Structure of Renal Glomeruli in Hypertensive NISAG Rats Reared by Normotensive Females

M. D. Shmerling, E. E. Filyushina, V. A. Lazarev, I. I. Buzueva, A. L. Markel'*, and G. S. Yakobson

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 131, No. 6, pp. 713-716, June, 2001
Original article submitted February 26, 2001

Morphometry of ultrastructural components of renal glomeruli in 3-week- and 6-month-old rats with hereditary stress-induced arterial hypertension (NISAG rats) reared by normotensive Wistar females showed deceleration of the development of arterial hypertension in NISAG rats and attenuated its negative consequences for kidneys as the target organs.

Key Words: *hypertension; cross rearing; renal glomeruli; ultrastructure*

It is known that the onset and progression of arterial hypertension (AH) depend on inherited predisposition and environmental conditions. Experiments on animals with hereditary AH help to clarify the mechanisms of this pathology and the modulatory effects of environmental factors. NISAG rats characterized by mild stress-induced AH developing in mature animals represent a model of hereditary AH [4,9].

Recent studies demonstrated that environmental conditions during early ontogeny can affect the formation of hypertensive status in hypertensive animal strains or even prevent it [2,7,10,11]. In particular, it was shown that cross-rearing modulate realization of genetically determined program of AH development in NISAG rats [2].

Kidney is simultaneously the target organ and the organ involved in the regulation of blood pressure (BP) and plays an important role in the pathogenesis of hypertension. Morphofunctional changes in the kidneys, in particular, in their glomerular system, reflect the stages of the disease and, therefore, can serve as morphological criteria of the stage, severity, and prognosis of the disease. Previous studies demonstrated considerable structural changes in the kidneys of NISAG rats (typical of hypertensive kidneys) in comparison with those of Wistar rats [3].

For evaluation of the effect of environmental factors on structural parameters of the target organs in animals with inherited AH we examined the renal glomerular system in NISAG rats reared by normotensive Wistar rats.

MATERIALS AND METHODS

Experimental groups consisted of 3-week- and 6-month-old NISAG rats (n=6) reared by Wistar rats; age-matched Wistar and NISAG rats (n=6 in each group) not subjected to cross-rearing served as the control. In adult animals BP was measured on the tail by sphygmography. The rats were sacrificed under ether narcosis. Kidney specimens were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, postfixed in 1% OsO_4, dehydrated, and embedded in epon-araldite mixture. The diameter and numerical density of renal glomeruli were evaluated on toluidine blue-stained semithin sections. Ultrathin sections were contrasted with uranyl acetate and examined under a JEM-100SX electron microscope. Stereophotometric analysis [1] of cellular and non-cellular components of renal glomeruli was performed. The data were processed statistically using Statgraphics 4.0 software. Significance of differences was estimated by Student's t test.

Institute of Physiology, Siberian Division of Russian Academy of Science; *Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Medical Science, Novosibirsk. ***Address for correspondence:*** m.d.shmerling@iph.ma.nsc.ru. M. D. Shmerling

0007-4888/01/1316-0605$25.00

RESULTS

In 3-week-old NISAG rats reared by Wistar females, most parameters of renal glomeruli little differed from those in age-matched Wistar and NISAG rat reared by their mothers (Table 1) except parameters characterizing the connective tissue components, *i.e.* the relative volumes of basal membranes and mesangium, which were higher in hypertensive rats compared to normotensive controls. In experimental animals these parameters decreased compared to control NISAG rats. Thus, the relative volume of basal membranes in experimental rats did not differ from normal, while the relative volume of the mesangium had intermediate values between values found in Wistar and NISAG controls (Table 1).

The data on increased content of the mesangial components in renal glomeruli of NISAG rats (most pronounced in control and less pronounced in experimental rats) agree with previous reports [8]. Immunochemical studies of fibronectin and collagen IV contents in renal glomeruli of 4-week-old SHR rats (*i.e.* before the onset of AH) revealed intensive proliferation of mesangial cells and disturbances in some biosynthetic processes, in particular, in fibronectin synthesis. These changes in proliferative and biosynthe-

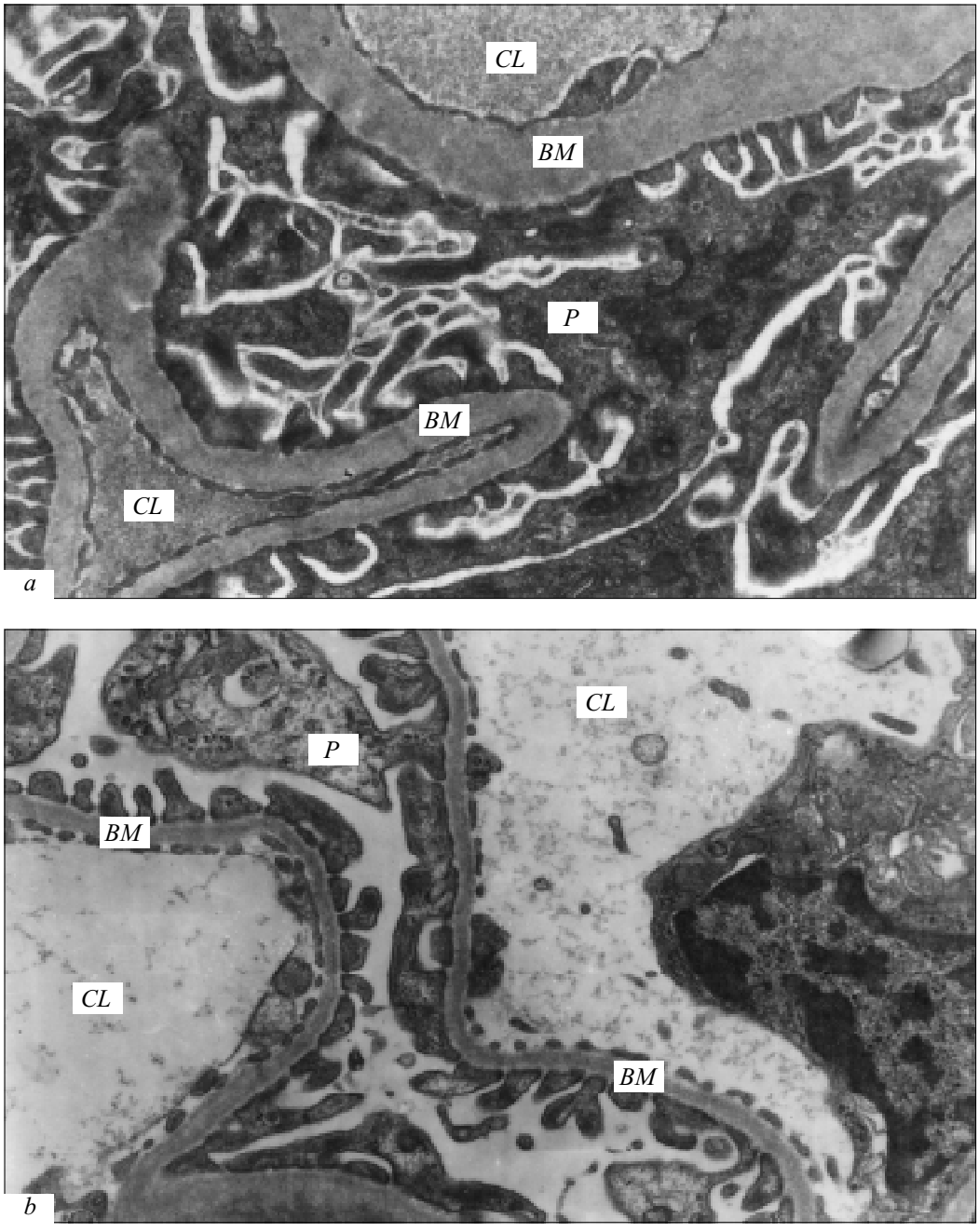

Fig. 1. Renal glomerulus of adult NISAG rats reared by NISAG (*a*) and Wistar females (*b*), ×15,000. *a*) narrowing of capillary lumen (*CL*) and thickening of basal membrane (*BM*). Podocytes (*P*) are characterized by short processes and more lengthy contacts with BM; *b*) structure of glomerular component is close to normal.

TABLE 1. Morphometric Parameters of Renal Glumeruli in Wistar and NISAG Rats of Different Age (*M*±*m*)

Parameters	3 weeks			6 months		
	Wistar	NISAG	NISAG-1	Wistar	NISAG	NISAG-1
Numerical density of glomeruli per 1 mm^2	17.1±0.9	16.6±1.2	17.8±0.4	7.6±0.4	6.9±0.4	8.5±0.6$^+$
Relative volume of glomeruli, %	5.51±0.27	5.98±0.39	5.23±0.56	6.15±0.38	6.51±0.38	5.33±0.42
Glomerulus diameter, μ	57.6±0.8	61.2±1.1	62.57±1.47	102.5±1.7	112.7±1.6*	99.3±2.7$^+$
Relative volume, %						
podocytes	33.12±1.21	30.78±1.39	31.18±1.10	33.50±1.38	35.90±1.24	33.30±1.13
capsular space	9.02±0.53	7.58±0.45*	10.59±0.56$^+$	11.10±0.51	12.90±0.77*	10.10±0.45$^+$
endotheliocytes	5.73±0.88	16.46±1.00	17.44±1.01	14.50±1.01	10.1±0.7*	16.10±0.84$^+$
capillary lumen	17.45±1.07	5.86±1.78	16.86±1.15	18.2±1.2	15.90±1.08	16.80±1.01
mesangium	3.26±0.64	7.44±0.89*	5.80±0.68*	7.16±0.71	9.25±0.76*	7.37±0.76
basal membranes	5.86±0.42	8.31±0.40*	6.30±0.27$^+$	8.48±0.39	12.20±0.44	9.93±0.34$^+$
Thickness of basal membranes, nm	140.50±4.35	155.00±6.58	152.20±6.72	198.30±5.64	292.60±13.09*	12.90±6.48$^+$
Length of podocyte processes-basal membrane contacts, nm	221.80±8.99	218.1±12.0	243.20±14.29	366.6±31.4	530.2±41.7*	378.6±20.3$^+$

Note. NISAG-1: NISAG rats reared by Wistar mothers. $p<0.05$: *compared to Wistar rats or $^+$normally reared NISAG rats.

tic processes of mesangial cells represent inherited abnormalities, rather than the consequence of increased BP, while the development of AH was related to predisposition to glomerular pathology.

Our experiments revealed similar mechanisms in NISAG rats. Increased content of mesangial components revealed in 3-week-old NISAG rats (experimental rats and controls) was not associated with increased BP. However, this hypothesis does not explain the reduced content of mesangial components in NISAG rats reared by Wistar females. This probably reflects the effect of environment factors on the realization of genetic program.

Sustained AH induced by mild stress developed in 6-month-old NISAG rats reared by their mothers. In NISAG rats reared by Wistar females BP increase was less pronounced (151.0±4.3 mm Hg compared to 160.0±3.3 and 130.0±3.5 mm Hg in control NISAG and Wistar rats, respectively).

Electron microscopy of kidneys from experimental and control rats revealed significant structural changes in the glomerular system. In contrast to control NISAG rats characterized by glomerulal hypertrophy, in experimental rats this parameter did not differ from that of Wistar rats (Table 1). Morphometric parameters of capillaries (relative volume of endotheliocytes and capillary diameter), podocytes, and the length of contacts of podocyte processes with the basal membrane were also close to normal. It should be noted that the length of podocyte processes—basal membrane contacts was higher in control hypertensive rats, which indicated decreased efficacy of filtration barrier of glumerular capillaries [5,6].

Connective tissue component of renal glomeruli in cross-reared rats was less pronounced than in controls. In adult NISAG rats increased content of mesangial components, enhanced proliferation of mesangiocytes, and thickening of the basal membranes (Fig. 1, *a*) indicated initial sclerotic changes in renal glomeruli. However, these changes were absent in rats reared by normotensive females (Fig. 1, *b*), while the relative volumes of mesangial components and basal membrane, as well as the thickness of the basal membrane did not differ from those in normotensive animals (Table 1).

Comparative analysis showed the absence of structural signs of renal glomerular hypertrophy, changes in blood circulation, functional strain of podocytes, and initial signs of glomerulosclerosis in NISAG rats reared by Wistar females. These findings suggest that despite AH developing in adult NISAG rats reared by normotensive Wistar mothers, its negative consequences for kidneys as the target organs were less pronounced compared to normally reared NISAG rats, probably, due to more effective adaptation and compensatory reactions of the organism.

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry* [in Russian], Moscow (1990).
2. S. Ya. Amstislavskii, N. K. Popova, Yu. E. Tomilova, *et al., Fiziol. Zhurn.*, **84**, No. 8, 783-789 (1998).
3. N. P. Kazarinov, M. D. Shmerling, A. L. Markel', and G. S. Yakobson, *Byull. Eksp. Biol. Med.*, **127**, No. 5, 576-579 (1999).
4. A. L. Markel', *Izvestiya AN SSSR*, No. 3, 466-469 (1985).
5. E. P. Mel'man and B. V. Shutka, *Kidney Morphology* [in Russian], Kiev (1988).

6. V. A. Titova, I. I. Pavlenko, A. Yu. Balakina, *et al., Byull. Eksp. Biol. Med.*, **127**, No. 3, 347-350 (1999).
7. M. A. Cierpial and R. McCarty, *Behav. Neural Biol.*, No. 3, 262-270 (1991).
8. J. B. Lopes de Faria, D. Zoukhri, and M. Lorenzi, *Kidney Int.*, **52**, No. 2, 387-392 (1997).
9. A. Markel, *Genet. Hypertension*, **218**, 405-407 (1992).
10. R. McCarty and C. Fields-Okotcha, *Physiol. Behav.*, No. 5, 839-844 (1994).
11. R. McCarty and J. H. Lee, *Ibid.*, No. 1, 71-75 (1996).