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AUGMENTATION BY CONVERTING ENZYME INHIBITION OF ACCELERATED ENDOTHELIN RELEASE FROM RAT MESENTERIC ARTERIES FOLLOWING NEPHRECTOMY

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SUMMARY: We investigated the release of endothelin-1 (ET) from rat mesenteric arteries to clarify its pathophysiological role in the sustained hypertension of spontaneously hypertensive rats (SHR) following nephrectomy and the regulatory mechanism of the ET release which might be modified by vascular angiotensins and bradykinins. Nephrectomy increased the plasma level of ET and enhanced the ET release in both SHR and Wistar-Kyoto rats (WKY). CV-11974, an angiotensin II receptor antagonist, did not affect the ET release from arteries of nephrectomized rats. On the contrary, infusion of captopril, a converting enzyme inhibitor, further enhanced the ET release in both intact and nephrectomized rats. These findings suggest that the release of ET from mesenteric arteries may be regulated by bradykinins, but not by angiotensins. This pressor substance does not contribute to the sustained hypertension because the enhanced production of ET observed in both SHR and WKY. However, there is a possibility that the exaggerated responsiveness of vascular ET may in part account for local vascular tone and vascular remodeling in renal dysfunction.

Contrary to the initial and theoretical expectation regarding endothelin-1 (ET), a potent vasoconstricting peptide, gene targeting in the ET knockout mouse failed to produce sustained hypotension (1). However, there is still a possibility that ET may play different physiological roles in addition to blood pressure regulation in various stages of development from fetus to maturity. In fact, a newly developed ET receptor antagonist showed a hypotensive effect in sodium-depleted squirrel monkeys (2). Therefore, we are still intrigued with the hypothesis that disturbances in the control of ET production may contribute to the pathogenesis of hypertension or may affect cardiovascular remodeling. While it is unlikely that ET regulates hypertension of spontaneously hypertensive rats (SHR) because plasma

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ET levels in SHR are lower than (3) or of the same as (4) those in normotensive Wistar-Kyoto rats (WKY), ET may play a role in the sustained hypertension of nephrectomized SHR because plasma ET level was elevated in patients with uremia (5).

Previously, we have reported that the production of angiotensin II (AngII), another important factor in the regulation of cardiovascular function, was regulated differently in SHR than in Sprague-Dawley rats and rabbits (6). We showed that nephrectomy caused augmentation of vascular AngII release in SHR and WKY, in sharp contrast to its reduction observed in Sprague-Dawley rats and rabbits. Furthermore, it has been reported that there are some interactions between the production of ET and AngII in vascular cells (7-9).

The present study was therefore designed to examine 1) whether nephrectomy affects vascular ET production, 2) whether endogenous AngII regulates the production of ET in mesenteric arteries, and 3) how the regulation of plasma and vascular ET differs between hypertensive and normotensive rats following nephrectomy.

MATERIALS AND METHODS

Animals and nephrectomy : Male 12-14 -week-old SHR and WKY were obtained from Charles River Japan, Inc. (Atsugi, Japan). Nephrectomy was performed through bilateral flank incisions using a retroperitoneal approach. Tight ligatures were tied around the renal pedicles, and the kidneys were removed after cutting. Only bilateral flank incision was performed in sham-operated rats as control of nephrectomy. Experiments were performed 48 hours after the operation. All procedures were in accordance with the institutional guidelines for animal experiments of Osaka University Medical School. The minimum number of animals necessary for validity was used in this investigation (5 per treatment group).

Preparation of blood samples and mesenteric arteries : Mesenteric arteries and a blood sample (2 ml) were prepared as described previously (10) from sham-operated and nephrectomized WKY and SHR. The tissues were perfused with Krebs-Ringer solution at a constant flow rate of 4.5 ml/min. Experiments were started after perfusion for an equilibration period of 30 minutes. Captopril (10^{-7} M), an angiotensin converting enzyme inhibitor, CV-11974 (10^{-6} M), or vehicle was infused with a microinfusion pump (model 501B, ATOM, Tokyo, Japan). CV-11974 is the active de-esterified metabolite of TCV-116 which is a newly developed potent, orally active and specific nonpeptide AngII type-1 receptor antagonist (11, 12) (donated by Takeda Chemical Industries, Osaka, Japan).

Determination of immunoreactive ET : ET released from the isolated perfused mesenteric artery preparation was trapped by an octyl minicolumn (Amprep-C8, 500 mg, Amersham, UK). The minicolumn was prewashed with 5 ml of methanol and then 10 ml of Krebs-Ringer solution. The column was exchanged at 15 minute intervals. After the minicolumn was washed with 10 ml of 0.1% trifluoroacetic acid (TFA) in distilled water, trapped peptides were eluted with 3 ml of methanol/water/TFA (80:19.9:0.1, vol/vol/vol). The eluate was dried in a

centrifugal concentrator, and the resultant residues were dissolved in 100 µl of 0.1% TFA. High performance liquid chromatography characterization was performed as previously described (13). In the appropriate fraction, samples were collected and dried in a vacuum centrifuge, and redissolved in 0.1 M Tris-acetate buffer (pH 7.4) containing 2.6 mM disodium EDTA-2Na, 1 mM phenylmethylsulfonyl fluoride, and 0.1% bovine serum albumin.

Immunoreactive ET was measured by radioimmunoassay using an ET antibody (IBL, Fujioka, Japan). The sensitivity of this assay was 0.25 to 64 pg/tube. The cross reactivity was 277% for endothelin-2 (ET-2), 0.1% for endothelin-3 (ET-3), 80.3% for big ET-1, 277% for big ET-2, and 0.1% for big ET-3. The ET antibody (50 µl) was added to both sample (300 µl) and standard tubes, which were incubated overnight at 4°C. The (3-[^{125}I] iodotyrosyl) ET (Amersham, UK) was reconstituted in 0.1 M Tris-acetate buffer and was added to each tube at a concentration of approximately 0.37 kBq/tube (50 µl) with further incubation overnight at 4°C. On the third day, 50 µl of 4.2% bovine γ-globulin was added to each tube and mixed, and 1 ml of 25% polyethylene glycol 6,000 was added and mixed once again. After centrifugation at 2500g for 30 min at 4°C, the supernatant was aspirated and the pellet was counted with a gamma-counter. We confirmed that neither captopril nor CV-11974 interferes with the measurement of ET with this method. Plasma ET concentration was measured as described above after passing a 0.8 ml sample through a C8 minicolumn.

Statistical analysis : The levels of ET release were constant for more than 90 minutes (data not shown), whereas AngII release from mesenteric arteries of kidney-intact rats, both WKY and SHR, was shown to be stable for only 60 minutes (7). Therefore, we calculated the mean ET release per minute from the data of the first three periods of 45 minutes in each experiment. Values are expressed as mean ± SEM. Statistical analysis of these data was performed using two-way analysis of variance followed by multiple comparison test (Duncan's test) when appropriate. Significance was defined as a P value of less than 0.05.

RESULTS

Effects of nephrectomy on plasma ET levels and the ET release from mesenteric arteries of WKY and SHR are shown in Fig. 1 and Fig. 2, respectively.

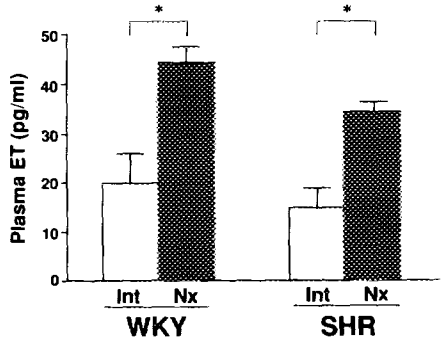

Fig. 1. Effect of nephrectomy on levels of plasma endothelin-1 (ET) in Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). "Int" and "Nx" represent kidney-intact and nephrectomized rats, respectively. Each column represents mean ± SEM of 5 experiments. *p<0.01.

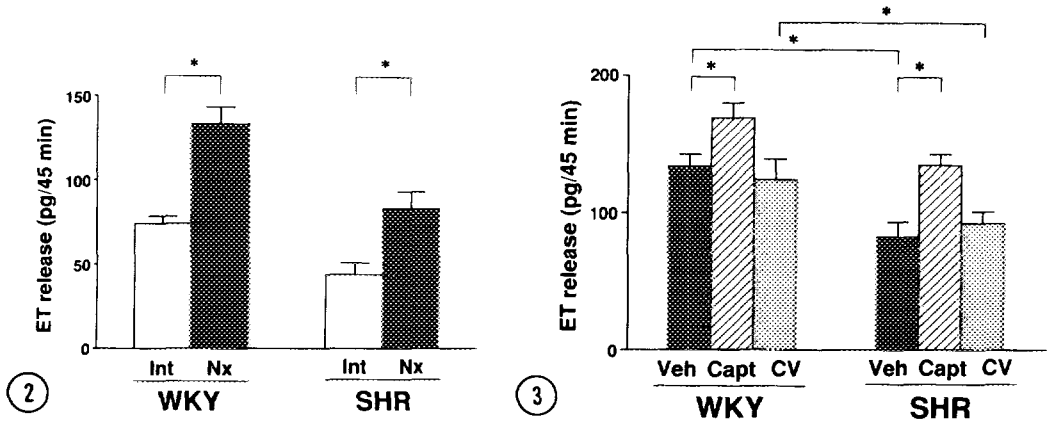

Fig. 2. Effect of nephrectomy on ET release from mesenteric arteries of WKY and SHR. Abbreviations are same with Fig. 1. Each column represents mean ± SEM of 5 experiments. *p<0.01.

Fig. 3. Effect of captopril and CV-11974 on the release of ET from mesenteric arteries of nephrectomized WKY and SHR. "Veh", "Capt" and "CV" represent vehicle, captopril and CV-11974, respectively, in the perfusion solution of mesenteric arteries. Each column represents mean ± SEM of 5 experiments. *p<0.05.

There was no significant difference in the basal plasma level of ET between WKY and SHR. Nephrectomy caused significant increases in both WKY (223%) and SHR (232%). The basal ET release from arteries in WKY was higher than that in SHR, and nephrectomy caused the enhancement of ET release to 180% in WKY and to 189% in SHR. CV-11974 did not affect the ET release from arteries of nephrectomized rats (Fig. 3). On the contrary, captopril infusion further enhanced the ET release in both intact (140% in WKY, 180% in SHR, data not shown on Figure) and nephrectomized rats (127% in WKY, 163% in SHR, Fig. 3).

The perfusion pressure was not different in these four groups and remained stable throughout the study (data not shown).

DISCUSSION

The present study clearly demonstrated that isolated mesenteric arteries of nephrectomized WKY and SHR released higher levels of ET compared with arteries of kidney-intact rats (Fig. 2) resulting in the higher levels of plasma ET (Fig. 1). This observation in an isolated vasculature system suggests that high levels of plasma ET in uremia (5) are, at least in part, due to an augmented production of ET from the vasculature.

What is the mechanism of the activated ET release following nephrectomy in WKY and SHR? Concerning the interaction between AngII and ET, it has been demonstrated that exogenous AngII stimulates the release of ET from cultured

endothelial cells (7) and smooth muscle cells (8). In fact, we have previously reported that the AngII release from mesenteric arteries was enhanced by nephrectomy in WKY and SHR (6). However, it appears unlikely that the enhanced ET release was directly or solely regulated by vascular AngII because in the present study there were no effects of CV-11974, an AngII receptor antagonist, on the ET release after nephrectomy. It appears that interaction between ET and AngII after nephrectomy is not bidirectional, but the augmented ET release may contribute to the stimulated AngII production from mesenteric arteries of nephrectomized WKY and SHR. The previous report (9) that exogenous ET stimulates the release of AngII from rat mesenteric arteries supports this possibility.

It is very interesting to note in the present study that captopril, which decreased the vascular AngII release (6), rather increased the ET release in both kidney-intact and nephrectomized rats. The observed difference between the effects of captopril and CV-11974 on the ET release after nephrectomy suggests that increased bradykinin due to kininase II inhibition by captopril may play an important role. Endothelial cells have two kinds of bradykinin receptors, B_1 and B_2 receptors. Momose *et al* (14). reported that bradykinin caused bifunctional effects on ET release from endothelial cells via these different types of receptors: bradykinin stimulates ET release via the B_1 receptor and inhibits it via the B_2 receptor. Contrary to our perfusion experiments, there are reports that angiotensin converting enzyme inhibitors inhibited ET secretion from cultured human endothelial cells (14,15). However, expression of different receptor subtypes can differ *in vivo* and *in vitro*, as reported for natriuretic peptides receptors (16). While it has not yet been clarified which type of bradykinin receptor is predominant in mesenteric arteries, captopril might enhance ET secretion through B_1 receptor stimulation.

This postnephrectomy activation of vascular ET does not account for the sustained hypertension after nephrectomy in SHR, because mesenteric arteries of WKY showed a similar activation of vascular ET generation. As discussed above, however, accelerated ET production may enhance the release of vascular AngII. ET is known to induce not only vasoconstriction but also vascular smooth muscle cell growth as does AngII (17,18). Therefore, the exaggerated responsiveness of vascular ET may contribute to vascular remodeling via enhancement of AngII and biological effects of ET itself in patients with renal failure.

In summary, these results suggest that after bilateral nephrectomy, the vasculature produces various pressor substances, such as AngII and ET. The release of ET was not regulated by AngII, however, it is of note that enhanced release of ET may be evoked by bradykinins. Although the precise pathophysiological role of this augmentation of vascular ET following nephrectomy remains to be elucidated, there is a possibility that the exaggerated ET responsiveness may in part contribute to vascular cell growth and local vascular tone.

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