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Wall Thickness and Constrictive Responses of the Caudal Artery in Rats with Renovascular Hypertension

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In rats with hypertension modeled by the one kidney-one clamp method, constrictory responses of the isolated caudal artery to norepinephrine differed under various perfusion conditions. Vascular reactions in hypertensive rats were more potent at a constant flow rate, and less potent at a constant pressure compared to those in normotensive rats. Previous experiments demonstrated similar changes in constrictory responses of the caudal artery in spontaneously hypertensive rats. It is assumed that these peculiarities of the vascular reactivity during hypertension are determined by thickening of the smooth muscle layer of the vascular wall.

Key Words: *renovascular hypertension; rats; vascular reactivity*

Arterial hypertension is accompanied by thickening of the walls of resistive vessels (RV) [3]. This should lead to elevation of vascular reactivity to constrictory stimuli. However, recent studies showed that vasoconstrictory responses in spontaneously hypertensive rats depend on experimental conditions [7,8]. During constant flow perfusion, the constrictory responses of isolated segments from the caudal artery in hypertensive rats were more potent than in normotensive rats. At the same time, at a constant pressure these reactions in hypertensive rats were less pronounced than in normotensive rats.

It remains unclear whether these peculiarities of the vascular reactivity are typical of spontaneously hypertensive rats, or they can be also observed during secondary hypertension (*e.g.*, renovascular hypertension, RVH). The mechanisms of thickening of RV walls are different in these cases [5,6,11]: hyperplasia of the vascular wall (VW) in spontaneously hypertensive rats [10] and hypertrophia in rats with RVH [5, 11]. If these peculiarities of the vascular reactivity in spontaneously hypertensive rats are determined by thickening of RV walls, but not by the mechanism of this process, vascular reactivity in rats with RVH should undergo similar changes. Here we tested this assumption.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 230-250 g. In Nembutal-anesthetized rats (40 mg/kg intraperitoneally), the left renal artery was clipped with a clamp (inner diameter 0.25 mm), and the right kidney was excised to produce RVH. The animals underwent removal of the right kidney served as the control. Systolic arterial pressure in alert rats was measured by plethysmography before and after surgery (weekly) [2]. Six weeks postoperation, the thickness of the smooth muscle layer in the caudal artery was estimated. Vascular reactivity to exogenous norepinephrine was assessed in perfusion experiments. It is

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known that rat caudal artery is a large muscular vessel similar to RV by its properties.

For morphometry, segments of the caudal artery from experimental and control rats were fixed in 10% buffered formalin (Lillie method), dehydrated, and embedded in paraffin by routine methods. Slices (5-7 μ) were stained with hematoxylin and eosin. The thickness of VW was measured using an Avtandilov triangular grid [1] under a Laborux S. Laica microscope equipped with a video device. The thickness and the diameters of VW in two perpendicular planes were measured. The arteries whose transverse sections had a round shape were examined.

The vessels were perfused with Krebs-Henseleit solution containing (in mmol/liter) 122.22 NaCl, 6.67 KCl, 2.57 $CaCl_2$, 1.25 $MgSO_4$, 25 $NaHCO_3$, 1.18 KH_2PO_4, and 8 glucose (pH 7.35-7.40) and aerated with 95% air and 5% CO_2 for 15 min. A segment of the caudal artery was thermostated at 37°C and perfused with physiological saline using an LKB peristaltic pump at a flow rate of 3.4 ml/min. The perfusion pressure was measured with a DDA-2 transducer. Perfusate flow rate was measured with a flow meter (Transonic Systems Inc.). The data were recorded and processed by an L-card precise analog-digital converter and a computer using original software.

The initial stabilizing perfusion was performed for 40 min, and the reactions to cumulatively increasing concentrations of norepinephrine bitartrate (Serva) were then examined in two perfusion regimens at constant pressure or constant flow (similarly to the experiments on spontaneously hypertensive rats [7,8]). During constant pressure perfusion, the pressure in a solution-containing chamber was set (by air flow) at 120 mm Hg, and norepinephrine-induced changes in the flow rate were recorded. The vessel was then washed for 30 min and perfused at a constant flow rate. The solution was delivered with an LKB peristaltic pump; the flow rate was so adjusted that perfusion pressure without norepinephrine was 120 mm Hg. Under these conditions, norepinephrine-induced vascular contraction elevated the pressure. Changes in the vascular resistance were calculated from the flow rate and pressure.

The results were analyzed by ANOVA.

RESULTS

Experimental (n=7) and control (n=8) animals had similar body weights (424±4 and 407±14 g, respectively). Arterial pressure remained unchanged in control rats. In rats with RVH this parameter increased over the first 4 weeks postoperation and after 5-6 weeks attained 190 mm Hg (50% higher than in the control, Fig. 1).

The thickness of VW in experimental rats and sham-operated animals was 92.4±0.5 and 75.0±1.3 μ,

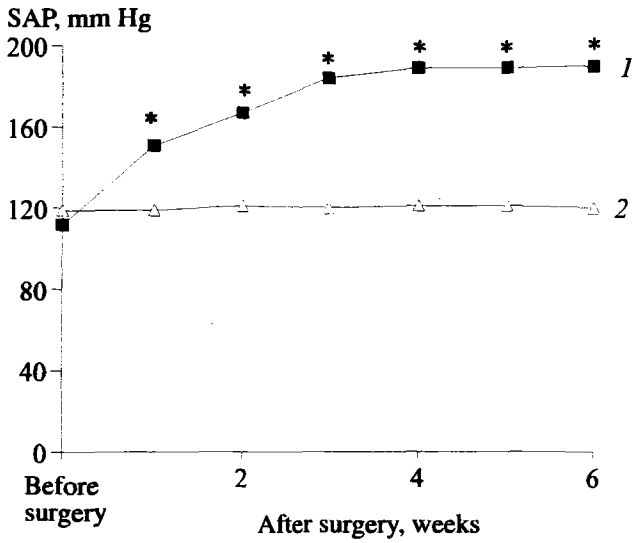

Fig. 1. Changes in systolic arterial pressure (SAP) in rats with renovascular hypertension (*1*) and in control animals (*2*). Here and in Fig. 2: *p<0.05 compared to normotensive rats.

respectively (p<0.05). Hence, the thickness of VW smooth muscle layer in rats with RVH increased, which is consistent with published data [5,11].

In the absence of norepinephrine, perfusate flow rates at 120 mm Hg were practically similar in hyper- and normotensive rats (11.0±2.1 and 11.5±0.5 ml/min, respectively). Reactions of thickened vessels in rats with RVH were different under various perfusion conditions. Vascular contractions in hypertensive rats were more potent under conditions of constant flow perfusion (Fig. 2, *a*) and less pronounced during constant pressure perfusion compared to those in normotensive rats (Fig. 2, *b*). It should be emphasized that the same vessel was perfused in both regimens and, therefore, these differences do not attest to various numbers of norepinephrine receptors in hyper- and normotensive rats.

Thus, changes in the vascular reactivity to norepinephrine were similar in rats with spontaneous hypertension and RVH: vascular reactions were more potent at a constant flow rate and less pronounced at a constant pressure compared to those in normotensive rats. These changes probably attest to different contribution of myogenic reactions (MR) to the contractile response. MR is a contraction of smooth muscles in response to pressure-induced stretching [4]. MR considerably elevates vascular reactions to constrictive stimuli [9]. The contribution of MR to the contractile response depends on the thickness of VW. The thickness or, more specifically, elasticity of VW determines the degree of vascular smooth muscle stretching. Since at the given pressure the stretching of smooth muscle layers in thick-wall vessels is lower than in vessels with normal walls, the myogenic response and, there-

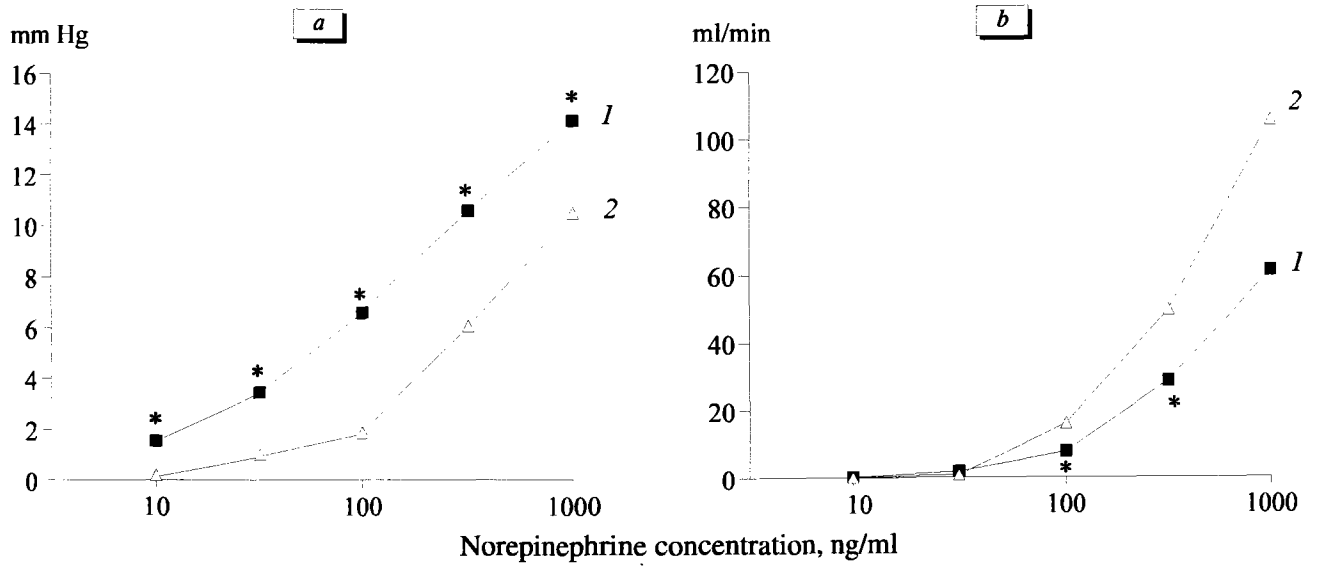

Fig. 2. Elevation of vascular resistance in hypertensive (*1*) and control (*2*) rats in response to cumulatively increasing concentrations of norepinephrine under conditions of constant flow (*a*) or constant pressure perfusion (*b*). Ordinate: changes in vascular resistance.

fore, myogenic potentiation of vascular reactions to constrictive stimuli is less pronounced. This assumption is confirmed by the data showing that after suppression of smooth muscle sensitivity to stretching, constrictive vascular responses in spontaneously hypertensive rats were more potent than in normotensive rats under both perfusion conditions [7,8].

The contribution of MR to the contractile response also depends on the conditions of vascular contractions, which markedly differ at various perfusion regimens. During constant flow perfusion, VW stretching considerably increases and MR activation approaches the maximum. In this case, MR increases the differences in vascular reactivity between hyper- and normotensive rats related to various weights of smooth muscle elements in VW. During perfusion at a constant moderate pressure (120 mm Hg), constrictive responses in hypertensive rats are weakened probably because this pressure is too low for induction of MR in their thick-wall vessels, whereas in normotensive rats we observed considerable myogenic potentiation of norepinephrine-produced vasoconstriction at this pressure.

These data show that VW thickness, but not the mechanism of its thickening (hypertrophy or hyperplasia), plays the major role in various vascular constrictive responses in hypertensive and normotensive rats.

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