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Different Expression of Renin-Angiotensin System Components in Hearts of Normotensive and Hypertensive Rats

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Summary

Tissue renin-angiotensin systems are known to behave differently from the circulating renin-angiotensin system (RAS). It has already been proposed that not only the circulating RAS, but also RAS localized in the cardiac tissue plays an important role in the heart failure. The objective of this study was to compare the gene expression of individual components of the renin-angiotensin system in hearts of normotensive and hypertensive rats. Two genetically hypertensive rat strains – spontaneously hypertensive rats (SHR) and hereditary hypertriglyceridemic rats (HTG) – were compared with Wistar-Kyoto (WKY) and Lewis (LEW) normotensive controls. In addition, developmental changes in gene expression of individual components of cardiac RAS were studied in 20-day-old fetuses, 2-day-old newborns and 3-month-old HTG and LEW rats. In our study, the angiotensinogen gene expression did not differ either among adult normotensive and hypertensive strains, or during development. In contrast, the renin gene expression was significantly increased in hearts of hypertensive compared to normotensive rats. Moreover, a 5-fold increase of renin mRNA was observed in hearts of HTG rats between day 2 and the third month of age. There was also an age-dependent increase of ACE gene expression in both HTG and LEW rats which was substantially delayed in HTG hearts. In conclusion, the results of our study suggest that overexpression of the cardiac renin gene in hypertensive strains could participate in the structural and functional changes of the heart during the development of hypertension.

Key words
Renin-angiotensin system • Development • Heart • Gene expression • Hypertension

Introduction

Many clinical and experimental studies suggest that the renin-angiotensin system (RAS) plays an important role in the pathogenesis of hypertension. Systemic hypertension induces cardiac hypertrophy, which is a structural adaptation of the heart to attenuate the systolic stress on the left ventricle (Hein *et al.* 1997). Angiotensin II (Ang II) represents one of the mechanisms that have been proposed to trigger cardiomyocyte growth and left ventricular hypertrophy. Ang II also acts, both directly and indirectly, on the heart and affects the heart rate, contractility and cell growth. Recent observations based on the clinical use of angiotensin-converting

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enzyme (ACE) inhibitors suggested the possible role of cardiac RAS at several pathological stages, i.e. chronic congestive heart failure, acute myocardial infarction, ventricular remodeling, etc. (Lindpaintner and Ganten 1991). The regulation of Ang II production is determined by components directly involved in its production, i.e. angiotensinogen, renin and ACE. Although an increasing body of evidence very strongly suggests important physiological and pathophysiological role for the cardiac RAS, very few experiments were designed to provide the direct evidence of physiological effects of intracardially synthesized Ang II. There is also an open question whether all components of RAS are synthesized in the cardiac tissue (Stock *et al.* 1995). The findings of mRNA for angiotensinogen (Campbell and Habener 1986, Lynch and Peach 1991), renin (Samani *et al.* 1988, Jurkovičová *et al.* 1999), ACE (Schunkert *et al.* 1990) and angiotensins (Dzau 1987) suggest the synthesis, instead of only uptake, of individual components of RAS in the cardiac tissue.

Hereditary hypertriglyceridemic (HTG) rats were originally developed as a genetic model of hypertriglyceridemia (Vrána and Kazdová 1990, Klimeš *et al.* 1995), but these rats also exhibit insulin resistance (Štolba *et al.* 1993), glucose intolerance (Vrána *et al.* 1993) and hypertension (Štolba *et al.* 1992, Lichardus *et al.* 1993). Spontaneously hypertensive rats (SHR) are the most frequently used animal model of essential hypertension (Okamoto and Aoki, 1963, Davidson *et al.* 1995). It has already been demonstrated that the generation of circulating angiotensin II as well as the expression of angiotensin receptors and angiotensinogen are higher in SHR than in the respective normotensive Wistar-Kyoto rats (Yongue *et al.* 1991).

In the present study, we measured the expression of individual components of cardiac RAS (angiotensinogen, renin and angiotensin-converting enzyme) in the two genetically hypertensive rat strains in comparison with the respective normotensive controls. Since the differences in gene expression of RAS components could be age-dependent, the expression of these genes was also studied in three selected developmental stages of rat heart, i.e. in 20-day-old fetuses, in 2-day-old newborns and in 3-month-old adults of both hypertriglyceridemic (HTG) and Lewis (LEW) rats.

It was shown that the major changes between hearts of normotensive and hypertensive strains are in the expression of renin gene. Furthermore, the expression of renin and ACE genes increased significantly after the birth in hypertensive HTG rats, while in the normotensive LEW strain only mRNA of ACE was significantly increased. The physiological relevance of this age-dependent change remains to be elucidated.

Methods

Animals

The protocol used in this study was approved by the Animal Care Committee of the Slovak Academy of Sciences (Bratislava, Slovak Republic) and by the Animal Care Committee of the Institute of Physiology ASCR (Prague, Czech Republic). Male spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats were obtained from Charles River Laboratories (Sulzfeld, Germany). Hypertriglyceridemic hypertensive (HTG) and normotensive Lewis (LEW) rats are bred in the Institute of Physiology, Prague. The rats were kept under standard conditions (23±2 °C, 12:12 h light-dark cycle) and were fed a standard pellet diet with free access to tap water.

Blood pressure was measured in adult animals either by tail-cuff plethysmography (SHR and WKY rats) or directly through a catheter inserted into the carotid artery (HTG and LEW rats). All experiments were done between 8:00 and 14:00 h on 3-month-old males. For the developmental studies, the day following overnight mating was taken as the first day of prenatal life. Twenty-day-old fetuses of both sexes were delivered by Caesarean section under light ether anesthesia. They were removed successively from the uterus and killed by decapitation. In addition, 2-day-old newborns of both sexes and 3-month-old males were also used in the developmental studies.

The body weight of animals and the weight of both ventricles were determined. Cardiac tissue was frozen in liquid nitrogen and stored at –80 °C until the RNA determination.

RNA preparation

RNA was isolated from rat hearts according to the procedure of Chomczynski and Sacchi (1987) using guanidine isothiocyanate (Fisher Scientific, USA) and phenol-chloroform extraction. Briefly, the cardiac tissue was homogenized in guanosine-thiocyanate with 2-mercaptoethanol, phenol, sodium acetate and mixture of chloroform : isoamylalcohol (24:1 v/v) and mixed thoroughly. After 15 min of the incubation on ice, the

homogenate was centrifuged for 20 min (12 000 rpm, 4 °C). An equal volume of isopropanol was added to the aqueous phase and kept at –20°C for 1 h. After the precipitation, the mixture was centrifuged for 20 min (12 000 rpm, 4 °C). The RNA pellet was dissolved in the RNase free water and extracted twice by phenol : chloroform (1:1 v/v). The last aqueous RNA phase was mixed with 1/10 volume of 3 mol/l sodium acetate and twofold volume of 96 % ethanol. RNA was precipitated overnight at –20 °C. Afterwards, the RNA pellets were washed twice with 75 % ethanol and dissolved in RNase free water. The concentration and purity of RNA was determined spectrophotometrically on Shimadzu UV-3000 (Kyoto, Japan).

Relative quantification of mRNA levels by RT-PCR

Reverse transcription was done using Ready-To-Go You-Prime First-Strand Beads (AP Biotech), with $pd(N)_6$ primer. Specific PCR for renin was done afterwards using RR1 (5′-TCT CAG CAA CAT GGA CTA TGT GC-3′) and RR2 (5′-TTA GCG GGC CAA GGC GAA CC-3′), the primers according to Pieruzzi *et al.* (1995), giving 190 bp fragment. PCR program included 35 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min and polymerization at 72 °C for 1 min. The primers used for angiotensinogen ($A_0$1: 5'-TTG TTG AGA GCT TGG GTC CCT TCA-3'; $A_0$2: 5'-CAG ACA CTG AGG TGC TGT TGT CCA-3') were giving 699 bp band after 35 cycles of PCR under the following conditions: denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and polymerization at 72 °C for 1 min (Shyu *et al.* 1995). ACE was detected after 35 cycles of PCR using primers AC1 (5'-CCT GAT CAA CAA GGA GTT TGC AGA G-3') and AC2 (5'-GCC AGC CTT CCC AGG CAA ACA GCA C-3') and annealing temperature at 58 °C, giving 320 bp band (Iwai *et al.* 1995). As a control for semi-quantitative evaluation of PCR primers for the housekeeper glyceraldehyde-3-phosphate dehydrogenase (GAPDH) - (GPH1: 5'-AGA TCC ACA ACG GAT ACA TT-3'; GPH2: 5'-TCC CTC AAG ATT GTC AGC AA-3') were used to amplify 309 bp fragment from each first strand sample. After denaturation at 94 °C for 5 min, 30 cycles of PCR were performed at 94 °C, 60 °C and 72 °C for 1 min each (Lou *et al.* 1995). Number of cycles was determined for each kind of RT-PCR separately, testing 15, 20, 25, 30, 35, 37, and 40 cycles. By this procedure we verified that under the described conditions the PCR amplification of each fragment was still in the linear range. PCR products were analyzed on 2 % agarose gels. The intensity of the individual bands was measured by Kodak camera, quantified using IMAGE software and compared relatively to GAPDH. As a negative control, amplification was done from mRNA omitting reverse transcription.

Table 1. Body weight (BW), relative heart weight (HW/BW) and mean arterial pressure (MAP) of genetically hypertensive (SHR, HTG) and normotensive (WKY, LEW) rats.

		n	BW (g)	HW/BW (mg/100g)	MAP (mm Hg)
SHR		(8)	273±6**	n.d.	167±5**
WKY		(8)	320±6	n.d.	105±4
HTG	F20	(12)	2.68±0.09**	408±11**	n.d.
	D2	(8)	6.70±0.17	536±24	n.d
	M3	(8)	254±3**	203±4	139±5**
LEW	F20	(10)	3.27±0.05	488±10	n.d.
	D2	(7)	7.13±0.85	491±18	n.d.
	M3	(8)	355±11	193±3	110±2

*Data are means ± S.E.M., n.d. - not determined, F20: 20-day-old fetuses, D2: 2-day-old rats, M3: 3-month-old rats, n - number of animals, **p<0.001 vs. corresponding control rats.*

Statistical analysis

Results are means ± S.E.M. from at least five animals. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Values of $p<0.05$ were considered significant. For multiple comparisons, an adjusted t-test with P values corrected by the Bonferroni method was used (Instat, GraphPad Software, USA).

Results

Table 1 summarizes the data about body weight, relative heart weight and blood pressure in hypertensive and normotensive strains. Body weight was lower while relative heart weight and blood pressure were higher in SHR in comparison to WKY controls. In HTG rats, body weight was significantly lower in 20-day-old fetuses and adult animals when compared to age-matched Lewis controls. Furthermore, mean arterial pressure was significantly higher in adult HTG rats.

The mRNA levels encoding particular components of the renin-angiotensin system were different in normotensive (LEW and WKY) rat hearts as compared to the hypertensive ones (HTG and SHR). Figure 1 shows the mRNA levels of angiotensinogen (AGT), renin and angiotensin converting enzyme (ACE). A typical PCR gel is given as an example for each fragment. Semi-quantification was expressed in relation to the housekeeper GAPDH gene. The cardiac angiotensinogen gene was expressed almost equally in all four strains tested (Fig. 1A).

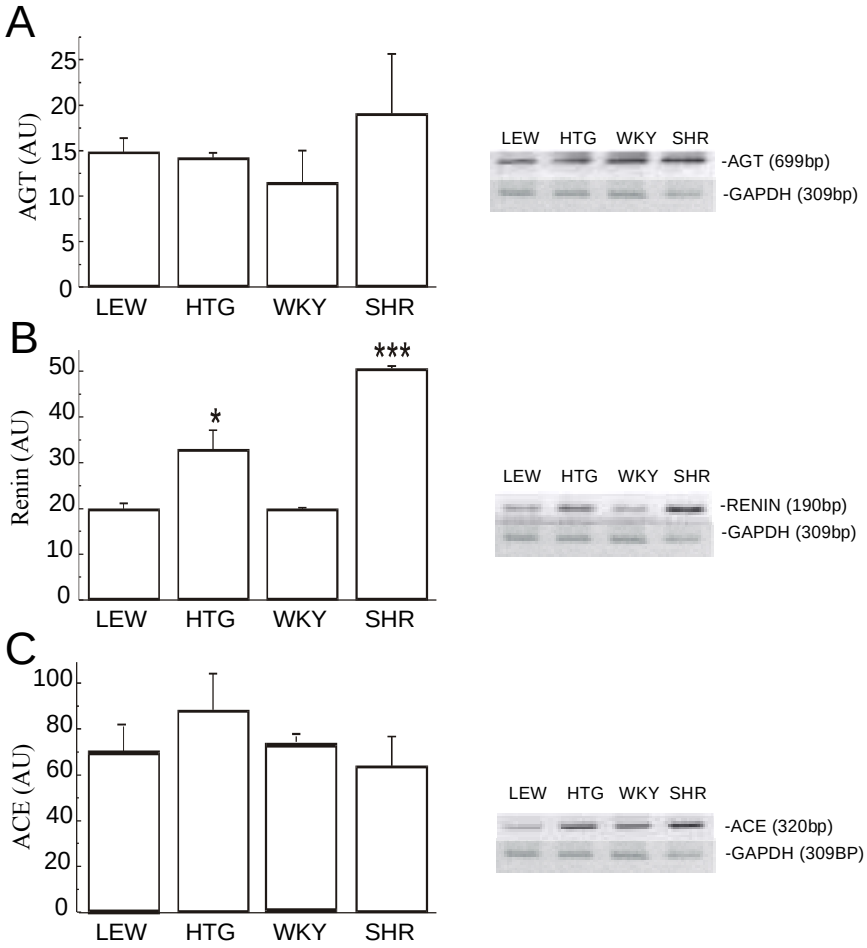

Fig. 1. *Semi-quantitative comparison of mRNA levels of the angiotensinogen (A, AGT), renin (B, Renin) and angiotensin-converting enzyme (C, ACE) in LEW, HTG, WKY and SHR rat hearts compared to the housekeeper GAPDH gene. On the right side, representative examples of the gels are given. Each column represents an average of measurements from at least five animals. Results are expressed as means ±S.E.M. * $p<0.05$, *** $p<0.0001$ significantly different from the corresponding normotensive controls.*

On the contrary, the renin gene was expressed in significantly higher quantities in hearts from hypertensive HTG and SHR rats (Fig. 1B), compared to normotensive LEW and WKY ones. The expression of ACE gene was also not significantly altered in hearts from hypertensive rats (Fig. 1C).

Developmental studies exhibiting the mRNA levels of cardiac RAS components in LEW and HTG rats are shown in Figure 2. Angiotensinogen expression was similar in all developmental periods in the two strains (Fig. 2A). The same was also true for the expression of renin gene in LEW hearts (Fig. 2B). On the contrary, a rapid increase in the renin gene expression occurred in HTG hearts between day 2 and 3 months of age. In HTG rat hearts, a developmental profile of renin gene was similar to that of the expression of cardiac ACE gene (Fig. 2C). Interestingly, in normotensive LEW animals, almost the maximal expression of ACE gene was already observed on the second day *post partum*, while in HTG rats the gene expression was delayed and reached the maximum in adult animals.

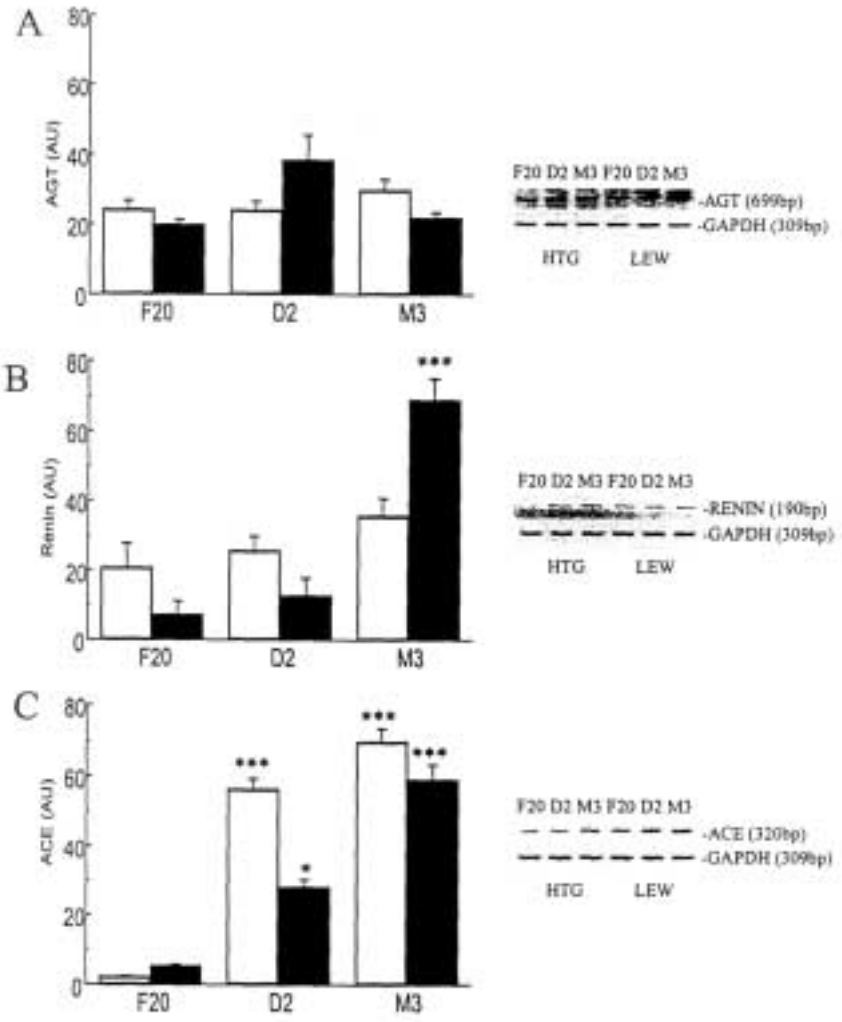

Fig. 2. *Semi-quantitative comparison of mRNA levels of the angiotensinogen (A, AGT), renin (B, Renin) and angiotensin converting enzyme (C, ACE) compared to the housekeeper GAPDH gene in LEW (empty columns) and HTG (full columns) rat hearts during development. The gene expression was measured in 20-day-old fetuses (F20), second day post partum (D2) and in 3-month-old (M3) male rat hearts. Each column represents an average of measurements from six animals. Results are expressed as means ± S.E.M. Statistical significance *p<0.05, ***p<0.0001 is compared to F20.*

Discussion

Our results have clearly demonstrated that the expression of the cardiac renin gene is significantly higher in both hypertensive (SHR and HTG) rat strains than in normotensive WKY and LEW controls. There were no significant differences in the expression of cardiac AGT and ACE genes among the strains studied. Increased mRNA for renin in ventricles of SHR rats compared to WKY was observed by Sano *et al.* (1998) and by Jurkovičová *et al.* (1999). Sano *et al.* (1998) suggested that the cardiac renin-angiotensin system may play an important role in collagen accumulation in hypertensive cardiac hypertrophy. This is in agreement with the postulate that it is not the circulating but activated tissue RAS that may exert a detrimental effect on cardiac function (Pinto *et al.* 1996). However, the physiological importance of the cardiac RAS is still under the investigation (Lindpaintner and Ganten 1991). It is known that Ang II itself enhances hypertrophy of myocardial cells (Miyata and Haneda 1994) as well as the proliferation of vascular smooth muscle cells (Geisterfer *et al.* 1988, Loukotová *et al.* 1998). A significantly increased expression of the renin gene in both hypertensive rat strains used in our study supported the idea that activated tissue RAS might contribute to the cardiac hypertrophy. This is in good agreement with the results of Flesch *et al.* (1997) who demonstrated higher levels of renin mRNA and Ang II concentrations in hypertrophic hearts of transgenic TGR(mREN2)27 rats.

Differences in the age-dependent expression of individual genes of cardiac RAS in HTG and Lewis rats have suggested the possibility of different role of local RAS either in the process of blood pressure increase or cardiac hypertrophy in this new model of genetic hypertension. While the expression of the angiotensinogen gene was similar and stable in the two strains during the whole developmental period, the expression of both renin and ACE genes progressively increased after birth. These major changes in gene expression seen after birth correspond to major changes of circulating RAS components (Jelínek *et al.* 1986) or to angiotensin II vascular receptors (Ghiani *et al.* 1988) during this critical developmental period. Nevertheless, our observations leave an open question about the physiological significance of cardiac RAS during development. It was demonstrated that the expression of genes for renin and angiotensinogen during embryogenesis might be involved in the growth and development of the chick myocardium (Chernin *et al.* 1990).

In conclusion, different amounts of mRNA for cardiac renin in adult hypertensive *vs.* normotensive rats could be related to higher blood pressure. Moreover, different expression of cardiac RAS genes in HTG and Lewis rats during prenatal and postnatal periods might be differently involved in the participation of RAS in the heart development of these two strains.

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