Structural Characteristics of Renal Glomerular System in ISIAH Rats under Conditions of Chronic Stress and Preventive Hypotensive Drug Therapy

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The effects of chronic stress on the structure and function of the renal glomerular system were studied in hypertensive ISIAH rats treated with hypotensive drugs during the early ontogeny. The morphometric parameters of the renal glomerular system, characteristic of each of the experimental animal groups treated by enalapril, losartan, or terazosin in early age virtually did not change under conditions of stress exposure. These results indicate a persistent delayed nephroprotective effect of these drugs.

Key Words: *arterial hypertension; hypotensive drugs; chronic stress; kidney; morphometry*

The development of arterial hypertension is paralleled by the development of pathological shifts in the kidney, primarily in its glomerular system, leading to the development of renal failure. The armory of drugs used in clinical medicine, reducing BP level and alleviating the manifestations of renal disease, is constantly enlarged. An important problem (particularly in terms of prevention of hereditary arterial hypertension) is to attain a stable hypotensive effect after preventive antihypertensive therapy in early age. Our experiments on ISIAH hypertensive rats showed that enalapril or losartan blockade of the renin-angiotensin-aldosterone system (RAAS) during the 2nd month of life leads to a lasting (up to 4 months) reduction of BP and prevent the development of pathological changes in the renal glomerular system [1,3]. Experiments with terazosin (α-adrenoreceptor blocker) gives similar results, but only if the drug is injected during earlier age.

We studied the preventive effects of these drugs under conditions of experimental chronic stress by their effects on the structure and function of the renal glomerular system, the most important functional system of the organ, after chronic stress under conditions of preventive hypotensive therapy.

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MATERIALS AND METHODS

The study was carried out on 4 groups of ISIAH rats, 5 males per group. Rat pups of the experimental groups received daily oral hypotensive drugs in fine suspension in 0.25-0.50 ml water. Group 1 received enalapril maleate (angiotensin-converting enzyme blocker; KRKA) in a dose of 25 mg/kg on days 28-58 of life. Group 2 rats received losartan (angiotensin receptor I blocker; E.I. du Pont de Numours & Co.) in a dose of 10 mg/kg on days 28-58 of life. Group 3 animals received terazosin hydrochloride (α1-adrenoreceptor blocker; Abbott Lab) in a dose of 2 mg/kg on days 20-30 of life. Controls (group 4) received water (placebo) on days 28-58 of life.

At the age of 6 months, all the animals were subjected to chronic stress (daily 60-min immobilization in a small cage for 10 days). Systolic BP was measured in all animals by the indirect (tail cuff) method at rest and after chronic stress under slight rausch narcosis in order to rule out the impact of mental stress.

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Experiments were carried out with due consideration for humanity philosophy recorded in the European Community Directive (86/609/EC). After experiments the rats were sacrificed by guillotine under ether narcosis in accordance with Regulations on Studies on Experimental Animals.

Kidney specimens for morphological studies were fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% osmium tetroxide, dehydrated, and embedded in epon and araldite mixture. Diameters and volume density of capillaries in the cortical matter, volume and numerical density of renal glomeruli were evaluated in semithin sections of the liver stained by Toluidine blue at ×640. Ultrathin sections contrasted with uranyl acetate and lead citrate were examined under a JEM-100SX electron microscope. Ultrastructural stereomorphometric analysis of cellular and other than cellular components of the renal glomeruli was carried out on the negatives obtained at ×8000 using a square test lattice (72 points) with a 8-μ step and a ruler with a 0.2-μ division value. The data were statistically processed using Statistica 6.0 software. The results were presented as the means and errors in the means ($M \pm m$). The significance of differences was evaluated by Student's t test for $p<0.05$.

RESULTS

In the controls (placebo) aged 6 months, the basal BP was significantly elevated (190±8 mm Hg; Fig. 1). Chronic stress in these animals led to elevation of BP to 205±5 mm Hg. Basal pressure in experimental rats treated with enalapril, losartan, or terazosin in the early ontogenesis was significantly lower than in the controls. The reactions to chronic stress were different in animals of different experimental groups. Chronic stress led to a significant elevation of BP after preventive losartan and terazosin treatment, but its level remained lower than in the control. Enalapril completely blocked the BP reaction to chronic stress. Hence, BP was lower under conditions of stress in all animals treated with hypotensive drugs than in the controls (Fig. 1).

Morphological studies showed that chronic stress exposure caused no acute structural disorders in the kidneys, but resulted in an increase of the volume of the capillary bed in the renal cortical matter in all the rats after placebo and hypotensive treatment (Table 1). Importantly that the structural characteristics of the renal glomerular system, typical of each experimental group at rest [1-3], virtually did not change in stress.

The renal glomeruli in the control ISIAH rats exposed to chronic stress retained the structure characteristics of the hypertensive status, similar to those

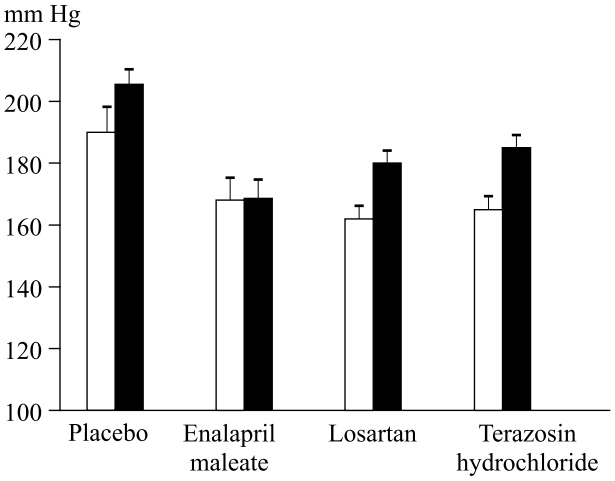

Fig. 1. Effect of chronic stress on BP of ISIAH rats after preventive hypotensive treatment. Light bars: at rest; dark bars: chronic stress.

described previously in adult rats of this strain [2]. The glomeruli were enlarged in comparison with those in normotensive Wistar rats, the glomerular capillaries were sharply stenosed or, vice versa, dilated; the mesangial volume was enlarged, basal membranes were thicker, hyperplasia of the membrane structures was seen in the podocytes, the cytopodias were flattened and the length of their contacts with the basal membrane was greater (Fig. 2). The complex of these signs indicated hemocirculatory disorders in the renal glomeruli, high functional strain of podocytes, and the initial stages of glomerulosclerosis.

A trend to normalization of some major morphometric parameters was noted in the renal glomeruli of experimental rats treated with antihypertensive drugs (enalapril, losartan, or terazosin) during the prepubertal period and exposed to chronic stress, similarly as in rats under common conditions [1,3] (Table 1). The diameters of the renal glomeruli were smaller in rats

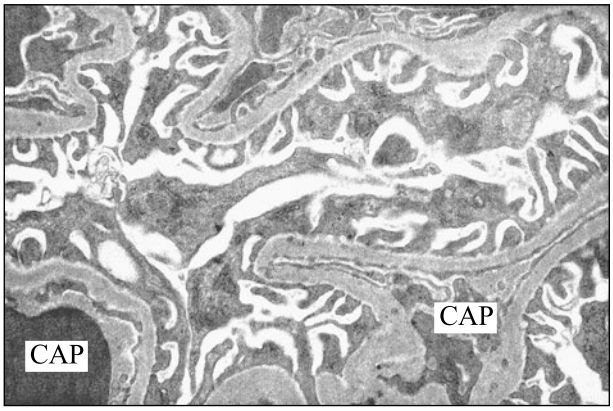

Fig. 2. Electronogram of the renal glomerulus of ISIAH rat, control (chronic stress after placebo), ×15,000. Initial signs of glomerulosclerosis. Signs of hemocirculation disorders, some capillaries (CAP) are stenosed, others are dilated and have erythrocytes in the lumen. Basal membranes are thick.

TABLE 1. Morphometric Parameters of the Renal Glomeruli of ISIAH Rats after Chronic Stress Following Preventive Antihypertensive Drug Therapy in Comparison with Wistar Rats (*M*±*m*)

Parameter	Wistar, 6 months	Placebo	Enalapril	Losartan	Terazosin
N_A of glomeruli per mm^2	7.6±0.4	6.14±0.64	6.5±0.8	6.42±0.57	6.26±0.48
Glomeruli, V_v	6.15±0.38	6.39±0.66	6.11±0.79	5.81±0.60	6.6±0.7
Diameter of glomeruli, μ	102.5±1.7	112.45±2.62*	101.22±2.52$^+$	99.09±4.45$^+$	106.31±2.80
Capillaries in cortical matter, V_v	6.24±0.25	6.86±0.54	6.93±0.43	7.06±0.35	7.13±0.67
Podocytes, V_v	33.52±1.38	35.39±1.58	28.00±1.26$^+$	26.23±1.05$^+$	25.37±1.25$^+$
Endotheliocytes, V_v	14.46±1.0	13.86±0.99	13.29±1.07	11.33±0.72	14.66±1.19
Urinary space, V_v	11.07±0.51	9.86±0.61	10.01±0.58	13.60±0.70	11.31±0.80
Capillary lumen, V_v	18.19±1.20	14.28±1.38	17.81±1.44	14.25±1.01	12.30±1.32
Mesangium, V_v	6.80±0.73	9.76±1.18*	8.86±0.92	8.16±0.89	7.96±0.97
Basal membranes, V_v	8.48±0.39	13.39±0.48*	11.49±0.42$^+$	11.56±0.41$^+$	12.21±0.50$^+$
Width of basal membrane, nm	198.30±5.64	297.28±13.89*	247.13±9.36$^+$	253.36±10.09$^+$	261.54±11.60$^+$
Length of cytopodia contacts with basal membrane, nm	366.60±31.40	660.09±62.48*	575.41±48.22	543.03±44.69	580±47

Note. N_A: numerical density; V_v: volume. $p<0.05$ in comparison with: *Wistar rats, $^+$ISIAH rats receiving placebo.

treated with enalapril and terazosin, these values being intermediate between those in the controls and Wistar rats. Moreover, this parameter in rats treated with losartan was virtually the same as in normotensive rats. The rats of these groups were also intermediate by such parameters as the length of the podocyte process contacts with the basal membrane and the width of basal membranes. The volume of basal membranes in the glomeruli of rats of these experimental groups was significantly less than in control animals. We regarded these structural changes in the renal glomeruli, indicating reduction of signs of the glomerular filter dysfunction, as an evidence of the nephroprotective effect of preventive hypotensive therapy [1,3]. On the other hand, the renal glomeruli in all these animals retained to a certain measure the structure characteristic of the renal hypertensive status, for example, manifest mesangium or signs of podocyte hypertrophy (Fig. 3). These data indicate a delayed nephroprotective effect of hypotensive drugs (enalapril, losartan, terazosin), used in the prepubertal period in ISIAH rats and persisting under conditions of chronic stress.

More rapid glomerular filtration under conditions of stress is realized due to higher intraglomerular pressure. Clinical studies showed a more pronounced vasoconstrictor effect of a stress exposure in hypertensive patients (similarly as in elderly people), this effect being longer than in normal subjects and associated with more pronounced morphofunctional disorders in the glomeruli [5,9].

The data accumulated by the present time indicate that antihypertensive treatment reduces the negative aftereffects of stress. The results of comparative analysis of the efficiencies of drugs with different mechanisms of action are particularly interesting in this respect. A clinical study in hypertensive patients showed hypotensive effects of terazosin and enalapril not only at rest, but in test stress as well. The efficiencies of these drugs were similar [4]. However, of the drugs alleviating the negative aftereffects of stress in hypertension, the renin-angiotensin system inhibitors

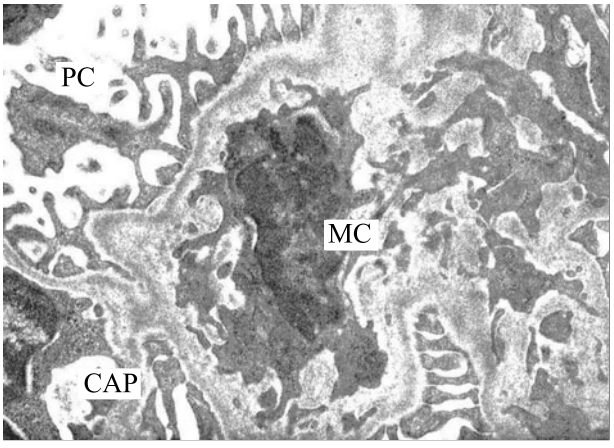

Fig. 3. Electronogram of the renal glomerulus of ISIAH rat, chronic stress after preventive enalapril, ×15,000. Normal structure of podocyte (PC) and its cytopodias. Mesangiocyte (MC) with numerous processes occupies the greater part of mesangium.

have attracted special attention in recent studies. The involvement of this functional system in stimulation of lipid peroxidation in the kidney in stress-associated conditions has been demonstrated [10]. Angiotensin II stimulates NADH-oxidase [7] and hence, the production of peroxide compounds and oxidative stress. Olmesartan treatment prevents this stress reaction, reduces the signs of sclerosis and apoptosis in the renal glomeruli. It has been found [8] that angiotensin II in SHR hypertensive rats plays a more significant role in the development of stress reaction than in normotensive Wistar rats and that AT1 receptor blockade with losartan stimulates the activity of antioxidant enzymes in hypertensive rats. Moreover, other authors hypothesized a direct antioxidant effect of losartan on the renal mitochondria [6]. These authors attribute renal dysfunction in SHR rats to changes in the mitochondrial oxidative capacity. Losartan alleviates these shifts, which (as the authors think) can be explained by the favorable antistress effect of the drug.

Our experiments showed that preventive administration of hypotensive drugs such as RAAS blockers enalapril and losartan and α-adrenoreceptor blocker terazosin young hypertensive ISIAH rats produced not only the delayed hypotensive effect, but also pronounced nephroprotective effect.

Virtually all morphometric parameters of the renal glomerular system, in particular, the main functional components of the filtration barrier (endotheliocytes, podocytes, and basal membranes) were preserved after chronic stress in animals of experimental groups. The fact that chronic stress caused no significant destructive changes in the kidneys and virtually did not modify the quantitative characteristics of the main structural components of the glomerular system in animals treated with losartan, enalapril, or terazosin during the early ontogeny suggests that the delayed nephroprotective effect of these drugs persisted under conditions of chronic stress.

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