Effect of Transmural Pressure on Constrictor Reactions of Caudal Artery in Hypotensive and Hypertensive Rats

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Clipping of the abdominal aorta distally to the renal arteries produces a persistent decrease in blood pressure in hindquarter vessels by 35-40%. On week 6-7 postoperation, the reactions of the caudal artery perfused *in vitro* under constant pressure to norepinephrine were studied. At transmural pressure of 150 mm Hg, the vascular responses in hypotensive rats were reduced compared to those in normotensive control. By contrast, the responses of hypertensive vessels were more pronounced at 75 mm Hg even after deendothelization.

Key Words: *rats*; *norepinephrine; regional hypotension; caudal artery; endothelium*

It is well established that long-term changes in transmural pressure induce structural changes in the vascular bed. The smooth muscle layer of the vascular wall becomes thinner during chronic hypotension [6, 8,9], which modifies the elastic properties of the vascular wall. Therefore, at the same transmural pressure the smooth muscle cells in vessels with different wall thickness are unequally stretched. Stretching of smooth muscle is a basic factor determining constriction capacity of the vessel, because the force developed by smooth muscle depends on its length [2]. Moreover, stretching of the smooth muscle increases its sensitivity to constrictor agents [11,12]. It should be emphasized that vascular reactivity during chronic arterial hypotension is less studied than in hypertensive states. Our aim was to compare the constrictor responses of vessels from hypotensive (HR) and normotensive (NR) rats to norepinephrine (NE) at different transmural pressure.

Chronic changes in arterial pressure affect not only smooth muscles, but also other components of the vascular wall, *e.g.* vascular endothelium [3]. It can be expected that changes in the endothelium-dependent regulation of the vascular tone are manifested not only during arterial hypertension [7], but also during long-term hypotension. Therefore, we studied the role of endothelium in constrictor responses of vessels from HR and NR.

MATERIALS AND METHODS

Experiments were performed on Wistar rats weighing 200-220 g. In nembutal-anesthetized rats (40 mg/kg intraperitoneally) a chronic drop of arterial pressure in hindquarter vessels was produced by clipping of the abdominal aorta distally to the renal arteries with a clamp (lumen diameter 0.25 mm). Sham-operated animals served as the control. The vessels were tested 6-7 weeks postoperation, when body weights of HR and NR were 394±6 g (n=17) and 409±7 g (n=17), respectively. Blood pressure measured in catheterized femoral artery of HR and NR under nembutal narcosis was 70.8±1.3 and 115.5±2.1 mm Hg, respectively (p<0.001).

After decapitation, the ventral caudal artery was isolated. Two metal cannulas (external diameter 0.8 mm) were inserted into both ends of a 7-8 mm vascular segment, which was thermostabilized at 37°C. The vessels were perused and superfused with Krebs—Henseleit solution containing (in mM): 122.2 NaCl,

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6.67 KCl, 2.5 $CaCl_2$, 1.25 $MgSO_4$, 25.0 $NaHCO_3$, 1.18 KH_2PO_4, 8.0 D-glucose, 96% O_2+4% CO_2, pH 7.4.

Perfusion was carried out under constant pressure. The pressure at the inlet and outlet of the vascular segment was measured with a DDA-2 transducers and perfusate flow rate was measured with a flowmeter (Transonic Systems Inc., model T106). Flow-type transducer was placed upstream of the input cannula. The data were recorded and processed with an L-card high-precision digitizer and original software with a sampling rate of 10 Hz.

Pressure difference before and after the vascular segment (50 mm Hg) was created hydrostatic alley. Since the input and output cannulas had equal hydraulic resistance, the transmural pressure was calculated as a mean between the input and output pressures. For modulation of the transmural pressure the input and output pressures were changed simultaneously by the same value (Table 1). The experiments were started after 40-min stabilization perfusion at 75 mm Hg, which resulted in complete relaxation of the smooth muscle. Then the vascular segment was perfused with solutions containing increasing concentrations of NE (Sigma) for 4-5 min per dose until attaining a stable flow rate.

In series I, the responses to NE were examined at 25, 75, 100, and 150 mm Hg. The vessel was washed for 30 min after each tests. In series II, the effect of NE was studied before and after air-stream destruction of the endothelium (30 sec at 75 mm Hg). This procedure completely eliminated the dilatory response to acetylcholine (10^{-6} M). In this series, the transmural pressure was 75 mm Hg.

The equivalent diameter of isolated vessel was calculated by Poiseuille formula:

$$d=\sqrt[4]{\frac{Q\times l}{\Delta P}},$$

where Q is perfusion rate, l the length of the vessel, and ΔP pressure difference before and after the vessel.

The response to NE was assessed by the decrease in its diameter (% of relaxed vessel).

The data were processed statistically using Mann—Whitney and Wilcoxon tests.

RESULTS

The flow rate in experimental (HR) and control (NR) vessels was virtually the same (Table 1). The increase in transmural pressure slightly increased the flow rate, which was probably determined by pressure-induced widening of the vessel.

The reactivity of blood vessels in HR and NR depended on transmural pressure (Fig. 1). There were no differences between groups at 25 mm Hg. However, at 75 mm Hg the vessel diameter in HR decreased in response to NE to a greater extent than in NR, which attests to higher reactivity of HR vessels to the constrictor agent. At 100 mm Hg, the reactions of HR and NR vessels were similar. At 150 mm Hg, NR vessels still responded to NE, while HR vessels virtually did not contract.

It was previously shown that in this model of regional hypotension, the cross-section area of the caudal artery media decreased by 40% [9]. Probably, the transmural pressure is the major factor affecting the vessel structure. The blood flow rate in major arteries of hindquarter regions, which initially decreased after clipping of the abdominal aorta, recovered two weeks postoperation [1]. It should be noted that we studied the responses of the same vessels in HR and NR at different transmural pressure, therefore the observed differences could not be explained by changes in the receptor apparatus of smooth muscle cells.

Thus, reactivity of thin-wall vessels in HR to constrictor stimulation was maximum at a pressure they were adapted to. However, they could not constrict at high pressure. We believe that the observed peculiarities of vascular reactivity in HR are determined by changes in the elastic properties of the vascular wall. It is known that adaptation of blood vessels to low pressure is accompanied by an increase in their distensibility [2]. The curve representing the force developed by vessel segment as the function of the applied load is also shifted to the left [2]. It can be hypothesized that at high pressure (150 mm Hg) the vascular

TABLE 1. Effect of Transmural Pressure on Flow Rate in Arterial Segments from NR and HR ($M\pm m$)

Inlet pressure, mm Hg	Outlet pressure, mm Hg	Transmural pressure, mm Hg	Perfusion rate, ml/min	
			NR (n=10)	HR (n=10)
50	0	25	7.74±0.49	7.90±0.22
100	50	75	8.81±0.76	7.91±0.88
125	75	100	9.71±0.45	9.72±0.81
175	125	150	9.52±0.84	10.02±0.79

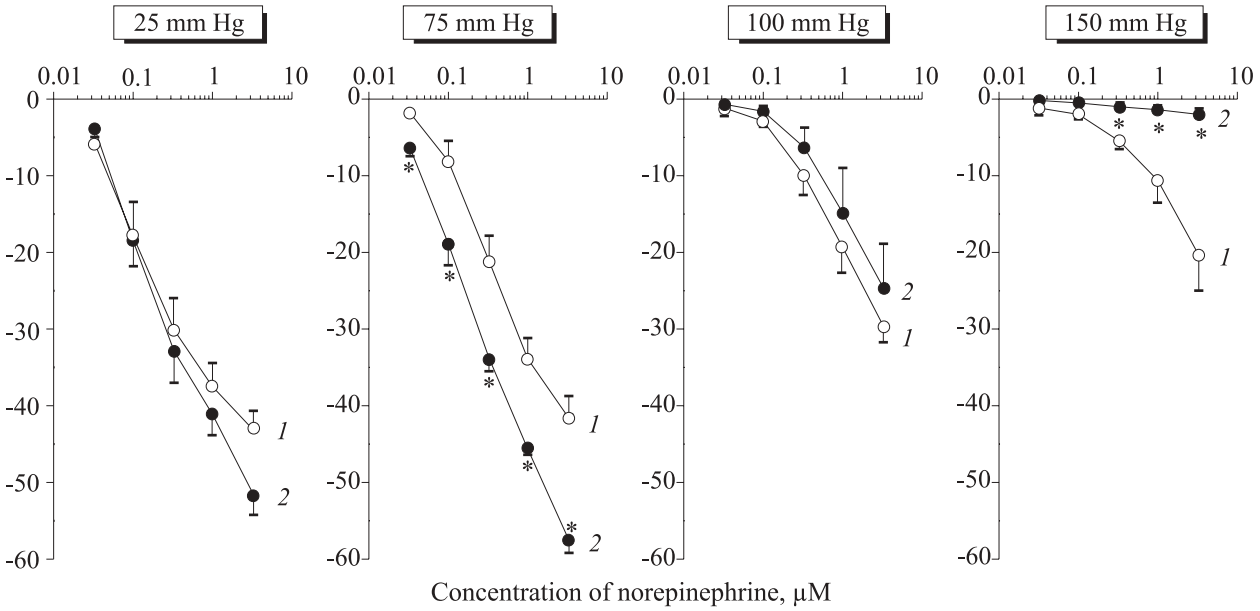

Fig. 1. Vascular reaction in normotensive (*1*, *n*=10) and hypotensive (*2*, *n*=10) rats to norepinephrine at different transmural pressure. Here and in Fig. 2: ordinates: changes in vessel diameter (% of relaxed vessel). **p*<0.05 compared to normotensive rats (*1*).

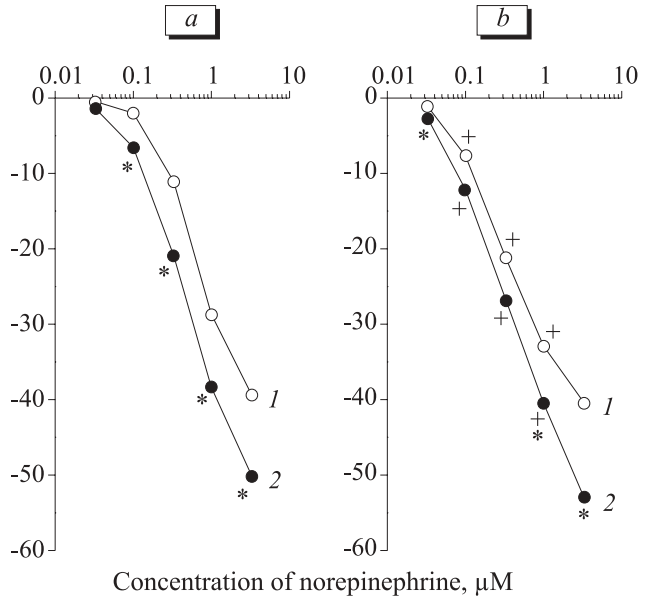

Fig. 2. Vascular reaction in normotensive (*1*, *n*=7) and hypotensive (*2*, *n*=7) rats to norepinephrine in control (*a*) and after deendothelization (*b*). +*p*<0.05 compared to the control.

smooth muscle in HR vessels is overstretched and cannot contract.

Increased reactivity of HR vessels at 75 mm Hg in comparison with NR vessels can be explained as follows. Moderate distension increases sensitivity of smooth muscle to vasoconstrictor agents [11,12]. In thin-wall vessels, the effect of distension is manifested even at lower pressures [9]. Probably, at 75 mm Hg, which is close to arterial pressure during hypotension, distension of the vascular wall potentiates the constrictor response only in HR. Therefore, the structural changes in blood vessels induced by hypotension make it possible to regulate blood flow at a lower blood pressure in comparison with the normal. At higher pressure (100 mm Hg) the effect of distension can manifest in rats of both groups.

In series II, deendothelization had practically no effect on the flow rate. During perfusion with NE-free solution, the flow rates before and after denudation in NR were 8.45±1.14 and 7.80±1.47 ml/min (*n*=7), respectively. In HR the corresponding values were 8.23±1.10 and 7.97±0.67 ml/min (*n*=7). In both groups, deendothelization potentiated the constrictor reactions to low and middle doses of NE (Fig. 2), which agrees with the views on anticonstrictor role of vascular endothelium. Under the action of mechanical stimuli (shear stress [10] or radial distension [5]), endothelial cells release factors moderating the contractile response. In our experiments, the constrictor reactions could be also diminished due to the action of NE on α_2-adrenoceptors of endothelial cells [4]. The anticonstrictor effect of the endothelium is manifested to approximately the same degree in NR and HR, so after removal of endothelium HR vessels remain more reactive to NE than NR vessels (Fig. 2, *b*).

Thus, our findings suggest that not only structure, but also function of arterial vessels adapt to chronic low pressure. Vascular reactivity during regional hypotension is determined by properties of the smooth muscle, rather than endothelium.

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