Sex Differences in Endothelium-Dependent Reactions of Rabbit Arteries to Elevated Blood Flow

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Acute experiments on anesthetized rabbits were performed to compare dilator responses of the carotid arteries in a wide range of blood flow rates. In males, the reactivity of vessels to changes in blood flow rate was higher than in females. Thirty percents of the revealed sex differences are due to various blood viscosities in males and females. The main factor determining these differences is a higher vascular tone in males.

Key Words: *endothelium; shear stress; vasodilation; blood flow rate*

Vascular occlusion and coronary heart disease are more common in men, than in women [4,12]. The endothelium-dependent and flow-sensitive relaxation of the coronary vessels receives much attention in studies of the pathogenesis of coronary heart disease [4,8,9,12,13].

Here we studied sex differences in endothelium-dependent reactions of rabbit carotid arteries to elevation of blood flow rate using criteria for this type of vascular reactivity.

MATERIALS AND METHODS

Acute experiments were conducted on rabbits weighing 3.5-4.0 kg and narcotized with ketamine (13 mg/kg intravenously, premedication with chlorpromazine). A 4-cm segment of the carotid artery was isolated, the thyroid artery was ligated, and the studied vessel was cannulated (the length was equal to that *in situ*). The blood was withdrawn from a large artery using a perfusion pump and then infused into the studied artery, which was connected via a plastic tube (hydraulic valve) to the tube, through which the blood was reinfused into the large vein. Blood pressure was stabilized as described elsewhere [7]. The method for perfusion and blood pressure stabilization was described previously [6]. The outer diameter of the artery was measured by a contact circular detector equipped with a high-linear capacitance differential transducer.

After 30-min stabilization, the artery was perfused with heparinized blood (nonpulsatile flow, flow rate 7.5 ml/min, transmural pressure 100 mm Hg). Blood flow rate increased in increments of 7.5 ml/min. Flow-dependent vasodilation was recorded to the moment, when the diameter of the artery did not increase in response to the elevation of blood flow rate. The following parameters were analyzed: the increase in vessel diameter; threshold blood flow rate; blood flow rate corresponding to 100% flow-dependent dilation (V_{Q100}); the increase in vessel diameter as a function of blood flow velocity; blood flow velocity corresponding to total vascular relaxation (V_{L100}); the initial diameter of arteries at the zero blood flow rate; and the diameter of vessels corresponding to maximum relaxation (D_{100}). The reaction of arteries was studied in a wide range of blood flow rate.

Flow-induce relaxation of arteries was controlled by their denudation with distilled water for 60 sec.

The results were registered on a KSP-4 automatic recorder equipped with a Shch1413 4-bit digital voltmeter. Blood viscosity was estimated on a VK-4 viscometer.

The results were analyzed by Student's t test.

RESULTS

The elevation of blood flow rate led to an increase in the diameter of the artery. This parameter returned to

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the initial level after normalization of blood flow rate (Fig. 1).

It was shown that the dependence of the artery diameter on blood flow rate is represented by an S-shaped curve (Fig. 2, *a*) [13]. The rise of blood flow rate to 15 ml/min led to a considerable increase in the diameter of the artery. The threshold blood flow rate did not differ between males and females (15 ml/min). Further increase in this parameter above the threshold value was accompanied by an increase in the diameter of the artery, which was proportional to the rise of blood flow rate and reached a plateau at 52.5 ml/min (both in males and females, $p>0.05$). There were no considerable sex differences in V_{Q100} in males and females. During perfusion with a subthreshold blood flow rate, the mean diameter of the carotid artery in males was lower than in females (2.100±0.013 *vs.* 2.300±0.006 mm, $p<0.05$, Fig. 2, *a*). At the same time, we observed no statistically significant sex differences in this parameter under conditions of maximum flow-dependent vasodilation. It is difficult to interpret these data, because the initial diameter of vessels at zero blood flow rate markedly varied, and vasodilation accompanying the increase in blood flow rate led to a decrease in blood flow velocity. Therefore, blood flow rate will not be an appropriate parameter for comparative analysis. The endothelium of arteries responds not to changes in blood flow rate, but to shear stress on the endothelium surface determined by blood viscosity and velocity [7]. Hence, the endothelium-dependent and flow-induced reaction should be evaluated from blood flow velocity (Fig. 2, *b*). This parameter in females and males was 5.77±0.5 and 6.9±0.16 cm/sec, respectively ($p<0.05$). V_{L100} in females and males were 21.2±0.69 and 23.25±1.62 cm/sec, respectively ($p>0.05$). After elevation of blood flow velocity to V_{L100}, the relative increase in the diameter of vessels in males and females was 11.58±1.23 and 7.05±1.72%, respectively ($p<0.001$, Fig. 2, *b*). It should be emphasized that the dependence of vessel diameter on blood flow velocity in males was steeper than in females. For further analysis these curves were standardized by D_{100} and V_{L100} (Fig. 3). The diameter of the carotid arteries in the absence of flow stimulus significantly differed in males and females ($p<0.01$). However, these sex differences disappeared at the maximum blood flow rate. Diameter-velocity curves were also similar in males and females, but in males this dependence was steeper than in females. Physiologically, different artery reactivity to increased blood flow rate in males and females are probably associated with different strength of flow stimuli formed on the endothelium due to differences in blood viscosities, mechanical sensitivity of the endothelium, production of endothelium-derived relaxing factor, and vascular smooth muscle contractility.

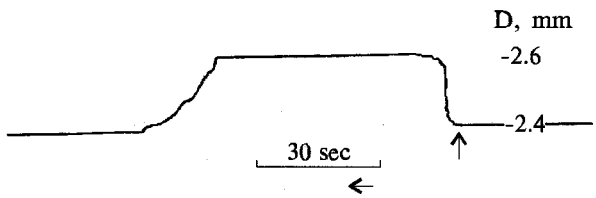

Fig. 1. Typical dilator response to maximum elevation of blood flow rate in the carotid artery. Arrows: the increase in flow rate and direction of recordings on an automatic recorder.

Shear stress is a stimulus triggering the endothelium-dependent and flow-induced reaction in arteries. This force exerted by the flow on a unit of tube lateral surface is proportional to fluid viscosity and flow viscosity [2]:

$$\sigma_t=\eta\times d\omega/dn, \quad (1)$$

where σ_t is shear stress, η is fluid viscosity, $d\omega$ is flow viscosity, and dn is the gradient along the flow normal. Equation (1) shows that at the same increase in flow rate, shear stress is greater in vessels with higher blood viscosity. Blood viscosity significantly differed between females and males (3.40±0.21 and 4.00±0.28 cP, respectively, $p<0.05$). Therefore, the flow stimulus in vessels of males was more intensive than in females. The flow-induced increase in the diameter of arteries in males was higher than in females by 64%. Blood viscosity in females and males differed by 17%. Thus, only 30% of sex differences in the increase in vessel diameter induced by elevated flow rate (the ratio between the percentage of an increase in the diameter of vessels and the difference in blood viscosity) are due to various blood viscosities in males and females. Seventy percents of these differences are related to other factors of flow reactivity.

At subthreshold flow rates, the artery diameter in males is lower than in females (Figs. 2, *a* and 3). A peculiarity of the endothelium-dependent flow-induced regulation is total relaxation of arteries at V_{L100}. Previous studies of biomechanical properties of arteries showed that the diameter of a vessel dilated at the maximum flow rate is equal to the diameter of this vessel treated with high doses of papaverine [5]. Since the mechanism of flow-dependent regulation in vessels can completely exhaust their relaxation capacity [5], this reaction (*i.e.*, the relative increase in the diameter of vessels) reflects the degree of vascular relaxation in the absence of flow stimuli. In a wide range of flow velocities (from the initial level to V_{L100}), differences in the diameter-velocity dependence curves are determined by endothelium-dependent flow-induced relaxation of smooth muscles. Therefore, a smaller diameter of arteries in males at a subthreshold flow

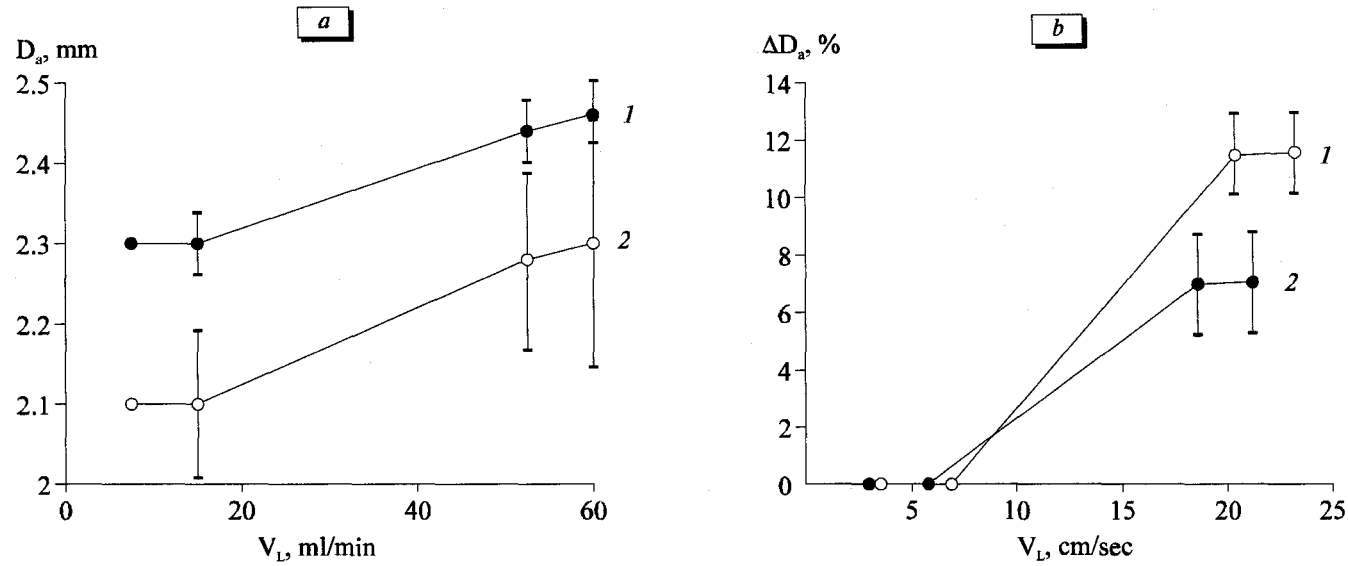

Fig. 2. Changes in artery diameter (D_a) as a function of blood flow velocity (V_L) and rate (V_{vol}). Here and in Fig. 3: females (*1*) and males (*2*).

rate indicates a greater smooth muscle tension (*i.e.*, higher vascular tone). Hence, the initial (zero) vascular tone of vessels in males is higher than in females.

Previous studies of the coronary arteries help to understand the mechanisms of these sex differences in the tone of the carotid arteries [4]. Experimental, instrumental, clinical, and biochemical assays indicate that estrogens (at various routes of administration) decrease the tone of intact and precontracted coronary arteries [9]. Estrogens rapidly change the vascular tone by modulating electric properties of biological membranes and transmembrane ion permeability [1]. Dilation of the coronary vessels induced by steroid and nonsteroid estrogens does not depend on the adrenergic, cholinergic, and histaminergic mechanisms, but is related to the blockade of transmembrane Ca^{2+} transport [13]. Experiments on the coronary arteries showed that estrogens do not modulate the endothelium-dependent formation and release of endogenous vasodilating compounds [8,9]. These data can explain lower vascular tone and slope of the diameter-velocity curves in females compared to males (despite the lower threshold sensitivity to flow rate changes in females).

It was shown that the aortic endothelium in males is more sensitive to prostaglandin $F_{2\alpha}$ [12]. Previous studies revealed no sex differences in the contractility of vascular smooth muscles. Testosterone increases the sensitivity of the vascular endothelium to prostaglandin $F_{2\alpha}$ in female rats, but does not affect the contractility of smooth muscles [12]. Sex differences in the reactivity of arteries to the thromboxane agonist U46619 and other cyclooxygenase products were reported [10]. At the same time, there are no sex differences in the reactivity of arteries to norepinephrine, serotonin, angiotensin, and KCl [11,12].

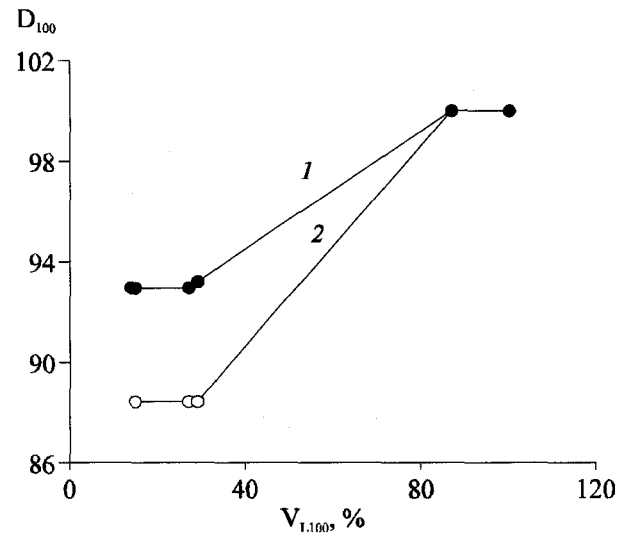

Fig. 3. Standardized diameter-blood flow velocity (V_L) curves. V_{L100}: blood flow velocity corresponding to 100% relaxation of the artery. D_{100}: maximum diameter corresponding to 100% relaxation of the artery.

Hence, estrogens modulate the state of smooth muscles and determine differences in the initial vascular tone and flow-dependent increase in vessel diameter between female and male rabbits.

REFERENCES

1. V. I. Kobrin, M. Manoakh, G. Gol'dberg, *et al.*, *Byull. Eksp. Biol. Med.*, **122**, No. 12, 611-613 (1996).
2. V. B. Kogan, *Theoretical Principles of Chemical Technological Processes* [in Russian], Leningrad (1977).
3. A. M. Mel'kumyants, S. A. Balashov, and S. P. Kartamyshev, *Ros. Fiziol. Zh.*, **82**, No. 4, 93-101 (1996).
4. P. V. Sergeev, A. N. Karachentsev, and A. I. Matyushin, *Kardiologiya*, No. 3, 75-78 (1996).

5. O. V. Filatova, *Topical Problems of Age, Applied, and Ecological Physiology* [in Russian], Barnaul (1991), pp. 102-108.
6. O. V. Filatova and V. D. Kiselev, *Ros. Fiziol. Zh.*, **84**, No. 5-6, 500-506 (1998).
7. V. M. Khayutin, *Vestn. Akad. Med. Nauk SSSR*, No. 6, 89-95 (1987).
8. C. W. Jiang, P. M. Sarrel, D. C. Lindsay, *et al.*, *Br. J. Pharmacol.*, **104**, No. 4, 1033-1037 (1991).
9. C. W. Jiang, P. M. Sarrel, D. C. Lindsay, *et al.*, *Eur. J. Pharmacol.*, **211**, No. 2, 163-167 (1992).
10. J. W. Karanian, F. M. Moran, E. R. Ramey, and P. W. Ramwell, *Br. J. Pharmacol.*, **72**, 10-12 (1981).
11. J. W. Karanian, A. L. Sintetos, F. M. Moran, *et al.*, *Cardiovascular Pharmacology of the Prostaglandins*, New York (1982).
12. T. Maddox, J. G. Falcon, M. Ridinder, *et al.*, *J. Pharmacol. Exp. Ther.*, **240**, No. 2, 392-395 (1987).
13. R. Raddino, E. Poli, G. Pela, and C. Manca, *Pharmacology*, **38**, No. 3, 185-190 (1989).