Changes in Vascular Responsiveness to Vasoactive Agents in the Hindlimb of DOCA-Salt Hypertensive Rats

Keizo SOGABE, Toshihiko UEMATSU, Hisakuni HASHIMOTO, Tohru OZAKI and Mitsuyoshi NAKASHIMA*
Department of Pharmacology, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan
Accepted May 22, 1987

Abstract—The responsiveness to vasoactive agents in the perfused hindlimb of DOCA-salt hypertensive rats was examined and compared with that of normotensive rats. The vasoconstrictor responses in the femoral vascular bed to norepinephrine and serotonin were markedly potentiated in DOCA-salt hypertensive rats as compared with those in normotensive rats, and no change was found in the responses to angiotensin II. On the other hand, the vasodilatory response in DOCA-salt hypertensive rats to isoproterenol was attenuated without any marked changes in responsiveness to acetylcholine, nitroprusside and papaverine. These results suggested that the reduced vasodilator responses as well as the increased vasoconstrictor responses occur to some specific vasoactive agents in DOCA-salt hypertensive rats.

An increased responsiveness to various vasoconstrictor stimuli has been demonstrated in experimental hypertensive animals with renal, deoxycorticosterone/NaCl (DOCA-salt) or genetic hypertension, and this may be closely related to the initiation of a rise in arterial blood pressure of such animals (1-6). This kind of increased responsiveness has been also demonstrated in patients with essential hypertension (7, 8). Although many authors have reported an increased responsiveness to vasoconstrictor stimuli, it has not been fully clarified whether the responsiveness to vasodilatory agents in DOCA-salt hypertensive rats would be altered or not.

In this study, the responsiveness to vasodilators as well as to vasoconstrictors in the hindlimb of DOCA-salt hypertensive rats were examined and compared with that in normotensive rats.

Male Wistar rats weighing 150-160 g were nephrectomized unilaterally under ether anesthesia. They were subsequently injected with deoxycorticosterone (DOCA) (3 mg, s.c.) twice a week and given 1% saline for drinking for five or six weeks. The control rats were nephrectomized unilaterally and given 1% saline for drinking in the same way with no administration of DOCA. Systolic blood pressures were measured by an indirect method using a tail cuff at weekly intervals following an administration of DOCA. Animals with systolic blood pressure of more than 180 mmHg were considered to be hypertensive.

A unilateral femoral vascular bed was perfused with a constant flow method. Blood was collected from 3 to 4 blood-donor rats, with weights of 500-550 g, through a catheter inserted into their carotid arteries under ether anesthesia and systemic heparinization. The blood thus obtained was mixed with an equal volume of Krebs-Ringer solution of the following composition (mM): NaCl, 120; KCl, 4.7; KH2PO4, 1.2; MgSO4, 1.2; CaCl2, 2.0; NaHCO3, 25.0; and glucose, 14. The solution was aerated with 95% O2 and 5% CO2 and kept at 37°C. A blood-recipient rat weighing 200-250 g, which was either normotensive or hypertensive, was anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and heparinized. With an

* To whom all correspondence should be addressed.
abdominal midline incision, the right common iliac artery and vein were carefully separated from the surrounding tissues and from each other. Through these vessels, polyethylene cannulae were inserted into the femoral artery and vein, respectively. An attempt was made to isolate the femoral vascular bed as entirely as possible, ligating the internal iliac, inferior epigastric vessels and their visible branches. Using the in situ perfusion of a rat hindlimb with a blood-containing solution, which was supplied at a constant flow of 2 ml/min with a roller pump (Atto, SJ-1211H), a perfusion pressure of as high as 100 mmHg could be obtained. Thus, a vasodilatory response could be easily observed as a decrease in the perfusion pressure without any application of vasoconstrictor substances into the perfusate. An outflow from the femoral vein was discarded. The perfusion pressure was measured with a pressure transducer (Nihon Kohden MPU-0.5 and RP-5) which was connected to a side arm of a T tube inserted into the circuit just before the inlet to the common iliac artery. The vascular response was recorded on an ink-writing oscillograph as a change in the perfusion pressure.

The vasoactive agents employed in this study were acetylcholine hydrochloride (Daiichi), angiotensin II acetate (Sigma), isoproterenol hydrochloride (Sigma), sodium nitroprusside (Katayama Kagaku), papaverine hydrochloride (Wako Pure Chemical) and serotonin creatine sulfate (Nakarai Chemical). All values were expressed as means±S.E. The data obtained were statistically analyzed by Student's t-test.

The dose-response curves of pressor responses in DOCA-salt hypertensive and normotensive rats to norepinephrine, serotonin and angiotensin II are shown in Fig. 1. The curves of norepinephrine and serotonin in DOCA-salt hypertensive rats shifted to the left of the corresponding curves of normotensive rats, showing that the responsiveness to both agents was significantly increased in the former as compared with that in the latter. No significant difference was found in the responsiveness to angiotensin II between the two groups. Figure 2 shows the dose-response curves to isoproterenol, acetylcholine and nitroprusside. The responsiveness to isoproterenol in DOCA-salt hypertensive rats was attenuated by more than 50% as compared with normotensive rats, while no marked differences in the responsiveness to nitroprusside and acetylcholine have been observed between both groups. The maximum dilatory responses of the femoral vascular bed to papaverine were not significantly different (82.7±4.9 mmHg in DOCA-salt hypertensive rats vs. 79.3±8.7 mmHg in normotensive rats).

An increased reactivity of vascular smooth muscles to vasoconstrictor stimuli is known
to be often observed depending on the agents employed. In this study, hyperreactivities to norepinephrine and serotonin were observed in DOCA-salt hypertensive rats, while there was no change in the responsiveness to angiotensin II. At the same time, the maximum dilation of the femoral vascular bed to papaverine was shown to be almost the same between the control and DOCA-salt hypertensive rats. Contrary to our observation, Bing et al. (9) reported an increased responsiveness to angiotensin II in DOCA-salt hypertensive rats; they showed a negative correlation between the hyperreactivity and the plasma renin activity. In other words, the hyperreactivity to angiotensin II might be caused by low renin-angiotensin levels in the blood and reciprocal high availability of unoccupied angiotensin II receptors. In this study, the femoral vascular bed was perfused with a solution mixed with blood, which was supplied from normotensive rats, and the apparent difference between the observation of Bing et al. (9) and ours could be attributable to this.

In contrast with the hyperreactivities mentioned above, the femoral vascular bed in DOCA-hypertensive rats showed less relaxation in response to isoproterenol than in normotensive rats. The responsiveness to nitroprusside and acetylcholine did not differ between both groups. These results are consistent with the report of Hagen and Webb (10), who showed that relaxations in response to isoproterenol and adenosine were exclusively reduced in the helical strips of coronary arteries excised from DOCA-salt hypertensive rats. From these observations including ours, it seems that reductions of responsiveness occur only to some specific vasodilators as well as in the case of increases in responsiveness. This markedly attenuated responsiveness to isoproterenol may be due to a reduction in the number of vascular beta-adrenoceptors and the resultant decrease in adenylyl cyclase response, which has been reported elsewhere (11, 12). The reduced responsiveness to isoproterenol does not seem to occur solely in DOCA-salt hypertensive rats because it has been also demonstrated in spiral strips of aorta excised from spontaneously hypertensive rats and renal hypertensive rats (13). In conclusion, the present results suggest that not only increased responsiveness to vasoconstrictors but also decreased responsiveness to vasodilators may contribute to the initiation and/or maintenance of hypertension in DOCA-salt hypertensive rats.

References


