Differential Remodeling of Carotid Artery in Spontaneously Hypertensive and Hereditary Hypertriglyceridemic Rats

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Summary
High blood pressure, increased level of cholesterol, diabetes, hypertriglyceridemia and obesity are risk factors accompanied metabolic syndrome. The aim of the study was to compare geometry of carotid artery (AC) of 3-week-old (3w) and 52-week-old (52w) hereditary hypertriglyceridemic rats (hHTG) and spontaneously hypertensive rats (SHR) which represent a genetic model of human essential hypertension with age-matched Wistar rats. After sacrificing the rats were perfused with a glutaraldehyde fixative under the pressure 90 mm Hg (3w) and 120 mm Hg (52w) for 10 min via cannula placed into left ventricle. Middle part of AC was excised and processed according to standard electron microscopy procedure. Geometry of AC was evaluated in light microscopy. SHR vs. Wistar rats: BP of 3w did not differ, in 52w it was increased; cardiac hypertrophy was found in both ages; wall thickness (WT) and cross sectional area (CSA) in 3w did not differ, in 52w both were increased; inner diameter (ID) in 3w and 52w was decreased; WT/ID was increased in both ages. Hereditary HTG vs. Wistar rats: BP was increased in both periods; cardiac hypertrophy was observed in 3w; wall thickness (WT) and cross sectional area (CSA) in 3w did not differ, in 52w both were increased; inner diameter (ID) in 3w and 52w was decreased; WT/ID was increased only in 52w. Discrepancies between development of BP, cardiac hypertrophy in SHR and hHTG rats were observed. Alterations of BP were not in harmony with alterations in geometry of carotid arteries in both SHR and hHTG rats. We suggest that BP is not the main stimuli evoked hemodynamic and structural alterations of cardiovascular system in ontogenic development of SHR and hHTG rats.

Key words
Morphology • Hypertension • Hypertriglyceridemia • Conduit Artery • Structure • SHR

Introduction
Hypertension is a heterogeneous disease that is multifactorial in origin. The basic concept is a resetting of control mechanisms at a higher than normal blood pressure level. It has long been considered a disease of the cardiovascular system that affects almost exclusively the arterioles and the heart. However, damages of the large arteries are clearly involved in cardiovascular morbidity and mortality associated with hypertension (Folkow 1978). Numerous animal and human studies have shown that sustained hypertension is associated with structural and functional alterations of both large arteries and arterioles, however, the fundamental causes of essential hypertension are still largely unknown.

Experimental models of human diseases are
frequently used to investigate the pathophysiology of disease as well as the mechanisms of action of therapeutics. Spontaneously hypertensive rats (SHR) represent a genetic animal model of human essential hypertension due to similarities in genetic predisposition to high blood pressure without specific etiology, increased total peripheral resistance without volume expansion, and similar responses to drug treatment. The development of hypertension in this strain is usually associated with the presence of vascular structural alterations (Mulvany et al. 1977) and impairment of endothelial function (Yang et al. 1991). Both these factors may contribute to imbalance between vasoconstriction and vasodilation (Rizzoni et al. 1997).

There is general agreement that hypertensive vessels are characterized by narrowed lumen as a result of increased contraction of the smooth muscle cells leading to an increased thickness of the arterial wall (Folkow et al. 1973).

Major risk factors for cardiovascular disease are besides high blood pressure diabetes, high cholesterol which is often accompanied by hypertriglyceridemia and obesity. Hypertriglyceridemia is a one of the most common metabolic disorder in the human population and is also recognized as an important independent risk factor for coronary heart disease (Jeppesen et al. 1973).

Prague hereditary hypertriglyceridemic rats (hHTG) were developed as an animal model of human hypertriglyceridemia and insulin resistance (Vrána and Kazdová 1990). They are characterized by the simultaneous appearance of hypertriglyceridemia (Vrána and Kazdová 1990). They are characterized by the simultaneous appearance of hypertriglyceridemia (Vrána and Kazdová 1990), insulin resistance (Štolba et al. 1993), glucose intolerance (Vrána et al. 1993), and by elevated blood pressure (Štolba et al. 1992, Lichardus et al. 1993). Their mild hypertension correlates positively with higher plasma triglyceride level (Štolba et al. 1992). This positive correlation was documented in F2 hybrids obtained from hHTG and normotensive Lewis progenitors (Kuneš et al. 1995). It is also worthwhile noting that ion transport alterations in hHTG rats results in an increased Na⁺ content in red blood cells (Kuneš et al. 1994, Zicha et al. 1995, 1997). Devynck et al. (1998) demonstrated several membrane abnormalities in hHTG rats. The close link between hypertension, hypertriglyceridemia and altered membrane electrolyte transport were supported by demonstration of their association in F2 hybrids of hHTG and Lewis rats (Zicha et al. 1997).

Despite numerous metabolic studies performed on these models of hypertension, no attention has been devoted to the potential conduit artery alterations. The main purpose of this study was to compare the ontogenic aspect of structural alterations in the arterial wall in spontaneously hypertensive and hereditary hypertriglyceridemic rats. Basic functional parameters of the cardiovascular system and geometry of carotid artery in SHR, hHTG and Wistar rats were determined at two points of ontogenic development: 3-week-old (3w) – at weaning period and at 52-week-old (52w) – at the later period of development of hypertension.

### Methods

The experiments were carried out in male Wistar rats, spontaneously hypertensive rats (SHR) and hereditary hypertriglyceridemic rats (hHTG) housed in a room with maintained temperature (22±2 °C), relative humidity (55±10 %), and 12 hour light / dark cycle (lights on at 6:00 AM). The animals had free access to standard chow and water. The procedures were approved by State veterinary and food administration of the Slovak Republic.

Wistar rats at the age of 3 weeks (3w) and 52 weeks (52w) and the age-matched SHR and hHTG rats were taken for the study.

Systolic blood pressure (SBP) was measured non-invasively in pre-warmed rats by the plethysmographic method on the tail artery. Body weight was recorded at the same time. At the end of experiment the animals were sacrificed by an overdose of anesthesia. The chest was opened, a cannula was placed into the left ventricle and the cardiovascular system was perfused at a constant pressure of 90 mm Hg (3-week-old rats), and 120 mm Hg (52-week-old rats) for 10 minutes with a fixative solution (300 mM glutaraldehyde in 100 mM phosphate buffer). The middle part of the carotid artery was excised and immersed in the same fixative, post-fixed with 40 mM OsO₄ in 100 mM phosphate buffer, stained en block with uranylacetate, dehydrated by graded alcohol series, and embedded in Durcupan ACM. Semi-thin sections were cut perpendicularly to the long axis and stained with a methylene blue. Both wall thickness (tunica intima + tunica media) and inner circumference were measured by light microscopy. The wall thickness was measured at 45° intervals around the circumference of the artery. The inner diameter, cross sectional area (tunica intima + tunica media), and wall thickness / inner diameter ratio were evaluated from these data.
For the statistical evaluation of differences between groups, one-way analysis of variance (ANOVA) was used and followed by Bonferroni post-hoc test. The differences of means were considered to be significant at p < 0.05.

**Results**

**General hemodynamic parameters**

Systolic blood pressure in 3-week-old SHR did not differ from the age-matched Wistar rats, but it was increased (p<0.01) in hHTG group (84.2±1.4 mm Hg, 83.3±1.9 mm Hg, 109.3±1.7 mm Hg, respectively). In 52-week-old rats SBP was increased (p<0.01) in both SHR and hHTG group in comparison to controls (189.7±2.4, 161.3±5.7 and 114.6±3.0 mm Hg, respectively).

Body weight (BW) in all three strains gradually increased with age. Body weight of SHR and hHTG rats was significantly lower as compared to controls. These findings confirm that the animals were not obese (data not shown).

Heart weight (HW) after perfusion with glutaraldehyde fixative of SHR at 3 weeks of age did not differ from the age-matched Wistar rats, but it was increased (p<0.01) in hHTG group (245±11 mg, 259±15 mg, respectively). The difference in this respect was found at 52 weeks of age (1991±90 mg in SHR vs. 1480±30 mg in Wistar, p<0.01). In hHTG in both studied ages HW was significantly lower (220±10 mg at 3w and 1190±30 mg at 52w, p<0.01) in comparison to control Wistar rats.

HW/BW ratio at 3-week-old animals was increased (p<0.01) in both experimental groups (7.71±0.15 in SHR, 5.7±0.09 in hHTG) compared with control rats (4.31±0.24). HW/BW ratio at 52 weeks of age was increased only in SHR (4.86±0.17, p<0.01) and no difference was observed in hHTG (3.17±0.11) when compared with age-matched Wistar rats (3.14 ± 0.09).

**Geometry of the carotid artery**

Wall thickness of the carotid artery of young SHR did not differ from that of Wistar rats; in hHTG rat it was significantly decreased. In 52-week-old SHR and hHTG rats wall thickness was increased in comparison with Wistar rats (Fig. 1).

Cross sectional area of arterial wall (tunica intima+tunica media) of 3-week-old SHR did not differ from that of Wistar rats. In hHTG rats this cross sectional area was significantly decreased in comparison with Wistar rats. In 52-week-old SHR it was increased and in hHTG rats it was decreased when compared with Wistar rats (Fig. 2).

Inner diameter of both 3- and 52-week-old SHR and hHTG rats was decreased in comparison with control Wistar rats (Fig. 3). Wall thickness/inner diameter ratio in 3-week-old SHR was significantly increased and in hHTG rats it did not differ from that in Wistar rats. In 52-week-old rats it was significantly increased in both SHR and hHTG rats (Fig. 4).

**Discussion**

In the present study we investigated basic physiological parameters and the geometry of the carotid artery in spontaneously hypertensive, hereditary hypertriglyceridemic rats, and normotensive Wistar rats aged 3 or 52 weeks. The findings revealed differences...
between experimental strains in blood pressure level and conduit artery wall properties proceeded in the two period of ontogenic development.

Blood pressure in 3-week-old SHR did not differ from the age-matched Wistar rats. These data corresponded with the original description of Okamoto and Aoki (1963), who indicated the existence of a prehypertensive period in the development of SHR. In hHTG group at this age, blood pressure was significantly higher. In 52-week-old rats, blood pressure was higher in both SHR and hHTG rats in comparison with their controls. The findings are in good agreement with characteristics of hHTG rats (Štolba et al. 1992) and of aged SHR (Marque et al. 1999). In hHTG strain hypertriglyceridemia and hyperinsulinemia are associated with development of hypertension. High plasma triglyceride concentrations in hHTG rats positively correlated with blood pressure levels (Štolba et al. 1992). A stimulation of the sympathetic nervous system could be one of the mechanisms responsible for this association (Rappaport et al. 1982).

In young hHTG rats the increase in blood pressure was accompanied by cardiac hypertrophy. Surprisingly, in SHR in spite of the same level of BP as in Wistar rats we also found cardiac hypertrophy. The findings suggest that in SHR there could exist genetic prediction for cardiac hypertrophy which is probably, at least in this ontogenic period, independent of blood pressure level. This is in good agreement with the findings of Pang et al. (1986) who demonstrated cardiac hypertrophy even in newborn SHR.

The morphogenesis of heart and vessels occurs mainly during the prenatal period, but it continues partially even after birth (Zicha and Kuneš 1999). In the period shortly after birth the heart grows more rapidly than the body so that heart weight to body weight ratio attains its maximum at 4-5 days of postnatal life and then declines (Clubb and Bishop 1984). Our results are in a good agreement with this observation. In SHR and hHTG rats, heart weight to body weight ratio was significantly higher at the age of 3 weeks than in 52-week-old rats. Nevertheless, the discrepancy between blood pressure and cardiac hypertrophy in SHR and hHTG was also found in 52-week-old animals. Though blood pressure was increased in both experimental groups, cardiac hypertrophy was observed only in SHR. The results suggest that blood pressure is not necessarily linked to the development of cardiac hypertrophy.

Studying the geometry of the carotid artery we found in both young and adult SHR decrease of inner diameter. Hypertrophy of the arterial wall accompanied by decrease of inner diameter is generally accepted. In adult SHR we observed pronounced hypertrophy of the arterial wall (increase of arterial wall mass – CSA). Hypertrophy of the arterial wall accompanied by decrease of inner diameter is generally accepted. In HTG arteries, accumulation of remnants of lipid metabolism in endothelial cells (Kristek et al. 1997) could decrease endothelial dependent relaxation of vessels to acetylcholine (Török et al. 2002, Čačányiová et al. 2005) and could be responsible for decreased inner diameter in both young and adult hHTG rats. Contrary to SHR, in adult hHTG rats we found hypotrophy of arterial wall (decrease of arterial wall mass – CSA) in spite of hypertension. Hypotrophy of thoracic aorta in 3-week-old SHR was also observed (Török et al. 2003, Cebová et al.)
The mechanisms responsible for changes in arterial wall mass in SHR and hHTG rats are still unknown. The following mechanisms may be considered: sympathetic nervous system, renin-angiotensin system, nitric oxide and apoptosis. Since the sympathetic nervous system in young SHR is increased (Nagatsu et al. 1976, Grobecker et al. 1975) and the sympathetic nervous system exerts a trophic effect on the blood vessels (Bevan 1975), it is quite possible that structural alterations of the blood vessels are secondary to the hyperactive sympathetic nervous system.

In adult SHR, increased activity of the sympathetic system is accompanied by increased activity of the renin-angiotensin system. Harrap and Doyle (1987) have shown that the elevated renal renin concentration of the SHR is linked to increased renal vascular resistance (Harrap et al. 1986). These renal abnormalities have been genetically linked to the development of high blood pressure in the SHR (Harrap et al. 1990). Inhibition of the renin-angiotensin system is associated with decreasing renal vascular resistance (Harrap et al. 1986, 1990) and a reduction in wall thickness (Wang and Prewitt 1990). An increased level of angiotensin II in SHR, beside its vasoconstrictor effect stimulates cell growth and proliferation of smooth muscle cells resulting in hypertrophy of conduit and resistant vessels (Brouwers-Celler et al. 1997). There is increasing evidence that the vascular hypertrophic effect of the sympathetic system and of the renin-angiotensin system is intimately related (Stassen et al. 1997).

In hHTG rats was also observed increased activation of the sympathoadrenal system documented by increase of both plasma norepinephrine and epinephrine concentration and blood pressure (Štolba et al. 1992). Increased activity of sympathoadrenal system is not associated with increased levels of renin and angiotensin II (Lichardus et al. 1993).

Thus, in SHR, increased activation of the sympathoadrenal system tightly cooperates with increased activity of the renin-angiotensin system, while in hHTG rats, normal levels of renin and aldosterone was found. We suggested that this difference could be one of the reasons leading to the absence of cardiac and vessel wall hypertrophy in hHTG rats. However, it cannot explain hypotrophy of the arterial wall in hHTG rats the cause of which remains to be elucidated.

The question is still open which part of the arterial wall was increased respective to decreased endothelial cells, smooth muscle cells, and/or extracellular matrix. Nevertheless, having in mind Laplace’s law, the changes in the geometry of the arterial wall (wall thickness, inner diameter, wall thickness/inner diameter ratio) observed in both young and adult SHR and hHTG rats exert probably a negative effect on physiological parameters of the cardiovascular system (wall tension, transmural pressure) and could negatively influence supply of nutritional demands of the respective areas.

In conclusion, our present study, performed using two genetic models spontaneously hypertensive and hypertriglyceridemic rats, indicates differences between the development of increased blood pressure, cardiac hypertrophy and geometry of carotid arteries in SHR and hHTG rats. The prehypertensive period in SHR was accompanied by cardiac hypertrophy and eutrophic remodeling, in spite of unchanged blood pressure. In a later period of ontogenic development in SHR we found hypertrophy of carotid arteries. In hHTG rats, blood pressure was increased from 3 weeks of age and hypotrophy of the arterial wall was found at both experimental ages. We suggest that blood pressure is probably not the main stimuli of changes during ontogenic development of SHR and hHTG rats.

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References


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