GENETICS

Gene Expression for the Renin System in the Myocardium of Hypertensive ISIAH Rats

L. A. Fedoseeva*, M. A. Ryazanova*, G. M. Dymshits*,, and A. L. Markel*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 10, pp. 430-433, October, 2009
Original article submitted May 22, 2009

The concentration of mRNA for angiotensin-converting enzyme (ACE) and angiotensin type 1A receptor (AT1A) in the myocardium of hypertensive ISIAH rats and normotensive WAG rats was measured by real-time PCR. Gene expression of the angiotensin type 1A receptor in the myocardium of 4-month-old ISIAH rats was lower than in WAG rats. The content of mRNA for angiotensin-converting enzyme in the myocardium of adult ISIAH rats was elevated by 80%. Therefore, the development of myocardial hypertrophy anticipates the increase in enzyme expression in the myocardium. Water deprivation (17 h) was accompanied by a decrease in the concentration of mRNA for angiotensin-converting enzyme in the myocardium of ISIAH rats, which did not differ from that in normotensive animals. Our results suggest that the decrease in cardiac preload and increase in plasma renin activity during dehydration reduce requirements for hyperactivity of the local cardiac renin—angiotensin system.

Key Words: *cardiac renin-angiotensin system; ISIAH rats; water deprivation; angiotensin-converting enzyme; angiotensin type 1A receptor*

Arterial hypertension (AH) is a disease of more than 20% adult people in industrially developed countries and has million complications, including strokes, myocardial infarctions, and renal disorders. A large body of evidence exists that genetic factors play an important role in the regulation of blood pressure (BP). These data form the basis for studying the pathogenesis of hypertensive states and efficacy of antihypertensive drugs. Much attention is paid to inhibitors of angiotensin-converting enzyme (ACE) and antagonists of type 1 receptor for angiotensin II.

Here we studied the expression of genes for ACE and angiotensin type 1A receptor (AT1A) in the myocardium of rats with inherited stress-induced AH (ISIAH). Basal BP in animals of this strain is elevated under rest conditions (160-170 mm Hg) and sharply increases during mild stress (200 mm Hg) [7]. Similarly to patients with essential hypertension [13], ISIAH rats are characterized by myocardial hypertrophy. This state is revealed at the age of 5 weeks [7]. There are data that cardiac hypertrophy is related to changes in the expression of components of the cardiac renin—angiotensin system (RAS) [6,8]. Our experiments showed that basal activity of RAS is reduced in ISIAH rats [1]. Therefore, these animals serve as the model of low-renin hypertension. However, gene expression for the local myocardial renin system can be regulated independently on renal RAS.

*Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk; **Novosibirsk State University, Russia. ***Address for correspondence:*** markel@bionet.nsc.ru. A. L. Markel

MATERIALS AND METHODS

Experiments were performed on hypertensive ISIAH rats and normotensive WAG rats at the age of 1.5 and 4 months. Isolation of myocardial RNA, reverse transcription, and study of gene expression were performed as described elsewhere [1]. Real-time PCR with SYBR Green I was conducted using the following primers: ACE-f, 5'-ATG GTA CAG AAG GGC TGG AA-3'; ACE-r, 5'-TTG TAG AAG TCC CAC GCA GA-3'; AT1A-f, 5'-AAA TGA GCA CGC TTT CTT ACC G-3'; and AT1A-r, 5'-TGA GGC AGG GTG AAT GGT CC-3'. The "housekeeping" *Rpl30* gene for ribosomal protein L30 was used as the reference gene. The following primers were used: Rpl30-f, 5'-ATG GTG GCT GCA AAG AAG AC-3'; and Rpl30-r, 5'-CAA AGC TGG ACA GTT GTT GG-3'.

The effect of short-term water deprivation was studied on adult animals aging 4 months. The rats were deprived of water (19.00-12.00), but had free access to food.

RESULTS

Gene expression for the AT1A receptor in the myocardium of 4-month-old ISIAH rats was 28% lower than in WAG rats. The content of ACE mRNA in the myocardium of ISIAH rats was elevated by 80%. No differences in gene expression were found between 1.5-month-old animals (Fig. 1).

Water deprivation for 17 h had no effect on *AT1A* gene expression in the myocardium of ISIAH and WAG rats. *Ace* gene expression in WAG rats (control) remained unchanged after water deprivation. The elevated level of ACE mRNA in the myocardium of ISIAH rats was shown to decrease and practically did not differ from that in normotensive animals (Fig. 2). Hyperactivity of cardiac RAS is related to an increase in functional load and resulting hypertrophy of the myocardium. It can be hypothesized that reduced activity of ACE after water deprivation is associated with a decrease in cardiac load. The observed changes can be explained by a decrease in the volume of fluid (*e.g.* plasma) and reduction of blood inflow to the heart (preload). Previous studies showed that water deprivation of this duration is followed by a 6% decrease in the volume of circulating plasma [4]. These changes are always accompanied by a decrease in the work for passage of reduced blood volume from the venous system to the arterial system.

Clinical observations [13] and experiments on Wistar rats with left ventricular hypertrophy due to aortic stenosis [12] revealed a relationship between ACE and myocardial hypertrophy. Conversion of angiotensin I to angiotensin II was increased in the hypertrophic myocardium. The content of ACE mRNA in the left ventricle of the hypertrophic heart was 4-fold higher than in the control [12]. The left ventricular mass index correlates with BP and ACE activity in human plasma. No relationship was found between the left ventricular mass index and concentration of angiotensin, renin, or prorenin [13].

Salt load is accompanied by the development of myocardial hypertrophy in hypertensive rats of the Dahl salt-sensitive strain. These changes are not proportional to the severity of hypertension. Myocardial indexes of ACE (gene expression and enzyme activity) in the left ventricle of these rats were higher than in animals feeding a normal diet or salt-resistant specimens receiving a high-salt or normal diet. Blockade of sympathetic activity prevented the development of hypertension, but had no effect on variations in the

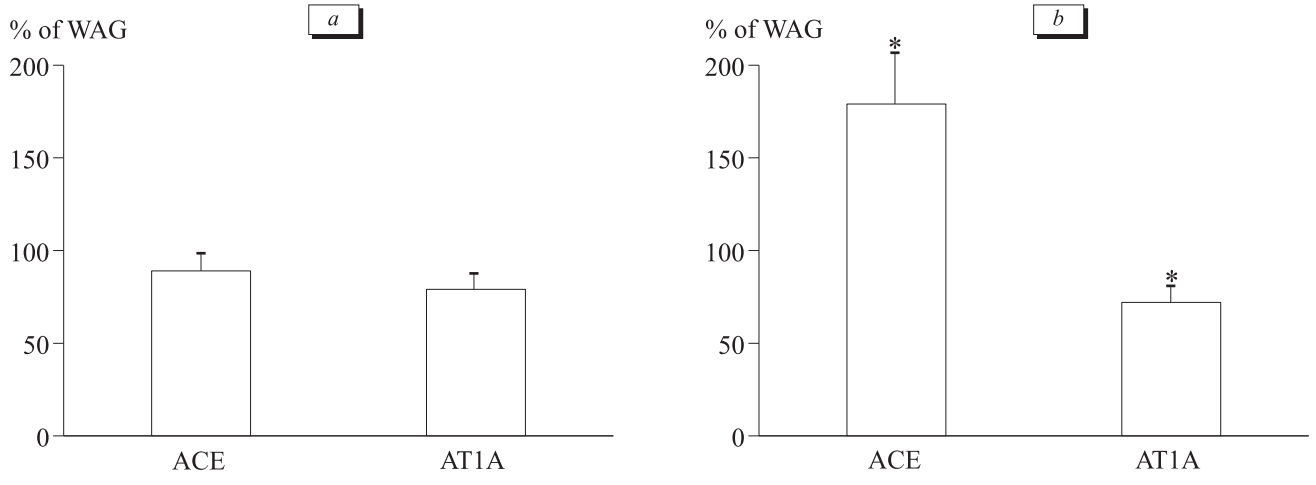

Fig. 1. Concentration of mRNA for RAS genes in the myocardium of hypertensive ISIAH rats and normotensive WAG rats. Animals at the age of 1.5 (*a*) and 4 months (*b*). *$p<0.05$ compared to Wistar rats (100%).

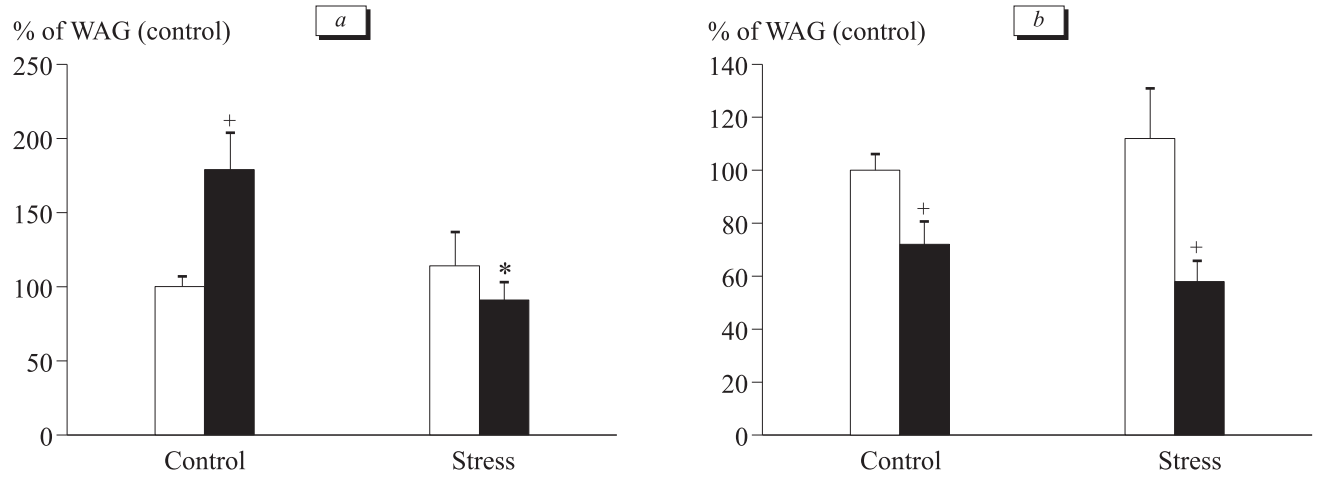

Fig. 2. Effect of dehydration stress on the concentration of mRNA for RAS genes in the myocardium of ISIAH rats (dark bars) and WAG rats (light bars). ACE (*a*) and AT1A (*b*). $p<0.05$: +compared to WAG rats; *significant effect of stress.

expression of ACE, enzyme activity, and weight of the heart. Salt load was not followed by the increase in angiotensin II content in the myocardium of Dahl salt-sensitive rats. Therefore, the increase in ACE is not associated with angiotensinergic mechanisms for myocardial hypertrophy [15].

The concentration of ACE mRNA and AT1A receptor mRNA in the myocardium increases in Dahl rats receiving a high-salt diet. On this model of low-renin and low-aldosterone hypertension, the development of myocardial hypertrophy, myocardial fibrosis, and hypertrophy of the coronary vessel wall is probably associated with activation of mineralocorticoid receptor by glucocorticoids [9].

Much attention was paid to the analysis of cardiac RAS in SHR rats. Some authors reported that the concentration of mRNA for angiotensinogen, ACE, and AT1A receptor in the heart of SHR rats is higher than in WKY rats (normotensive control). Other authors did not reveal these differences [10].

Some investigators believe that myocardial hypertrophy is related to the increased level of angiotensin II (AT1A-dependent mechanism) [5]. However, many experiments showed that there is no direct relationship between the development of cardiac hypertrophy and function of AT1 receptors. For example, transgenic rats (mRen-2) with increased expression of renin are characterized by hypertension and cardiac hypertrophy. The number of AT1 receptors in the aorta, atrium, and ventricle of these rats is lower than in Sprague-Dawley rats (by 40-50%), which contributes to the reduced response of the heart and aortic wall to stimulation with angiotensin II [11]. At the same time, the mice with AT1A receptor gene mutation were characterized by continuous activation of this receptor (but not by myocardial hypertrophy). This state was accompanied by a persistent moderate increase in BP (by 20 mm Hg) and elevated pressor response to angiotensin II. Other parameters (low production of renin and normal level of aldosterone) were similar to those observed during low-renin hypertension [3]. Experiments on AT1 receptor-knockout mice showed that the development of hypertrophy in response to hemodynamic load does not depend on AT1 [14].

As differentiated from hypertensive SHR rats and Dahl salt-sensitive animals, adult ISIAH rats were characterized by a significant increase in *Ace* gene expression and decrease in AT1A receptor gene expression in the myocardium. These indexes did not differ in young ISIAH rats (1.5 months) and normotensive control specimens. However, myocardial hypertrophy in ISIAH rats was observed in the earlier period [7]. It can be hypothesized that morphological changes in the heart are not associated with functional variations in the local RAS during hypertension. BP in 4-week-old ISIAH rats considerably surpasses the control level. ACE expression in the heart is elevated in the later period. These changes probably maintain the content of endogenous angiotensin II under conditions of low expression and reduced activity of plasma renin.

Our previous studies showed that the expression of the type 2 cyclooxygenase gene in the kidneys of ISIAH rats increases significantly after water-deprivation stress [1]. Type 2 cyclooxygenase is a key enzyme for the synthesis of prostaglandins that stimulate renin secretion from juxtaglomerular cells. The increase in activity of plasma renin (key enzyme of circulating RAS) during dehydration [2] probably reduces the requirements for hyperactivity of the local cardiac RAS. These changes can be followed by a decrease in the expression of myocardial ACE.

This work was supported by the Russian Foundation for Basic Research (grant No. 08-04-00533) and

Russian Ministry of Education and Science (grant No. 3N-319-09).

REFERENCES

1. L. A. Fedoseeva, G. M. Dymshits, G. S. Yakobson, and A. L. Markel, *Byull. Eksp. Biol. Med.*, **147**, No. 2, 134-138 (2009).
2. O. P. Cherkasova and V. I. Fedorov, *Vestn. TGU*, No. 21, 167-168 (2006).
3. S. Billet, S. Bardin, S. Verp, *et al*., *J. Clin. Invest*., **117**, No. 7, 1914-1925 (2007).
4. L. A. Jr. De Luca, Z. Xu, G. H. Schoorlemmer, *et al*., *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **282**, No. 2, R552-R559 (2002).
5. T. C. Herzig, S. M. Jobe, H. Aoki, *et al*., *Proc. Natl. Acad. Sci. USA*, **94**, No. 14, 7543-7548 (1997).
6. Y. A. Lee, C. S. Liang, M. A. Lee, and K. Lindpaintner, *Ibid*., **93**, No. 20, 11,035-11,040 (1996).
7. A. L. Markel, L. N. Maslova, G. T. Shishkina, *et al*., *Development of the Hypertensive Phenotype: Basic and Clinical Studies. Handbook of Hypertension*, Eds. R. McCarty *et al*., Amsterdam (1999), pp. 493-526.
8. T. Morgan, *Hypertens. Res*., **26**, No. 5, 349-354 (2003).
9. K. Nagata, K. Obata, J. Xu, *et al*., *Hypertension*, **47**, No. 4, 656-664 (2006).
10. Y. Naito, T. Tsujino, Y. Fujioka, *et al*., *Ibid*., **40**, No. 6, 827-833 (2002).
11. G. Nickenig, U. Laufs, P. Schnabel, *et al*., *Br. J. Pharmacol*., **121**, No. 1, 134-140 (1997).
12. H. Schunkert, V. J. Dzau, S. S. Tang, *et al*., *J. Clin. Invest*., **86**, No. 6, 1913-1920 (1990).
13. H. Schunkert, H. W. Hense, M. Muscholl, *et al*., *Heart*, **77**, No. 1, 24-31 (1997).
14. K. C. Wollert and H. Drexler, *Cardiovasc. Res*., **43**, No. 4, 838-849 (1999).
15. X. Zhao, R. White, J. Van Huysse, and F. H. Leenen, *J. Hypertens*., **18**, No. 9, 1319-1326 (2000).