Changes in Inducible NO Synthase in the Pial Arteries of Different Diameters in Hypertensive Rats

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Biomicroscopy of pial arteries of branching orders 1-5 in Wistar rats with induced renovascular hypertension revealed a common trend to vasoconstriction. Up to week 4 of arterial hypertension the diameters decreased mainly in arterial branches of orders 1-2, while at weeks 8-10 the reaction was significantly more manifest in branches of orders 4-5. During weeks 12-16, vessels with solitary club deformations or several alternating dilated and stenosed portions appeared in rats with renovascular hypertension. Immunocytochemical studies of the vessels showed hyperexpression of inducible NO synthase in these deformations. The enzyme activity was also detected in leukocytes in the vascular lumen and in perivascular tissue, as well as in cells fixed to the inner endothelial surface.

Key Words: *inducible NO synthase; pial arteries; arterial hypertension*

Nitric oxide (NO) plays an important role in the maintenance of adequate bloodflow. It is formed in the endothelium and serves as the signal molecule in the cardiovascular system. Three NO synthase isoforms are involved in its synthesis: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS), catalyzing production of NO from L-arginine into [5]. The expression of eNOS in the endothelial cells is stable; it maintains the basal level of NO production and some its functions, including endogenous vasodilatation [11].

The possibility of iNOS involvement in vascular reactions is disputed. According to some data, NO formed under the effect of iNOS (about 1000-fold more than in eNOS expression) plays the key role in suppression of bacterial and tumor cell activities, while its effects on vascular endothelium is negligible [3]. Other authors have noted pronounced vascular effects of iNOS, while the development of sustained arterial hypertension is attributed to changes in activity of this enzyme [2]. The expression of iNOS in the vascular wall in vascular disease has been proven: in myocytes, but not in the endothelium [9]. That is why its effects, as the authors suppose, are less pronounced in large arteries with well-developed tunica muscularis. However, according to other data, iNOS activity manifests only in the small parenchymatous vessels exclusively under conditions of impaired structure of the endothelium [13].

We studied reactivity of pial arteries of different diameters and changes in iNOS activity in them during the development of renovascular hypertension (RVH) in rats.

MATERIALS AND METHODS

The study was carried out on 30 adult male Wistar rats (200-240 g) with induced RVH [7] 4, 6, 8, 10, 12, and 16 weeks after the operation. Six sham-operated male Wistar rats kept under the same conditions served as the controls. The study was carried out on the middle cerebral artery branches of branching orders 1-5 by biomicroscopy [8] and immunocytochemical methods [14]. The rats were narcotized with urethane (125 mg/100 g). Systolic BP was measured by ML U/4c501 system (MedLab) for noninvasive monitoring of blood pressure in rats by the tail cuff method. The BP values

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in the controls were 118.5±5.7 mm Hg, in rats with RVH 138.4±6.4 mm Hg during week 4 postoperation, 151.7±7.4 mm Hg during week 6, 166.3±6.5 mm Hg during week 8, 164.2±6.4 mm Hg during week 10, 167.6±7.4 mm Hg during week 12, and 158.8±8.4 mm Hg during week 16.

In order to detect iNOS, the preparations were incubated during 15-17 h at ambient temperature with rabbit polyclonal antibodies to TH (Chemicon; 1:200) and to iNOS (Cayman; 1:200). After washing the material was incubated during 1 h at ambient temperature in biotinilated goat antiserum to rabbit IgG (Vectastain) in 1:200 dilution. Buffered saline with 0.03-0.10% Triton X-100 and 1-3% normal goat serum served as the solvent. The sections were then incubated during 1 h with avidin-biotinilated horseradish peroxidase (ABC, Vectastain) in 1:50-1:100 dilution in buffered saline with 0.1% Triton. Horseradish peroxidase activity was detected in 0.03% diaminobenzidene (Sigma) solution with 0.01% H_2O_2 in 0.05 M Tris buffer (pH 7.6).

The diameters of arteries and number of cell adhesion acts per mm vessel length were measured by Allegro-MC automated image analysis system [1].

The significance of differences between the groups was evaluated by Student's *t* test. The differences were considered significant at $p<0.05$.

RESULTS

Biomicroscopy showed that the pial arterial network in the controls formed as a result of dichotomic division of the main trunk into smaller branches of the first-fifth branching orders (Fig. 1, *a*) – short and more or less even portions of vessels with diameters of 118.0±6.1 μ (first order branches) to 14.0±2.3 μ (fifth order branches). No iNOS was detected in the blood cells of the arterial branch walls in these animals (Fig. 2, *a*).

The pial arteries developed a common trend to vasoconstriction in RVH. The degree of vasomotor reaction depended on the diameter of an artery and time elapsed after the intervention (Fig. 3). Up to week 4 of RVH development the decrease of the diameter (by 5-8%) was observed mainly in the first-second order arterial branches, while on week 6 the diameters of smaller arteries decreased by about the same value. During weeks 8-10 the reaction of the fourth-fifth order branches was more intense, their diameters decreasing by 28-37%, while in the first-third order branches the decrease was no higher than 7-14%. These changes in the structure of arteries were paralleled by a decrease in the density of pial vascular network (Fig. 1, *b*). It was noted not once that stenosis of pial arteries in

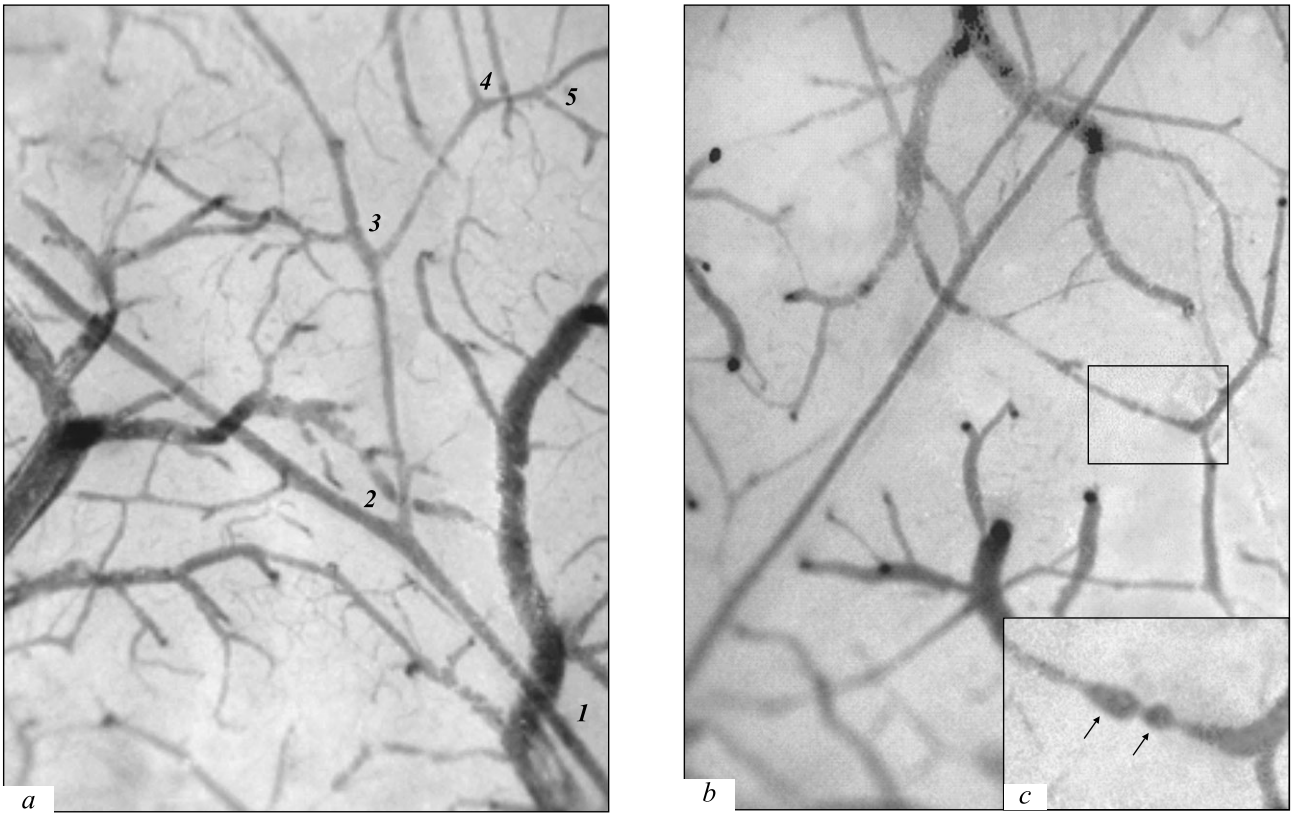

Fig. 1. Biomicroscopy of the rat brain pia mater. *a*) pial arterial network of a control animal (*1-5*: arterial branches of respective orders); *b*) week 10 after intervention; *c*) destructive changes in a vessel, the sausage phenomenon; ×100 (*a*, *b*), ×200 (*c*).

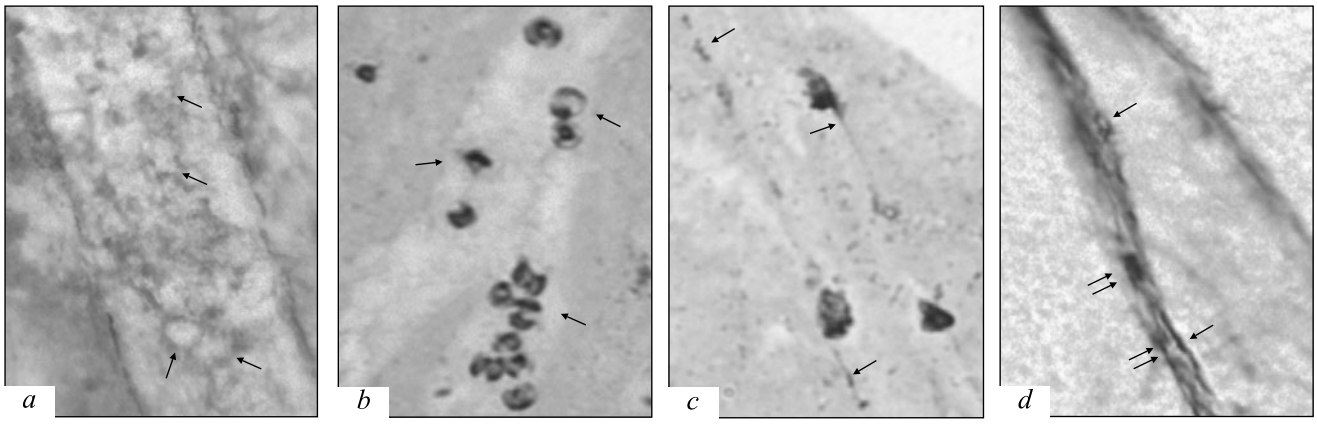

Fig. 2. iNOS in the rat pia mater. *a*) no iNOS in vascular wall and leukocytes (arrows: leukocyte shadows in vascular lumen); *b*) iNOS in leukocytes (arrow) in vascular lumen, week 6 postoperation; *c*) iNOS in leukocytes fixed to apical surface of endothelium (week 10), arrows show iNOS in endothelium; *d*) iNOS in endothelium (arrows) and vascular tunica muscularis (double arrows). Immunocytochemical method. ×100 (*a*, *b*), ×200 (*c*), ×400 (*d*).

hypertension developed as a result of autoregulatory processes maintaining constant cerebral bloodflow at high BP [7]. According to modern data, eNOS plays the key role in intricate mechanisms of this reaction. The enzyme provides the production of NO which, in turn, stimulates the endothelium-dependent component of the vasomotor reaction and some other protective vascular effects, preventing leukocyte adhesion and platelet aggregation [12].

The diameters of arteries did not change much during weeks 12-16 of RVH development in comparison with the previous period (Fig. 3). Vessels (usually of small diameters) with signs of destruction appeared (Fig. 1, *c*). Solitary club-shaped swelling or several alternating dilated and stenosed deformations of different size and shape were seen along these vessels. This phenomenon was called "sausage" [4] and its development could be due to stimulation of iNOS in vascular wall, paralleled by a significant and lasting elevation of NO level, sharp vasodilatation, and increase of vascular permeability [10].

Immunocytochemical methods detect iNOS in the pial arterial wall not before week 10 of RVH development. However, enzyme activity has been detected in some leukocytes in the vascular lumen as early as during week 6 (Fig. 2, *b*). The counts of these cells increase significantly between weeks 8 and 10, often causing obturation of the small branches lumen and inhibiting or arresting the bloodflow in them. During week 10 the iNOS labeling precipitate is deposited in leukocytes fixed to the apical surface of the endothelium, and iNOS is stimulated in these sites of endothelial lining (Fig. 3, *c*). Leukocyte adhesion is usually observed in the fourth-fifth order arterial branches and rarely in larger vessels. The number of leukocyte adhesions during this period is not high, no more than 6-8 per mm vessel length. During weeks 12-16 of RVH the count of leukocytes with the enzyme activity increases significantly, as well as of adhesion cases (more than 3-fold). During this period iNOS is expressed also in perivascular leukocytes. The most intense deposition of the reaction product is detected in sites of local dilatation of the arterial wall, where it labels the endothelium and sometimes the muscle cells (Fig. 3, *d*). The activity of iNOS in other sites of the vascular bed is not so high.

Our data indicate that the expression of iNOS is linked with not only leukocytes in vascular lumen and perivascular tissue, as other authors have noted [9,10], but also with sites of arterial wall deformation, where enzyme activity is especially high, which promotes disorders in the endothelial adhesive characteristics and increases endothelial permeability. These

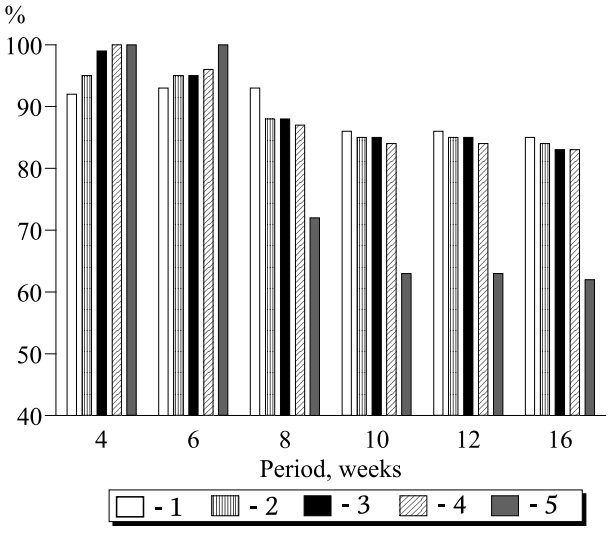

Fig. 3. Changes in diameters of pial arteries of branching orders 1-5 at different terms after surgery. The diameter of arterial vessels of respective order in control animals is taken for 100%.

events initiate further expression of iNOS with subsequent development of inflammatory reaction and deeper involvement of the endothelium [10]. In other words, dysfunctional and destructive changes in the pial hemocirculation system under conditions of stubborn arterial hypertension are largely caused by iNOS stimulation induced by disorders in NO synthesis in the vascular endothelium.

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