Age-Associated Characteristics of Vasomotor Regulation of the Pia Mater Arteries in Rats

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The endothelium-dependent and myogenic reactions of pia mater arteries of the 1st-5th branching orders were studied in 1-, 3-, and 24-month-old rats by biomicroscopy method. The endothelium-independent (myogenic) reaction predominated in the 1st-3rd order branches and the endothelium-dependent vascular reaction in the 5th order branches of 3-month-old rats. Both regulatory mechanisms were equally developed in the 4th order branches. In 1-month-old rats, the endothelium-dependent reaction was more active in the majority of branches. In 24-month-old rats, this reaction was significantly higher than the endothelium-independent one only in the 4th and 5th order branches. In contrast to 3-month-old rats, the myogenic reaction of 24-month-old animals predominated in the 1st and 2nd order branches and the endothelium-dependent one in the 4th and 5th order branches. Vascular reactivity of 24-month-old rats was lower than in younger rats in all cases.

Key Words: *biomicroscopy; pia mater arteries; age-associated characteristics; bloodflow regulation*

The regulatory mechanisms of the pia mater arteries often attract the attention of scientists [3,4]. The vascular endothelium is now more and more often assumed to play the key role in hemodynamic regulation [10,13]. However, other data suggest that the role of some regulatory mechanisms increases and that of others decreases with decreasing the diameters of pial arteries with their branching [3,8]. Presumably, the significance of the mechanisms regulating vascular functions is changing with age, though no proofs supporting or denying this hypothesis were obtained until present.

We studied age-associated characteristics of vasomotor regulation of pial arteries of different diameters in rats.

MATERIALS AND METHODS

The study was carried out on outbred albino rats of 3 age groups: 1) young (1 month), 2) adult (3 months), and 3) old (24 months). The animals were narcotized by intramuscular nembutal (5 mg/100 g). The rats were then fixed in a stereotaxic stand for head fixation in the horizontal plane. The 1st-5th branching order arterial vessels of the pia mater were examined by reflected light biomicroscopy through a 1 cm^2 opening in the cranial parietal area. The dura mater in the opening was removed and the brain was covered with a transparent film protecting it from drying and edema. At least 5 animals of each age group were examined.

The endothelium-dependent and endothelium-independent (myogenic) vascular reactions were studied by pharmacological tests as described previously [14]. The endothelium-dependent vasodilatation (EDVD) was evaluated by acetylcholine test, endothelium-independent vasodilatation (EIVD) by the nitroglycerine test. Vascular reactions to acetylcholine or nitroglycerin were evaluated 5 min after injection of L-NAME (NO-synthase blocker; 2 mg/kg) into the femoral vein. Arterial reactivity was evaluated using Allegro-MC automated system for image analysis by changes in the vascular diameters before and after treatment. The

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method of quantitative biomicroscopy was previously described in detail [1]. For more convenient comparison of vasomotor reactions of the vessels in different age groups, the changes in the diameters of the respective branching orders were expressed in percent of their initial values taken as 100%.

The results were processed by methods of variation statistics. 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 universal organization of the arterial network in the pia mater in the middle cerebral artery basin in rats of all age groups. Branches of the 1st-5th orders originate from the main artery forming as a result of successive dichotomic division. Part of the blood volume flows into the thickness of the brain via the precortical arterioles, the other part is shunted due to numerous anastomoses with the adjacent vessels without getting into the intracerebral network. This organization of pial vessels provides rapid redistribution of the blood and adequate blood supply to neurons in case of local changes in the functional activity of the brain [3,4].

Acetylcholine test showed a significant dilatation of arteries ($p<0.05$) in animals of all age groups (Fig. 1, *a*). The intensity of the reaction increases in arteries with increasing their branching order and decrease of their diameters (Fig. 2, *a*). The vasomotor reaction was more pronounced in young animals than in rats of other age groups, which was seen from greater dilatation of the arteries in response to acetylcholine (Fig. 2, *a*; 3). The vasomotor characteristics of group 1 rats were observed irrespective of arterial branching order or the initial diameter of arteries. Our results are in line with the data according to which NO synthesis is the most active in young age, because during this period NO is involved in not only vascular tone regulation, but also in proliferative activity of smooth muscle cells [11,15]. This characteristic of NO becomes par-

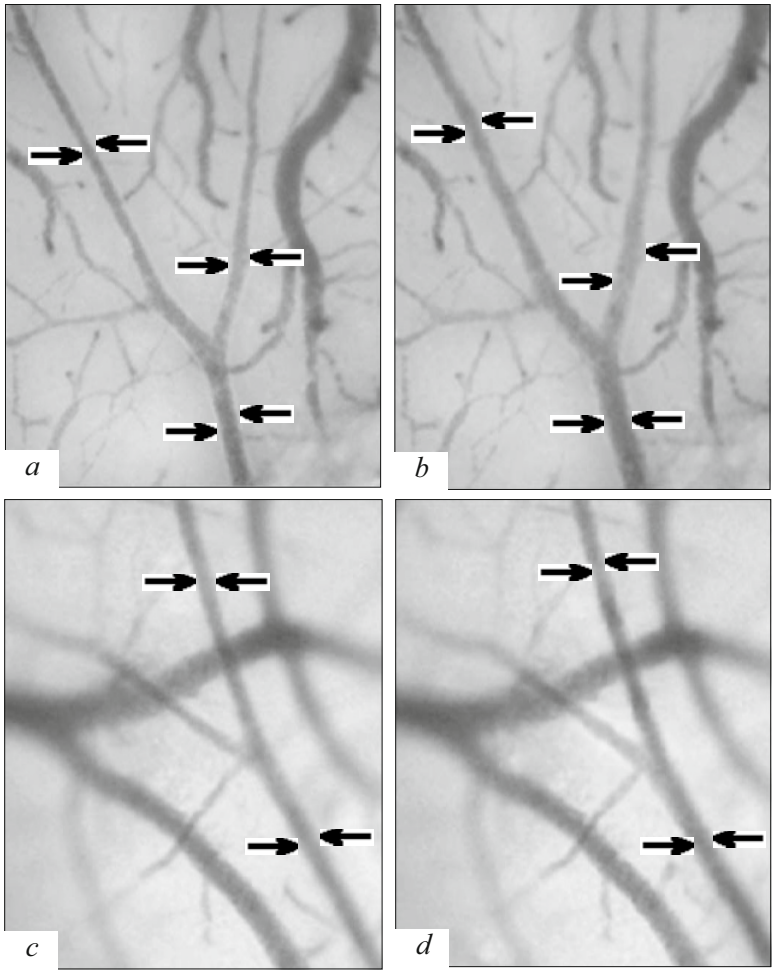

Fig. 1. Arterial branches of the 2nd-3rd branching order (arrows) in the pia mater of adult rats before (*a*, *c*) and after acetylcholine (*b*) and nitroglycerin (*d*). Biomicroscopy, ×80.

ticularly important during the period of structural transformations of the vascular wall. With aging the percentage of this regulatory molecule in the vascular wall decreased [12], which is presumably a factor contributing to reduced vasomotor reaction in old animals (Figs. 2, *a*; 3). The parameter characterizing EDVD in group 3 rats is reduced greater than in group 2.

The reaction to intravenous L-NAME generally tended to vasoconstriction. The reaction was more pronounced in vessels with lesser diameter in rats of all age groups (Fig. 2, *b*). It is clear that reduction of the

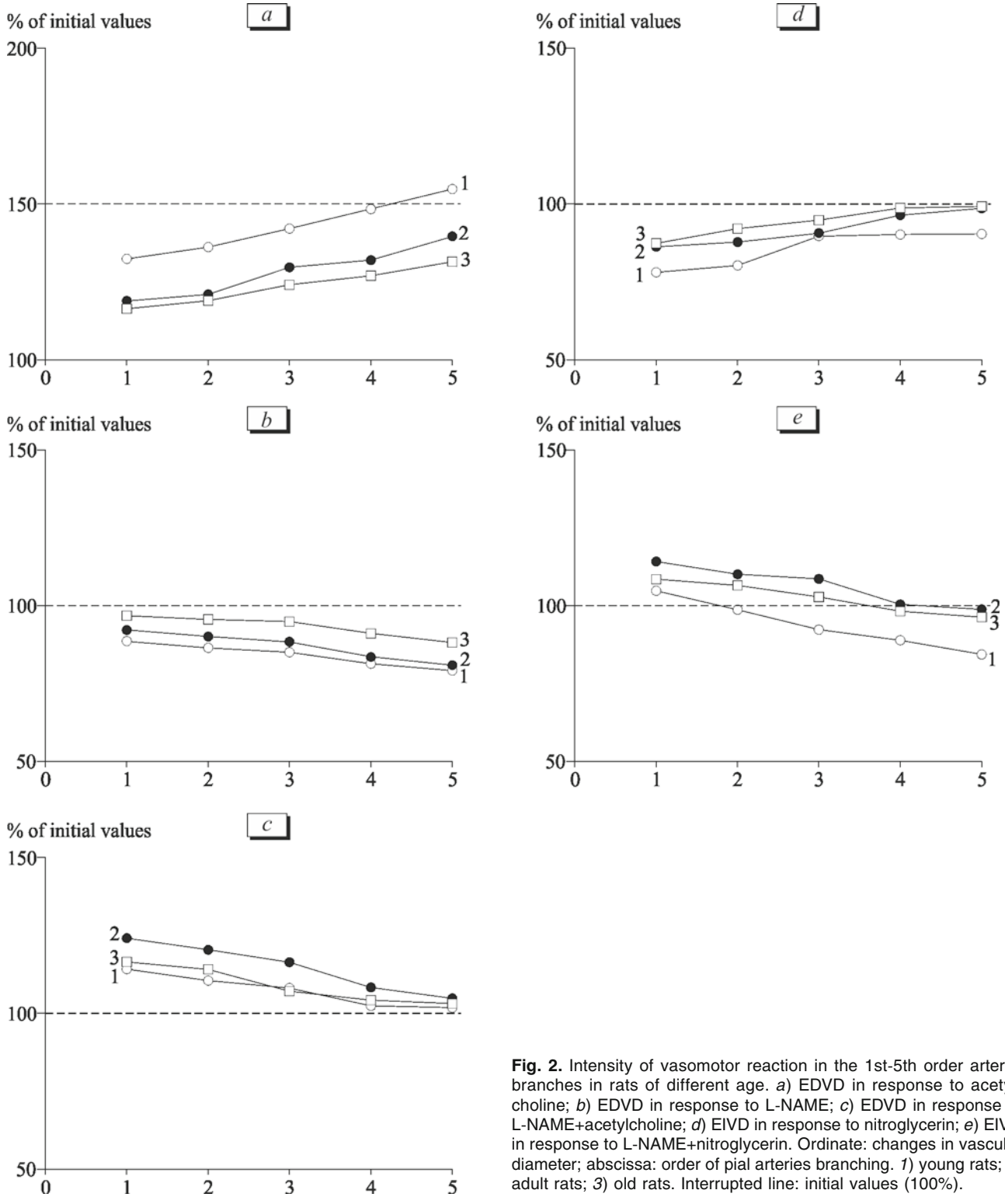

Fig. 2. Intensity of vasomotor reaction in the 1st-5th order arterial branches in rats of different age. *a*) EDVD in response to acetylcholine; *b*) EDVD in response to L-NAME; *c*) EDVD in response to L-NAME+acetylcholine; *d*) EIVD in response to nitroglycerin; *e*) EIVD in response to L-NAME+nitroglycerin. Ordinate: changes in vascular diameter; abscissa: order of pial arteries branching. *1*) young rats; *2*) adult rats; *3*) old rats. Interrupted line: initial values (100%).

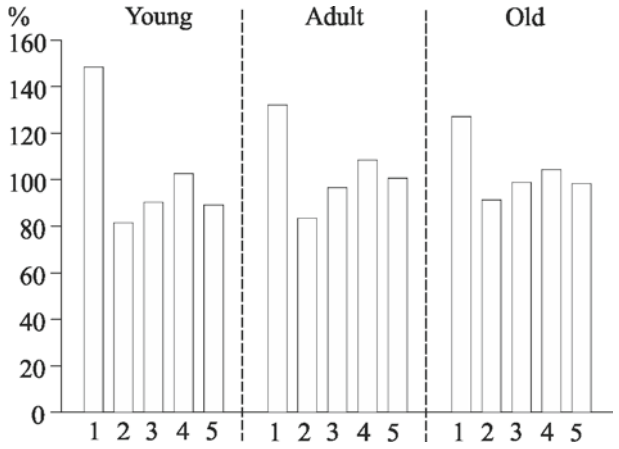

Fig. 3. Diameters of 4th order vessels in animals of different age. *1*) EDVD in response to acetylcholine; *2*) EDVD in response to L-NAME; *3*) EDVD in response to L-NAME+acetylcholine; *4*) EIVD in response to nitroglycerin; *5*) EIVD in response to L-NAME+nitroglycerin. Ordinate: vasomotor reaction.

concentration of so potent a vasodilator as NO, commonly leveling the effects of vasoconstrictors, causes an increase in the tone of resistant vessels and of blood pressure [5,7]. The contribution of endothelium-dependent mechanism to vasomotor changes is different in vessels of different diameter. The resistance of pial vessels to NO-synthase blocker increases mainly at the expense of thin vascular branches with limited vascular component and more active endothelium. Changes in vascular reactivity to this treatment were more pronounced in young compared to adult and old rats. The 1st and 2nd order arteries in group 3 rats were least sensitive to NO-synthase inhibitor. The diameters of these arteries decreased by 3-4% *vs.* 9-12% for the 4th and 5th order branches ($p<0.05$).

Vascular reaction to acetylcholine evaluated at the peak of L-NAME effect differed significantly from that to any of these substances alone (Figs. 2, *a-c*; 3). Though the diameters of the arteries remained less than in the control in all cases, these differences did not exceed 3-7% for the 1st and 2nd order arterial vessels ($p<0.05$) compared to virtually complete blockade of vascular reaction to acetylcholine by NO-synthase blocker in the 4th and 5th order branches (Fig. 2, *c*). Vasoconstriction in group 1 animals was more pronounced than in animals of groups 2 and 3 for all vessels. Hence, L-NAME failed to cancel acetylcholine-induced vasodilatation of all pial arteries (which disagrees with some authors [2,15]), but was effective only for the smallest vessels. Our data indicate that NO produced *in vivo* in the endothelium of larger arteries is not the only mediator in realization of acetylcholine-induced vasodilatation.

Nitroglycerin-induced EIVD in adult rats was clearly seen mainly in the 1st-3rd order branches with a well-developed muscular layer. The vasomotor reactions of smaller vessels were virtually negligible or did not manifest at all (Figs. 1, 2, *d*). This is explained by the fact that the effect of nitroglycerin depends on NO release in the vascular smooth muscle cells. As an NO donator, nitroglycerin stimulates guanylate cyclase and elevates the level of cyclic GMP, which leads to relaxation of the arterial smooth musculature [7,9]. As lesser amounts of NO are formed in the arteriolar walls with a thin unstable layer of smooth myocytes, normally the arterioles and precapillary sphincters relax less intensely than the large arteries. In group 1 rats, a pronounced reaction to nitroglycerin in nitroglycerin test was observed only for the 1st order branches ($p<0.05$), in which vasodilatation was rapid, but not so significant as in animals of groups 2 and 3 (Fig. 2, *d*). With increasing the branching order, the vascular diameters change less and less, being just about 2% for the 4th and 5th order branches in comparison with the control ($p>0.05$). The dynamics of vasomotor reactions in old rats is largely similar to that in adult animals. However, a statistically significant increment in the vascular diameters in group 3 rats was less pronounced than in adult animals and was observed only in the 1st and 2nd order branches ($p<0.05$).

Preinjection of L-NAME modified the vascular reaction to nitroglycerin. Vasodilatation was observed only in large vessels in rats of all age groups; however, this dilatation was less manifest than in nitroglycerin test and just slightly surpassed the control values (Fig. 2, *e*). The most clear-cut reaction in adult and old animals was observed for the 1st-2nd order arteries, a slight reaction for 3rd-4th order vessels, and no reaction was detected in the 5th order vessels. In young animals, a slight (about 5%; $p<0.05$) increase of the diameters was detected only for the 1st order branches. In the smaller arteries, the value dropped below the control and differed little from the diameters of the branches of the corresponding branching order in response to the NO-synthase blocker alone (Fig. 2, *b*; 3).

Hence, the "vascular regulation gradient" underlies the vasomotor regulation in the pia mater [4,6] in animals of all age groups; in other words, redistribution of the significance of this or that regulatory mechanisms with changes in the vascular diameters. Myogenic EIDV reaction predominates in the 1st-3rd order arterial branches in adult animals, while the EDVD predominates in the 5th order vessels. Both mechanisms are about similarly developed in the 4th order branches, which corresponds to their role in regulation of the cerebral hemodynamics [3]. A more pronounced EDVD was observed in pial arteries of young animals. In old animals it was significantly higher than the myogenic one only in the 4th and 5th

order branches. In contrast to adult animals, the EIVD in old animals markedly predominated only in the 1st and 2nd order branches and EIVD in the 4th and 5th order vessels. Vascular reactivity of old rats was lower than in animals of other age groups in all cases.

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