Humoral Factor(s) Circulating in the Blood of Spontaneously Hypertensive Rats Increases Vascular Tone and Reduces Constrictive Responses of Isolated Rat Caudal Artery to Stimulation of Sympathetic Fibers of the Vascular Wall

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It is shown that the perfusion pressure in isolated rat caudal artery rises and constrictive responses to electrical stimulation decrease when the blood of normotensive Wistar-Kyoto rats perfusing the vessel was replaced by the blood of spontaneously hypertensive rats. The norepinephrine release from the sympathetic terminals is reduced.

Key Words: *isolated vessel; humoral factors; electrical stimulation; norepinephrine release; spontaneously hypertensive rats*

Both pro- and antihypertensive humoral factors which are known to circulate in the blood of spontaneously hypertensive rats [3,4] are able to modulate the arterial pressure by affecting the tone and reactivity of vessels. However, the mechanism of action of many humoral factors remains unclear, since the effects of nervous and humoral factors on the cardiovascular system are hard to distinguish in the whole organism.

A new method we proposed previously, namely perfusion of an isolated segment of rat caudal artery with blood from a conscious donor rat, allowed us to study the humoral factors circulating in the blood of spontaneously hypertensive rats (SHR) and their influence on the constrictive responses to stimulation of sympathetic fibers in the vascular wall. A modification of this method supplemented by measurement of the norepinephrine (NE) release from presynaptic terminals of sympathetic fibers has enabled us to investigate the possible mechanism whereby the observed effect is realized.

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MATERIALS AND METHODS

The experiments were carried out on 6-7-month-old normotensive Wistar-Kyoto rats (WKY) and SHR weighing 250-350 g. The animals were used as blood donors. To this end the rats were narcotized with Nembutal (40 mg/kg, intraperitoneally), and the carotid and femoral arteries and the femoral vein were catheterized. In the course of the experiment blood for perfusion of the isolated caudal artery was drawn by means of a roll pump through the catheter introduced in the carotid artery and returned to the donor through the catheter introduced in the femoral vein, and the arterial pressure and heart rate in the donor rats were recorded using the catheter implanted in the femoral artery. The animals were conscious and moved freely in the cage throughout the experiment.

The isolated segments of the caudal artery were obtained from decapitated WKY. The segment

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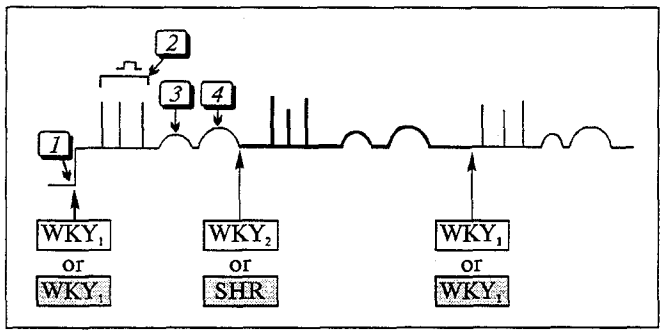

Fig. 1. Scheme of experiment. *1*) changing the perfusion from physiological saline to blood; *2*) electrical stimulation of sympathetic endings of the vessel wall; *3*) injection of α_1-adrenoreceptor agonist to donor rats; *4*) injection of α_2-adrenoreceptor agonist to donor rats; WKY – Wistar–Kyoto rats, SHR – spontaneously hypertensive rats.

was mounted in a thermocontrolled chamber with constantly replaced modified Krebs–Henseleit physiological solution containing (in mmol/liter): 122.2 NaCl, 6.7 KCl, 2.1 $CaCl_2$, 1.3 $MgSO_4$, 1.1 KH_2PO_4, and 14.9 glucose, and aerated for 20 min with carbogen (96% O_2 and 4% CO_2). The procedure for perfusing the isolated caudal artery with blood from conscious donors has been described previously [1,2].

In our experiments the vascular segment was successively perfused with blood from a normotensive WKY, then from an SHR, and again from the normotensive WKY, while in the control, the vessel was perfused with blood from different normotensive WKY (schemes WKY_1–SHR–WKY_1 and WKY_1–WKY_2–WKY_1, respectively, Fig. 1).

Two experimental series were carried out according to the above schemes. In both series electrical stimulation of the isolated segment was performed with pulses of the following parameters: frequency 1-16 Hz, voltage 50 V, duration 0.08 msec, and current strength 0.1-0.4 and 0.01-0.2 A for series I and II, respectively. In series I the donor rats were intravenously injected with phenylephrine (0.01-0.02 mg/kg, Sigma) and (-)3,4-dihydroxynorephedrine (0.1-1 μg/kg, Sigma), agonists of α_1- and α_2-adrenoreceptors, respectively. In series II the release of labeled NE from presynaptic endings of the sympathetic nerves of the vessel wall was measured. The electrical stimulation and addition of α-agonists raised the perfusion pressure, i.e., produced vasoconstriction, which was estimated as a percentage with respect to the tone of the blood-perfused vessel.

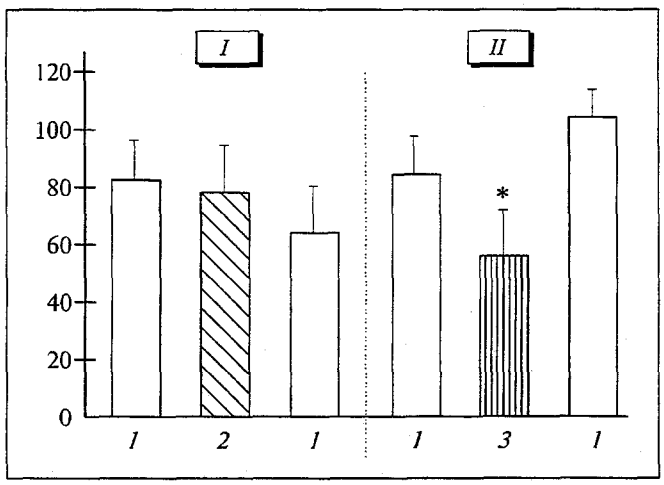

Fig. 2. Changes in perfusion pressure in isolated segment of rat caudal artery induced by electrical stimulation of sympathetic endings of vessel wall. *I*) control series. Isolated segment was perfused with blood from different normotensive Wistar–Kyoto rats (WKY) (*1*, *2*). *II*) experimental series. Isolated segment was successively perfused with blood from a normotensive WKY (*1*), a spontaneously hypertensive rat (SHR) (*3*), and again a normotensive WKY (*1*). Ordinate: changes in perfusion pressure, % of initial tone; *$p<0.05$.

In series II the isolated caudal artery from WKY was preliminarily saturated with labeled NE (^{3}H-NE, Izotop, St. Petersburg) in a final concentration of 0.25 μCi/mol and placed in a special chamber so designed that the superfusate bathing the vessel may be gathered in a fraction collector. In the estimation of the NE release induced by the electrical stimulation the background radioactivity was not taken into consideration. The mean NE release induced by electrical stimulation from the vessel perfused with blood of WKY_1 (Fig. 1) was taken as 100% and the NE released when the vessel was perfused with blood from the successive rat (WKY_2 or SHR) was normalized to this value.

RESULTS

Perfusion of the isolated vessel with blood from SHR raised the initial perfusion pressure, i.e., it increased the vascular tone by 13.3 mm Hg ($p<0.05$) in comparison with perfusion with blood from normotensive WKY, the constrictive responses to electrical stimulation being reliably decreased (by 28.3%, Fig. 2).

No changes in the tone and reactivity of the vessel segment were noted in the control experiments (perfusion with blood from different WKY). Since the isolated segment was taken from a normotensive WKY, these effects may be attributed solely to humoral factors circulating in the blood of SHR. The drop in the neurogenic constrictive responses may result from the influence of a humoral factor either directly on the smooth muscle, or on the release of NE from sympathetic endings.

For elucidation of this question the following two experimental series were performed. In series I we determined the magnitude of vasoconstriction caused by agonists of α_1- and α_2-adrenoreceptors for consecutive perfusion of the vessel with blood from WKY_1, SHR, and WKY_2.

In series II we measured the release of labeled NE from sympathetic endings in response to electrical stimulation, replacing blood from WKY perfusing the vessel with blood from SHR.

The constrictive responses of the isolated segment induced by injection of α_2-adrenoreceptor agonist to the donor rats tended to decrease when the vessel was perfused with blood from SHR. No such changes were produced by α_1-agonist. A humoral factor circulating in the blood of SHR probably somehow partly inhibited constrictive reactions mediated through the α_2-adrenoreceptors on vascular smooth muscles, thus reducing the neurogenic vasoconstriction.

However, since we observed only a tendency toward a decreased response of the isolated segment to α_2-agonist, these postsynaptic receptors presumably contribute little to the studied phenomenon. To determine whether the antihypertensive factor circulating in the blood of hypertensive rats affects the release of the transmitters, we measured the release of labeled NE from the presynaptic endings of sympathetic fibers in the vessel wall.

These experiments revealed a 33.5% decrease in the release of labeled NE when the vessel was perfused with blood from an SHR in comparison with perfusion with blood from a normotensive rat ($p < 0.05$). In the control (blood from different WKY) no reliable decrease was noted in the release of labeled NE.

Thus, our findings suggest the presence of both pro- and antihypertensive factors in the blood of SHR. The antihypertensive factors diminish the neurogenic constrictive responses of the isolated segment of the rat caudal artery by reducing the release of labeled NE from presynaptic endings of sympathetic fibers and, partially, by reducing the reactivity of α_2-adrenoreceptors of the postsynaptic membrane.

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