INTRODUCTION

The sympathetic nervous system (SNS) is mutually related to other regulatory systems and, via α1 adrenoceptors, actively participates in the control of the functions and structure of the arterial tree (smooth muscle activity, distensibility, and trophicity, among others) from early ontogeny. It is generally accepted that a sustained increase in resting sympathetic activity in this period plays an important role in systolic blood pressure (BP) elevation in spontaneously hypertensive rats (SHR). According to the majority of investigators and including the original data presented by Okamoto and Aoki (1), BP of 4-week-old SHR did not differ from that of normotensive Wistar rats. Compared to normotensive rats, the most rapid acceleration of the cardiovascular alterations in SHR, mainly a rise in BP and remodelling of the arterial wall of conduit and resistant arteries, occurs between the 4th and 10th week of age. Four-week-old animals were used: Wistar rats, SHR, and Wistar rats and SHR receiving prazosin (10 mg/kg/day in tap water) by gavage. Blood pressure (BP) was measured weekly by the plethysmographic method. After six weeks under anaesthesia, the carotid artery was cannulated for BP registration, and the jugular vein was cannulated for administration of drugs. Afterwards, the animals were perfused with a glutaraldehyde fixative at a pressure of 120 mmHg. The septal branch of the left descending coronary artery was processed using electron microscopy. The prazosin administration evoked the following results in both groups: a decrease of BP and heart/body weight ratio, enhancement of hypotensive responses to acetylcholine (0.1 µg, 1 µg, and 10 µg), and an increase in the inner diameter of the coronary artery without changes in wall thickness, cross sectional area (CSA) (tunica intima+media), CSA of smooth muscle cells, and extracellular matrix. In the SHR group, a reduction was observed in BP increase after noradrenaline (1 µg) application. CSA of endothelial cells which was decreased in the SHR (compared to the control Wistar rats) was increased after prazosin treatment (up to control value). Long-term prazosin administration from early ontogeny partially prevented some pathological alterations in the cardiovascular system of SHR.

Key words: blood pressure, cardiovascular system, coronary artery, prazosin, spontaneously hypertensive rats
the behaviour of the cardiovascular system in adulthood. To the best of our knowledge, the data on the effect of long-term alfa 1 adrenoceptors inhibition by prazosin on structure of coronary arteries in SHR are insufficient.

MATERIALS AND METHODS

The procedures followed the guidelines given in the Guide for the Use of Laboratory Animals (Ethics Committee for Experimental Work, Slovak Academy of Sciences, 1995). The animals were housed at a temperature of 22-24°C under a 12 h light/dark cycle and fed a regular pellet diet (Bonagro, Czech Republic).

Four-week-old Wistar rats were used for the study. The animals were divided into four groups of 10 animals each: 1) Wistar rats, 2) Wistar rats receiving prazosin, 3) SHR, and 4) SHR receiving prazosin. Prazosin was administered by a gavage in a dose of 10 mg/kg b.w./day dissolved in drinking water for 6 weeks. BP was measured noninvasively in pre-warmed animals by the plethysmographic method on the tail artery in all groups each week.

After 6 weeks of treatment, animals from each group were subjected to in vivo investigation and then for morphological investigation.

In vivo study

The animals were anaesthetised with ketamine (0.25 ml/100 g b.w.) and xylazine (0.1 ml/100 g b.w.) (Zentiva, Czech Republic) 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. After stabilisation (about 10 min), noradrenaline (Sigma, Germany), in a dose of 1 µg dissolved constantly in 0.1 ml of physiological salt solution, was administered through the jugular vein over a constant period of 10 s. Similarly, acetylcholine (Zentiva, Czech Republic) in doses of 0.1 µg, 1 µg, and 10 µg was administered through the jugular vein. The drugs were administered in a random order. Individual stimuli were applied in a 10-15 min interval after the BP returned to basal level and was stabilised.

Morphological study

The animals were sacrificed by an overdose of anaesthesia. The chest was opened, and the cardiovascular system was perfused with a fixative (300 mmol/L glutaraldehyde in 100 mmol/L phosphate buffer) at a constant pressure of 120 mmHg for 10 min via a cannula placed in the left ventricle. After perfusion, the heart was excised and weighed. The upper part of the septal branch of the left descending coronary artery (RS) was excised, cleaned, divided into four segments about 1 mm long and processed for electron microscopy. Three randomly selected blocks of the artery were cut perpendicular to the longitudinal axis. The inner circumference and arterial wall thickness (tunica intima and tunica media) were measured using light microscopy. The inner diameter and cross sectional area (tunica intima and tunica media) were calculated (for details, see 7).

Using electron microscopy, volume densities of cellular (endothelial cells and smooth muscle cells) and extracellular components of the coronary wall (tunica media and tunica intima) were estimated using the point counting method according to Weibel et al. (8). In short, the grid was placed on the respective section randomly, and 5000 points were counted. Sections from three randomly selected blocks from vessels of control and experimental animals were processed in the same way. From the volume densities, the cross sectional areas of individual parts of the arterial wall were counted.

The data obtained were expressed as means ±S.E.M. ANOVA and the Bonferroni test for unpaired variables were used to assess statistical significance. Results were considered significant when P<0.05.

RESULTS

General cardiovascular parameters

At the beginning of the experiment, the BP of 4-week-old Wistar rats and SHRs did not differ. In the SHR group, the BP continually increased from the 5th week of age until the end of the experiment (by 37%). Administration of prazosin to Wistar rats resulted in BP decreases only at the end of the experiments (by 8%). In the SHR treated with prazosin, the BP increased continually from the 5th week of age. Nevertheless, the increase was significantly slower than in the corresponding SHR group during the whole experiment. At the end of the experiment, it
was decreased by 7% in comparison to the SHR but still remained significantly higher (by 22%) compared to untreated Wistar rats (Fig. 1).

Heart weight (HW) (heart weight after perfusion with fixative is higher than heart weight without perfusion due to presence of the fixative in the open arterial tree) of the SHR did not differ from the HW values of control Wistar rats. Administration of prazosin decreased heart weight in both the Wistar rats and SHR (Fig. 2).

Heart weight/body weight ratio (HW/BW) in the SHR was significantly increased compared to the Wistar rats. Administration of prazosin to both the Wistar rats and SHR resulted in a decrease of HW/BW. The decrease in the SHR+prazosin group was up to the HW/BW levels in the Wistar rats (Fig. 2).

**Integrated responses**

Integrated responses of the cardiovascular system in the SHR did not differ after acute administration of increasing doses of 0.1 µg, 1 µg, and 10 µg of acetylcholine. The application of the same doses of acetylcholine to either Wistar rats treated with prazosin or SHR treated with prazosin significantly improved the acetylcholine-induced decrease of systemic BP (Fig. 3).

Noradrenaline (1 µg) application to the SHR evoked a significant increase of the integrated response of the cardiovascular system when compared to the Wistar rats. In comparison to the Wistar rats, no difference was observed when noradrenaline was applied to Wistar rats treated with prazosin. Noradrenaline applied to SHR treated with prazosin evoked a decrease in the increase in BP in comparison to untreated SHRs. This decrease in BP was comparable to the corresponding value for the control Wistar rats (Fig. 3).

**Geometry of the coronary artery**

Wall thickness (WT) (tunica intima+media) of the coronary artery was increased in the SHR compared to the Wistar rats. Administration of prazosin to both the Wistar rats and SHR did not evoke changes in WT (Fig. 4A). Cross sectional area (CSA) (tunica intima+media) in the SHR was enlarged in comparison to the Wistar rats. No difference in this respect was observed between either Wistar rats and Wistar rats receiving prazosin or between SHR and SHR treated with prazosin (Fig. 4A).

Inner diameter (ID) of the artery in the SHR did not differ from that in the Wistar rats. Administration of prazosin evoked
a significant increase of ID in both the Wistar rats and SHR (Fig. 4B).

Wall thickness/inner diameter ratio (WT/ID) increased in the SHR compared to the Wistar rats. Administration of prazosin to the Wistar rats did not evoke changes of WT/ID. WT/ID of the coronary artery in the SHR decreased to the control value after prazosin treatment (Fig. 4B).

Composition of the coronary artery

Structural analysis of the individual components of the coronary wall (tunica intima+media) revealed a decrease in endothelial cell CSA in the SHR compared to the Wistar rats. No difference in this respect was observed between the Wistar rats and Wistar rats receiving prazosin. The CSA of endothelial cells in the SHR after prazosin administration was increased up to the control value (Fig. 5).

The CSA of smooth muscle cells in the SHR was significantly enlarged in comparison to the Wistar rats. Long-term administration of prazosin evoked no changes in the CSA in either Wistar rats or SHR (Fig. 5).

The CSA of the extracellular matrix in the coronary wall (tunica intima+media) was increased in the SHR compared to the Wistar rats. No difference in this respect was found between either the Wistar rats and Wistar rats treated with prazosin or the SHR and SHR treated with prazosin (Fig. 5).

DISCUSSION

Long-term α1-adrenoceptor inhibition with prazosin resulted in a decrease in BP in both Wistar rats and SHR. The results correspond to the literary data documenting that α1-adrenoceptor antagonist administration from early ontogenic development results in a decrease in pathological alterations in adulthood. Nevertheless, after prazosin administration, systolic BP in the SHR decreased only partially, and at the end of the experiment, it was still significantly elevated compared to the Wistar rats. Lower decreases of BP can be associated with the beginning of antagonist administration. Long-term BP reduction was found in adult SHR treated with the α1-adrenoceptor antagonist terazosin from the third postnatal week (9); however when the blockade started later (from the age of 4 weeks), BP development was not affected (10, 11). To achieve more pronounced effects of the α1-adrenoceptor antagonist (prazosin) on BP, the treatment was supported by sympathectomy.
Neonatal sympatectomy itself can prevent hypertension and reduce vascular alterations (12, 13), and the combination with prazosin treatment lasting until puberty can completely avoid cardiac hypertrophy, and normalised vascular resistance persists even in adult SHR (14).

In our experiment, BP decreases in both groups after prazosin administration seems to have a positive correlation with decreases in cardiac hypertrophy. The effect of the treatment in both groups was more significant on the heart when compared to the effect on BP (heart/body weight ratio in the SHR did not differ from that observed in the normotensive Wistar rats after prazosin administration).

Hypertrophy of the coronary wall (tunica intima+media) observed in the SHR compared to the Wistar rats is in agreement with general findings on other types of conduit and resistant arteries in adult SHR (15-18). The structural analysis of the coronary artery revealed that the hypertrophy is evoked by increased mass of smooth muscle cells and extracellular matrix. Similar increases were found in coronary arteries of 16-week-old SHR (17). In spite of a remarkable increase in the arterial wall mass, the circumferential stress (blood pressure x inner diameter/wall thickness) in the SHR did not differ from that in the Wistar rats. The data suggest that rapid acceleration of BP, increasing from the 5th to the 9th weeks of age, in the SHR was compensated by a similar increase of arterial wall thickness or vice versa. Arterial wall hypertrophy is in a good relation to the amplified BP response to noradrenaline. Our previous experiments documented that the amplified BP response to noradrenaline lasted until the later ontogenic periods (19). Prazosin administration did not affect the wall thickness and total CSA of coronary arteries in the Wistar rats and SHR. Neither the mass (CSA) of smooth muscle cells nor the extracellular matrix were affected in either group with prazosin administration. In spite of this, we observed that the decrease of noradrenaline evoked a vasoconstrictory response of the resistant part of the vascular tree after prazosin administration only in the SHR group. Considering these data, we suppose that the BP decrement in this group was probably due to blunted hyperactivity of resistance vessels or supersensitivity to alfa 1 agonists (both are suspected in SHR (20, 21)). The findings agree with the suggestion that hypertension in SHR is evoked mainly by increased noradrenergic innervations and higher activity of the sympathetic nervous system, resulting in increased myogenic tone of small arteries already in the prehypertensive phase (22-25).

Structural analysis of coronary arteries in the SHR revealed (contrary to smooth muscle cells and extracellular matrix) mild hypotrophy of the endothelial cells in comparison to the Wistar rats. The cause of this phenomenon is not known. Endothelial cells are in close contact with blood, and their hypotrophy in SHR might be associated with acceleration of BP increase, changes in shear stress, blood flow, pulse wave velocity, among other factors, in early ontogeny. Surprisingly, the decrement in CSA of endothelial cells in the SHR did not result in a decrease of vasorelaxant responses to increasing doses of acetylcholine (the effect of acetylcholine is associated with the NO released from endothelial cells). The values did not statistically differ when compared to the Wistar rats (though the absolute values were lower, they were not significant). Nevertheless, the effect of NO in SHR is ambiguous. Acute and chronic NO deficiency evoked by L-NAME administration decreased endothelium-dependent relaxation of conduit arteries to acetylcholine in vivo; however, NO deficiency after L-NAME treatment did not decrease endothelium-dependent relaxation in vitro (26). Increase in NO levels due to long-term NO donor administration did not have a beneficial effect on the cardiovascular system of SHR (27). These results and data from other studies suggest that NO deficiency is not a primary reason for the development of hypertension in SHR. Moreover, possible decrement of NO (if any) from hypotrophied endothelial cells in SHR could be replaced by NO production from other sources. It was documented that NO can be produced in a sufficient concentration by smooth muscle cells (28, 29). On the other hand, Cosentino et al., (30) reported impairment of endothelial NO synthase in prehypertensive SHR. They suggest that endothelial NO synthase requires higher amounts of tetrahydrobiopterin that produces less NO but more superoxides. Thus, dysfunctional NO synthase may be, in prehypertensive SHR, a contributor to the development of hypertension and its vascular complications. In addition, alfa 1-adrenoceptor stimulation via NADPH oxidase increases the production of intracellular oxygen radicals, leading to hypertension of smooth muscle cells (31, 32). It seems that rather than NO deficiency due to insufficient NO synthesis, increased superoxide production contributes via NO inactivation to hypertension in SHR. Similar significant decreases of NADPH-dependent superoxide anion production increased by cocaine administration were observed after prazosin administration (33). Prazosin administration can preserve endothelium dependent relaxation in hypercholesterolemia where production of reactive oxygen species in vascular wall is stimulated (34, 35). The likely
beneficial effect of prazosin on the development of endothelial cells in SHR (complete prevention of hypertrophy) and improvement of acetylcholine-induced relaxation could be related to oxygen radicals.

Decrease of BP, increased relaxation of the arterial tree after acetylcholine, and increased inner diameter of the coronary artery after prazosin administration seems to be in good harmony. Nevertheless, the effect of prazosin is a result of multiple interactions of the sympathetic nervous system with other regulatory systems, such as kidney function (the relationship between sodium excretion and renal perfusion pressure is shifted to the right in SHR as young as 3 weeks of age SHR - 36, in SHR, but not in normotensive rats, periarterial nerve stimulation significantly augmented angiotensin II-induced changes in perfusion pressure - 37, mineralocorticoid receptor activation participates in hypertension-associated renal damage - 38, NO dependent mechanism - 39, 40, 41, and renin-angiotensin system (42, 43), among others. Endothelium-derived hyperpolarising factor presented mainly in resistant parts of the arterial tree (44) probably plays an important role as well.

In conclusion, long-term administration of prazosin from early ontogeny decreased BP and trophicity of the heart, improved integrated responses to acetylcholine, and evoked relaxation of coronary arteries in both Wistar rats and SHR. It also decreased noradrenaline-evoked vasoconstrictor responses of resistant parts of the vascular tree and significantly improved development of the endothelial cells in SHR.

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