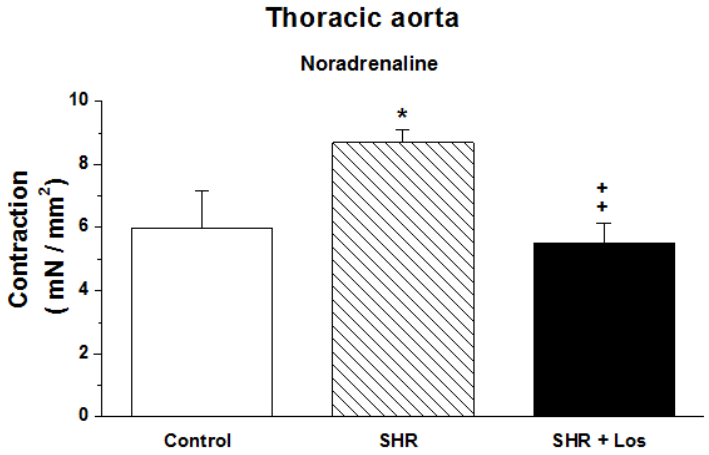

FIGURE 5: MAXIMUM CONTRACTILE RESPONSES OF THORACIC AORTA INDUCED BY NORADRENALINE IN CONTROL WISTAR RATS, SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND SHR TREATED WITH LOSARTAN. AORTIC RINGS FROM SHR EXPOSED TO LOSARTAN ELICITED A LESSER MAXIMUN CONTRACTION TO NORADRENALINE THAN UNTREATED SHR. VALUES ARE MEANS ± S.E.M. *p<0.05 vs. WISTAR RATS, $^{++}$p<0.01 vs. SHR.

The role of AT_1 receptors in sympathetic regulation of vascular response were studied in isolated mesenteric arteries. Electric field stimulation of perivascular nerves elicited frequency-dependent vasoconstriction (neurogenic contractions) in the control Wistar rats. In SHR contractile responses were significantly higher than in Wistar rats. In losartan-treated SHR, they were reduced at higher frequencies of electrical stimulation compared to SHR nevertheless they were still significantly higher than those in control Wistar rats (Figure 6).

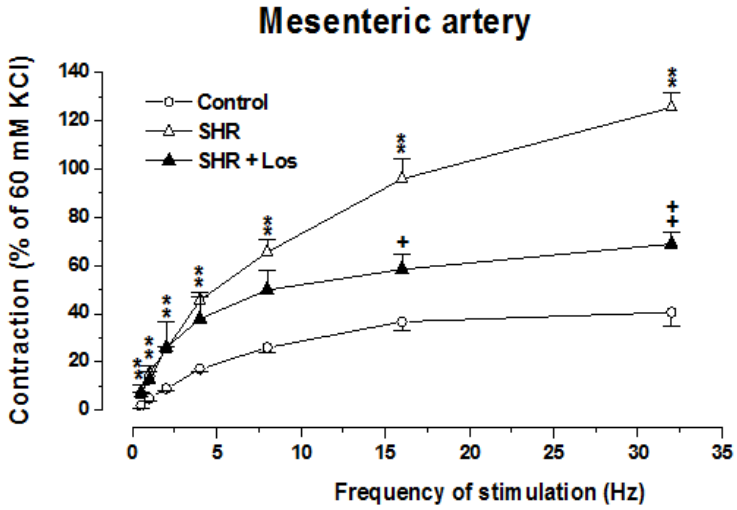

FIGURE 6: CONTRACTILE RESPONSES INDUCED BY ELECTRICAL STIMULATION OF PERIVASCULAR ADRENERGIC NERVES IN RINGS OF MESENTERIC ARTERY IN CONTROL WISTAR RATS, SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND SHR TREATED WITH LOSARTAN (SHR+LOS). CONTRACTION WERE EXPRESSED AS A PERCENTAGE OF 60 MM KCL - INDUCED CONTRACTION. VALUES ARE MEANS ± S.E.M. **p<0.01 VS. WISTAR RATS, $^{++}$p<0.01, $^{+}$p<0.05 vs. SHR.

3.4 Geometry of the thoracic aorta

Wall thickness (WT) (tunica intima+media) of the thoracic aorta was decreased (p<0.01) in SHR compared to Wistar rats. Administration of losartan to SHR did not evoke any changes of WT in SHR (Figure 7A). Cross sectional area (CSA) (tunica intima+media) in the SHR was reduced in comparison to the Wistar rats. No difference in this respect was observed between SHR and SHR treated with losartan (Figure 7A). Inner diameter (ID) of the aorta in the SHR did not differ from that in the Wistar rats. Administration of losartan evoked a significant increase of ID in SHR (Figure 7B). Wall thickness/inner diameter ratio (WT/ID) did not differ between Wistar rats and SHR. Administration of losartan to SHR decreased WT/ID in comparison with SHR. (Figure 7B).

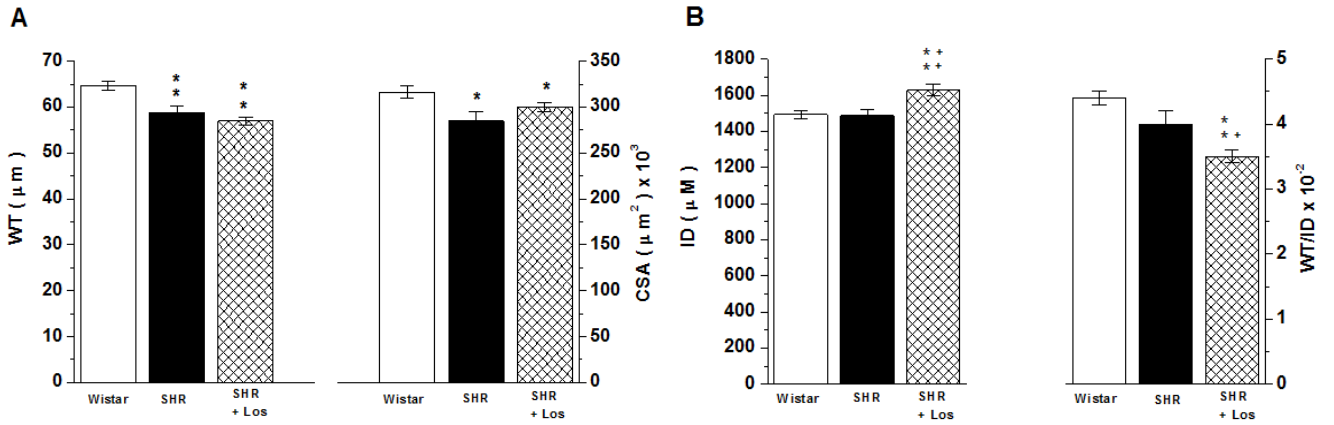

FIGURE 7. A - WALL THICKNESS (WT) AND CROSS SECTIONAL AREA (CSA), B - INNER DIAMETER(ID) AND WT/ID IN WISTAR RATS (WISTAR), SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND SHR TREATED WITH LOSARTAN (SHR+LOS). VALUES ARE MEANS ± S.E.M. *P<0.05, **P<0.01 vs. WISTAR RATS, $^{+}$P<0.05, $^{++}$P<0.01 vs. SHR.

IV. DISCUSSION

The present study demonstrates that 5 week-lasting administration of losartan, the AT_1 receptor antagonist, to 4-week-old spontaneous hypertensive rats, prevented development of hypertension, cardiac hypertrophy, impairment of acetylcholine-induced relaxation in aortic rings leading to diminished endothelial dysfunction, and prevented structural pathological changes in cardiovascular system. These effects are supposed to be beneficial outcome of drug treatment on the cardiovascular system during hypertension.

Systolic blood pressure increase and cardiac hypertrophy found in 9-week-old SHR as well as the process of blood pressure rise from the prehypertensive period observed represent the developing phase of hypertension. Blocking the action of angiotensin II, losartan and its metabolite cause blood vessel dilatation and thereby reduce blood pressure in hypertensive individuals (18). Nevertheless, blood pressure remained still significantly elevated compared to the Wistar rats. The findings are fully in agreement with reports from other laboratories and our previous data (19,14). In other study permanent treatment of SHR from conception onwards with AT_1 antagonists prevented hypertension and accompanying left ventricular hypertrophy (20,21). It is interesting that in 9 week-old not treated SHR thickness of aorta was lower that in Wistar controls. Administration of losartan to the SHRs did not resulted in any changes of wall thickness of aorta in SHR but it prevented hypertrophy of the heart. Decrease of cardiac hypertrophy in the losartan administered rats could be evoked by decreased peripheral resistance (myocardium had to surmount lower resistance) along with antiproliferative effect of losartan on myocardial cells (22). The hypotensive effect of losartan and the attenuation of relative heart weight observed in losartan-treated SHR are in good agreement with the earlier observations (14,16,23).

In SHRs, the lowering blood pressure after losartan administration was accompanying by increase of internal diameter of the aorta. Similar effect of losartan treatment has been observed by Rizzoni et al. (24) in mesenteric resistant vessels without affecting arterial wall hypertrophy. Partial decrease of blood pressure accompanied by adequate increase of inner diameter enable to preserve the delicate balance among the blood pressure, internal diameter and arterial wall thickness which results in relative stable circumferential stress in the artery. The disturbance in the circumferential stress should negatively influence the functional parameters of the vascular tree (16).

Administration of acetylcholine caused a reduction of systemic blood pressure. Magnitude of the blood pressure drop was similar in Wistar and SHR, at all concentrations used. In the SHR group treated with losartan, acetylcholine induced marked dose-dependent decrease of systemic blood pressure. Similarly, Schiffrin et al (25) observed reduction of pressure using pressurized precontracted mesenteric arteries isolated from losartan pretreated SHR. They described it to activation of angiotensin II AT_2 receptors (with an opposite effect to AT_1 receptors), which are not blocked by losartan. It is likely that beside the AT_2 receptors is involved protective role of some metabolites of renin-angiotensin system (RAS) as angiotensin-(1-7) and angiotensin-(1-9) on endothelial dysfunction and cardiac remodeling (26). The effect of individual metabolic products of RAS axis is a matter of intensive investigation.

In our experiments using isolated aortic rings from spontaneously hypertensive rats treated with losartan prevention of impairment of acetylcholine-induced relaxation was found. Li et al., (27) observed even more enhanced endothelium-

dependent relaxation of pressurized small (mesenteric) arteries after AT_1 blockade in SHR. Acetylcholine-induced endothelium-dependent relaxation of the thoracic aorta in mouse model of Marfan syndrome was improved after 3 months of losartan treatment, but such improvement disappeared with longer duration of treatment (28). These findings suggest that inhibition of the RAS might lead to amelioration of impaired vascular function.

Alfa-adrenergic receptors contribute to the control of vascular tone after sympathetic nerve activation. In the SHR, smooth muscle responsiveness to exogenous angiotensin II is enhanced (29). The increase of the contractile action of angiotensin II has been related to the enhancement of the facilitatory effect of angiotensin II on noradrenaline release in sympathetic endings (30). It is possible to speculate that hypotensive effect of losartan could be partially due to the reduction in sympathetic neural function that is augmented in hypertension (10).We found that AT_1 receptor antagonist losartan attenuated maximum contractile response to noradrenaline in SHR aortic rings, suggesting that angiotensin II through AT_1 receptor plays a mediatory role in these contractions.

In this study we describe the effect of chronic treatment with the losartan on sympathetic mechanisms regulating the vascular tone of the SHR mesenteric artery. Electric field stimulation, which stimulates the release of NA from perivascular sympathetic endings (31), elicited higher contractile responses in mesenteric artery of SHR than in control Wistar rats. This difference in contractions might be due either to an increase of the postsynaptic effect of noradrenaline or/and to a higher release of the transmitter from noradrenergic endings. Neurogenic contractions of mesenteric artery in losartan-treated SHR were at higher frequency of stimulation significantly reduced. These results are in harmony with findings that acute or chronic pretreatment with losartan suppressed the facilitatory role of angiotensin II on noradrenaline release from isolated caudal artery (23).

It has been reported that sympathetic nerve activity increased in patients with chronic renal failure was reduced by chronic (long-lasting, 6 weeks) treatment with losartan, this also supports the idea that angiotensin II is involved in the pathogenesis of the sympathetic hyperactivity (11). In accordance with these observations, we found in our study that the AT_1 receptor antagonist losartan attenuated the contractile respopnse to noradrenaline in SHR aortic rings, suggesting that angiotensin II through AT_1 receptor plays a mediatory role in these contractions.

In SHR, the administration of losartan to 4-week-old SHRs had no effect on the thickness of arterial wall and CSA. On the other hand losartan produced an increase in the inner diameter of thoracic aorta and caused a decrease of WT/ID ratio. Increased inner diameter was caused probably by reducing stiffness and media/lumen ratio of arteries as was before shown on other type of artery after treatment with losartan (32).

Our study was aimed at investigating the effects of prevention of hypertension, rather than treatment, since antihypertensive therapy was started in young SHR before the development of overt hypertension.

V. CONCLUSION

Long-term treatment of young spontaneously hypertensive rats with losartan, angiotensin II receptor antagonists, partially prevented the elevation in blood pressure in the SHR and reduced relative heart weight, prevented impairment of acetylcholine-induced relaxation in aortic rings leading to diminished endothelial dysfunction,, normalized sensitivity of aorta to exogenous noradrenaline,reduced the maximum contraction in aorta induced by exogenous noradrenaline, suppressed the facilitatory role of angiotensin II on enhancement of neurogenic contractions in mesenteric artery, reduced structural changes in aortic wall (increase of inner diameter and reduction of wall thickness/inner diameter ratio). Losartan and other nonpeptide angiotensin II AT_1 receptor antagonists besides their short-term reduction of blood pressure have oppened the tremendes potential of substantial target-organ protection.

ACKNOWLEDGEMENTS

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