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Renin System of the Kidney in ISIAH Rats with Inherited Stress-Induced Arterial Hypertension

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The renal renin system was studied in ISIAH rats with inherited stress-induced arterial hypertension. The expression of genes for renin (Ren1) and cyclooxygenase (Cox-2) was evaluated in renal tissue of ISIAH and WAG rats (normotensive control). Basal gene expression for Ren1 and Cox-2 in ISIAH rats was much lower than in WAG rats. Water deprivation for 11 h was followed by a 4-fold increase in Cox-2 gene expression in ISIAH rats. The increase in gene expression was insignificant in WAG rats (by 30%). Renin gene expression in renal tissue of ISIAH and WAG rats remained practically unchanged after water deprivation. We conclude that a change in Cox-2 gene expression after short-term water deprivation serves as a reliable criterion for functional strain of the renal renin system in hypertensive ISIAH rats.

Key Words: *ISIAH rats; renal renin system; water deprivation; renin; cyclooxygenase*

Previous studies revealed that ISIAH rats with inherited stress-induced arterial hypertension are characterized by hyperfunction of the hypothalamic-hypophyseal-adrenocortical [3] and sympathoadrenal system [1]. Transcriptional activity of genes encoding corticotropin-releasing hormone and pro-opiomelanocortin in the hypothalamic-hypophyseal-adrenocortical system was shown to increase under resting and stress conditions [9]. The glucocorticoid response increases during exposure to several stress factors (*e.g.*, zoosocial factors). ISIAH rats are probably characterized by suppression of the negative feedback (decrease in the expression of hippocampal genes for glucocorticoid receptors), which contributes to the increase in stress reactivity of hypertensive animals. As regards the sympathoadrenal system, epinephrine concentration increases in the adrenal glands of ISIAH rats. Plasma norepinephrine concentration during mild emotional stress (handling) is higher in ISIAH rats than in WAG rats (normotensive control). ISIAH rats have the increased content of mRNA for tyrosine hydroxylase, which serves as a key enzyme for catecholamine biosynthesis in the adrenal glands and several brain regions (bodies of noradrenergic neurons, *Locus coeruleus*).

By contrast, functional activity of the renin-angiotensin-aldosterone system (RAAS) decreases in these specimens. For example, renin activity in the plasma and renal tissue tends to decrease in ISIAH rats [6]. During repeated measurements, plasma aldosterone concentration in ISIAH rats was reduced or did not differ from that in normotensive WAG and Wistar rats. Angiotensin-converting enzyme (ACE) activity in lung tissue and blood plas-

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ma of ISIAH rats did not differ from that in normotensive animals [5]. Under stress conditions, ISIAH rats are characterized by a greater increase in lung ACE activity (restriction stress) and plasma aldosterone concentration (mild stress of blood loss). These data indicate that the response of RAAS to certain stimulatory factors is preserved in ISIAH rats. This system is probably involved in the formation of stress-induced arterial hypertension.

In the present work, renin gene expression was measured in the renal cortex. We studied the gene for cyclooxygenase Cox-2 (Ptgs2, prostaglandin-endoperoxide synthase 2), whose function in renal tissue is closely related to renin biosynthesis. The gene for Cox-2 is expressed in renal cells of the *macula densa*, adjacent convoluted tubules of the ascending Henle's loop, and interstitial cells. Our experiments revealed that Cox-2 plays a role in stimulation of renin synthesis and secretion by cells of the juxtaglomerular apparatus in the kidneys.

MATERIALS AND METHODS

Experiments were performed on 4-month-old male hypertensive ISIAH rats and normotensive WAG rats. The control group consisted of intact animals (7 specimens of each genotype) that were narcotized with ether and decapitated. The remaining animals (7 specimens of each genotype) were decapitated after stress of water deprivation, which lasted from 19.00 to 12.00. These rats had free access to food. Control specimens had free access to water.

Total RNA was isolated from the renal cortex by the standard method. The tissue sample was homogenized in a mixture of water-saturated phenol (10:1 by tissue volume) and 0.5% sodium dodecyl sulfate (5:1 by tissue volume) using an ice bath. Sodium acetate (2 M, pH 4.2, $^1/_8$ of mixture volume) was added. Incubation was performed at 0°C for 5 min. The homogenate was put in plastic tubes (1.5 ml) and centrifuged for 15 min (Eppendorf Centrifuge 5414, 12,000 rpm, 9000*g*). The aqueous phase was placed in clean tubes and extracted two times with an equal volume of a phenol-chloroform mixture (1:1 v/v) and equal volume of pure chloroform. After each session of extraction, the aqueous phase was removed by centrifugation at 9000*g* for 5 min. RNA was precipitated by treatment with 2.5-3 volumes of 96% ethanol and subsequent incubation at -70°C for 30 min.

Admixtures of genomic DNA were removed by DNase I (Promega) according to the manufacturer's recommendations. This treatment was followed by repeated extraction with a phenol-chloroform mixture and pure chloroform and precipitation with ethanol (as described previously). After centrifugation, the precipitate of RNA was dissolved in deionized water. The quality of RNA was determined by electrophoresis in 1% agarose gel. RNA concentration was evaluated from optical density at 260 nm.

RNA (10 mg) and hexamer primers (random N_6, 0.5 mg) were mixed in 11 ml water to obtain cDNA. The mixture with reverse transcriptase (19 ml) was added after denaturation of RNA (65°C, 5 min) and annealing of primers (37°C, 5 min). The final solution consisted of reverse transcription buffer (20 mM Trtis-HCl, pH 8.3; 10 mM dithiothreitol; 100 mM KCl; and 5 mM $MgCl_2$), 500 mM deoxynucleozide triphosphates, and 40 U reverse transcriptase MoMLV (Biosan). cDNA was synthesized as follows: at 37°C for 1 h; at 42°C for 30 min; and at 50°C for 10 min. The enzyme was inactivated by heating of this mixture at 75°C for 5 min. The polymerase chain reaction (PCR) was performed with 0.25-0.50 ml cDNA.

Equal volumes (3 ml) of cDNA samples were taken and mixed to prepare "standard" cDNA. The standard solution was used to construct calibration curves. The relative content of cDNA for target genes and reference gene in experimental samples was evaluated from calibration curves.

Real-time PCR was performed in the presence of SYBR Green I (Molecular Probes) using an iCycler iQ4 amplifier (Bio-Rad Laboratories) to evaluate

TABLE 1. Primers

Gene name	Primer sequence	T_{ann}
Ren1-f	5' — CAC TCA TCA AGG GCT CTG TGT C — 3'	66°C
Ren1-r	5' — TCT ATA CAG AGT TCG ACC GGC A — 3'	
Cox-2-f	5' — GCC ACC TCT GCG ATG CTC T — 3'	62°C
Cox-2-r	5' — GTG TTT GGG GTG GGC TTC A — 3'	
Rpl30-f	5' — ATG GTG GCT GCA AAG AAG AC — 3'	62 or 66°C*
Rpl30-r	5' — CAA AGC TGG ACA GTT GTT GG — 3'	

Note. Annealing of primers for the reference gene and target gene was performed at the same temperature.

the expression of genes for renin and Cox-2. The "housekeeping" Rpl30 gene for ribosomal protein L30 was used as a reference gene. The sequence of primers is shown in Table 1. The reaction mixture (20 ml) consisted of the standard buffer for PCR (67 mM Tris-HCl, pH 8.9; 16 mM $(NH_4)_2SO_4$; 0.01% Tween 20; and 10 mM b-mercaptoethanol), $MgCl_2$ (3 mM for Rpl30 and Ren1; and 4.5 mM for Cox-2), 0.2 mM dNTPs, SYBR Green I (dilution 1:20,000), 10 nM fluorescein, primers (150 nM for Rpl30 and Cox-2; and 450 nM for Ren1), and 0.4 U Taq polymerase (Institute of Cytology and Genetics). The reaction was performed under the following conditions: preheating at 95°C for 3 min; 5 "long" cycles (denaturation at 95°C for 30 sec; annealing for 30 sec (Table 1); and elongation at 72°C for 60 sec); and 35 main cycles (denaturation for 20 sec; annealing for 20 sec; elongation at 72°C for 30 sec; and data acquisition from fluorescence of Rpl30 (84°C, 10 sec) and target samples (Ren1 and Cox-2 at 88 and 86°C, respectively, for 10 sec). The specificity of this reaction was estimated from melting curves after PCR. Samples of test cDNA with primers for the target gene (4 repeats for the cDNA sample), samples with primers for the reference gene (4 repeats), and standard cDNA (1:1-to-1:16 dilution) with the same primers (2-3 repeats) were placed to one plate in each experiment. PCR was performed at least two times for each sample of cDNA. The basal content of test cDNA was evaluated from standard calibration curves (relative to standard cDNA). cDNA concentration for the target gene was divided by cDNA concentration for the reference gene. This ratio reflected the difference between gene expression in animals of various strains, as well as in intact and treated specimens of the same strain.

RESULTS

A control study showed that the basal mRNA level for genes of renin and Cox-2 is much lower in ISIAH rats. Stress (11-h water deprivation) had little effect on renin gene expression in animals of both strains. However, this treatment was followed by a significant increase in Cox-2 gene expression. These changes were more pronounced in ISIAH rats than in WAG rats (increase by 4 times and 30%, respectively). After stress exposure, the concentration of Cox-2 mRNA in ISIAH rats was higher than in WAG rats. It should be emphasized that Cox-2 mRNA level in ISIAH rats was 2-fold lower than in WAG rats under resting conditions.

These data indicate that functional activity of the Cox-2 gene is a reliable criterion for the state of the renal renin system. Stress-induced variations in the Cox-2 gene occur in an earlier period than the modification of renin gene expression. Previous experiments showed that plasma renin activity in rats increases after 24-h water deprivation. These changes are accompanied by a slight increase in renin activity in the renal cortex [4]. Studying the dynamics of gene expression suggests that the basal activity of the renal renin system is suppressed in ISIAH rats. However, activity of this system increases significantly under stress conditions. The initial stage of this process includes an increase in Cox-2 gene expression. Our results are consistent with published data [4]. Basal renin activity in the kidneys of ISIAH rats was much lower than in WAG rats. However, interstrain differences in renin activity were not observed after stress exposure. Renin activity increased in ISIAH rats, but remained unchanged in WAG rats [4].

To evaluate the regulatory role of Cox-2 in renin secretion, plasma renin activity in rats was

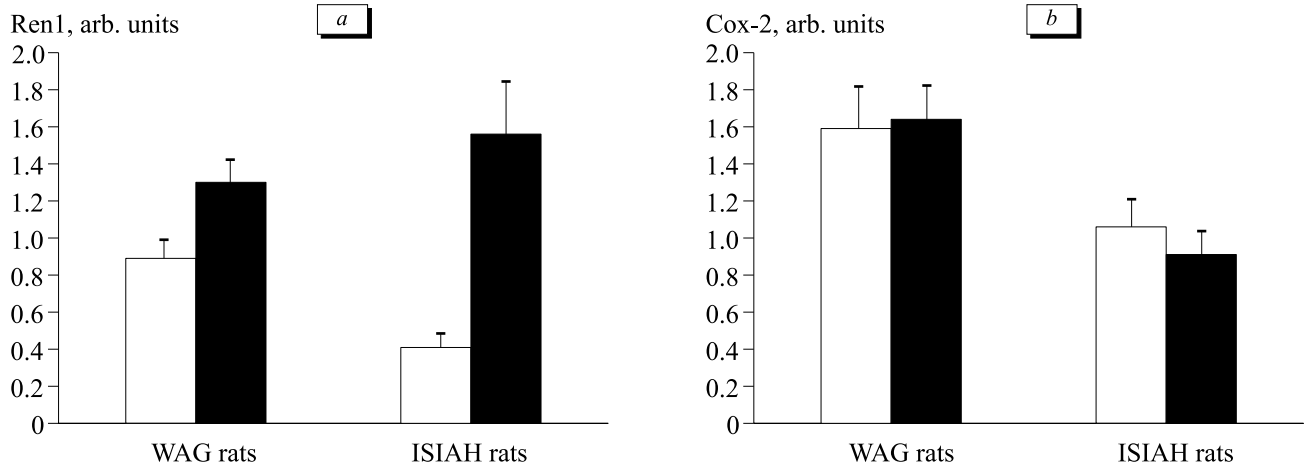

Fig. 1. Expression (mRNA) of genes for Ren1 (*a*) and Cox-2 (*b*) in the renal cortex of normotensive WAG rats and hypertensive ISIAH rats under resting conditions (light bars) and during stress (dark bars; *M*±*SE*, *n*=7 in each experimental series).

measured upon treatment with an ACE inhibitor after specific blockade of Cox-2 activity. Blockade of ACE is usually followed by a significant increase in renin secretion. However, these changes are not observed after the inhibition of Cox-2 [11]. The inhibition of Cox-2 was also accompanied by suppression of renin secretion under a low-salt diet and renovascular hypertension. Cox-2 blockade had an inhibitory effect on renin secretion in the isolated perfused preparation of the juxtaglomerular apparatus from rats [8]. According to the results of an immunohistochemical study, renin is absent in renal tissue of rats with deletion of the Cox-2 gene [12]. However, Cox-2 expression in renal tissue was shown to decrease upon the reduced volume of extracellular liquids and impairment of water-electrolyte balance [13]. Hence, the regulatory system for expression and function of renal Cox-2 is an important sensitive mechanism of the regulation and activity of the renal renin complex.

Our experiments demonstrated that the basal activity of the renin system is low in hypertensive ISIAH rats. These data should be considered from the viewpoint of low-renin arterial hypertension. A pathogenetic mechanism for the "low-renin" state during arterial hypertension remains unknown. A change in water-salt metabolism (hypervolemia and hypernatremia) and adverse effect of elevated blood pressure (negative feedback mechanism) probably play a role in these changes [7]. In the latter case, blood pressure elevation should be mediated by the mechanisms that do not depend on renal renin. In ISIAH rats, these mechanisms probably include an increase in activity of the sympathoadrenal and hypothalamic-hypophyseal-adrenocortical system.

A similar decrease in the basal activity of the renal renin system was found on other experimental models of arterial hypertension (SHR and LH rats). LH rats are believed to be a typical model of "low-renin arterial hypertension" [10]. Activity of the renal renin system in SHR rats is increased over a short ontogenetic period. Aging and development of the hypertensive state in these animals are accompanied by a decrease in renal renin (renin gene transcription and enzyme activity). These data suggest that an increase in functional activity of the renal renin system is an essential stage in the development of the hypertensive state during early ontogeny. In the follow-up period, blood pressure remains high with no increase in renin secretion in the kidney. This state is probably typical of ISIAH rats [2]. Previous experiments showed that blockade of the renin system in these specimens during early ontogeny is accompanied by the delayed development of the hypertensive state and smaller increase in blood pressure with aging.

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