ORIGINAL

Effect of endothelin-1 (1-31) on the renal resistance vessels

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Abstract : Human chymase produces not only angiotensin II but also endothelin(ET)-1(1-31). We previously reported that ET-1(1-31) had several biological activities in vascular smooth muscle cells. In this study, we investigated the vasoconstrictor effect of ET-1(1-31) on the renal resistance vessels using *in vitro* microperfused rabbit afferent and efferent arterioles. ET-1(1-31) decreased the lumen diameter of the afferent and efferent arterioles dose-dependently. ET-1(1-31)-induced afferent arteriolar vasoconstriction was not affected by phosphoramidon, an ET converting enzyme inhibitor. ET-1(1-31)-induced renal arteriolar vasoconstriction was inhibited by BQ123, an ET_A receptor inhibitor, but not by BQ788, an ET_B receptor inhibitor. These results suggest that ET-1(1-31)-induced renal arteriolar vasoconstriction may be mediated by ET_A-like receptors. J. Med. Invest. 50 : 87-94, 2003

Keywords : *ET-1(1-31), ET-1, afferent arteriole, efferent arteriole,*

INTRODUCTION

Human endothelin(ET)-1 is a 21-amino acid polypeptide, and is generated from the 38-amino acid precursor, big ET-1, through cleavage of Trp^{21}-Val^{22} bound via the action of a membrane-bound metalloprotease, ET-converting enzyme (ECE) (1, 2). ET-1 induced powerful and long-lasting vascular smooth muscle cell contractile responses in various systems with particularly potent actions on renal vascular beds (1, 3-6). ET-1 also exhibits various physiological actions, such as cardiac hypertropyh (7), vascular thickening (4) and mitogenesis (8). On the other hand, Nakao *et al.* (9) reported that human mast cell chymase, unlike rat mast cell chymases, selectively cleaves big ETs at the Tyr^{31}-Gly^{32} bond to produce novel trachea-constricting 31 amino acid ETs, ETs(1-31), without any further degradation products. ET-1(1-31) was found in human blood (10), granulocytes (11) and lung (12). ET-1(1-31) also exhibits various physiological actions, such as vascular contraction (3), cell proliferation (10, 13), and chemotactic effects (14).

Exogenous ET-1 caused potent vasoconstriction and prolonged the elevation of blood pressure. Thus, endogenous ET-1 is assumed to modulate vascular tone and regional blood flow as a circulating hormone, or to exert its actions locally within the vascular wall and on the endothelium in an autocrine or paracrine fashion. ET-1(1-31) may also modulate vascular tone and regional blood flow. However, the effect of ET-1(1-31) on the resistance vessels, which regulate the organ circulation, has not yet been examined. In renal circulation, preglomerular afferent and post glomerular efferent arterioles are crucial vascular segments to the control of glomerular hemodynamics (5). The balance of vascular tone between the afferent and efferent arterioles critically affects glomerular capillary pressure, and thereby the glomerular filtration rate, as well as renal excretory function. In this study, we examined the effects of synthetic ET-1(1-31) on the lumen diam-

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eter of isolated microperfused rabbit afferent and efferent arterioles.

MATERIALS AND METHOD

Materials

Human ET-1 and phosphoramidon (N-(α-rhamnopyranosyloxyhydroxyphosphinyl)-L-Leucyl-L-tryptophan) were obtained from the Peptide Institute (Osaka, Japan). ET-1(1-31) was synthesized using a solid-phase procedure at the Peptide Institute. Bovine albumin fraction V was purchased from Seikagaku Kogyo Co., Ltd.(Tokyo, Japan). Medium 199 was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). BQ123(cyclo-(D-Try-D-Asp(ONa)-Pro-D-Val-Leu)) (15) and BQ 788(N-cis-2,6-dimethylpiperidinocarbonyl-L-γMeLeu-D-Trp(COOMe)-D-Nle-ONa) (16) were gift from Banyu Pharmaceutical Co (Tsukuba, Japan). All other chemicals used were commercial products of reagent grade.

Isolation and microperfusion of the rabbit afferent and efferent arterioloes.

We used a method similar to that described previously (17, 18). Briefly, male New Zealand white rabbits were anesthetized with intravenous sodium pentobarbital (25 mg/kg) and given an intravenous injection of heparin (500U). The kidney was exposed through a retroperitoneal flank incision, and the renal pedicle was clamped and cut. The kidney was quickly removed and placed in iced medium 199. Then, the kidney was sliced along the corticomedullary axis. A thin slice was transferred to a dish containing chilled medium 199 and microdissected under a stereoscopic microscope (SZH, Olympus, Tokyo, Japan) using thin steel needles and sharpened forceps (No. 5, Dumont, Basel, Switzerland) at 4°C. The superficial glomerulus, with afferent and efferent arterioles, was dissected free from the surrounding tissue and all tubular fragments were removed. Great care was taken to avoid touching the vessels and exerting longitudinal or transverse tension on them. An afferent arteriole, with its glomerulus and efferent arteriole, was severed from the interlobular artery by cutting it with a disposable 27-gauge injection needle (TOP, Tokyo, Japan). The final preparation was transferred with a micropipette to a temperature-regulated chamber (ITM, San Antonio, TX, USA) and the camber was mounted on the stage of an inverted microscope with Hoffman modulation (Diaphot, Nikon, Tokyo, Japan). The volume of the chamber was 1 ml. For drainage, fresh bath medium (medium 199) was supplied to the bottom right side of the chamber at 0.5 ml/min, and the medium was gently aspirated from the top of left side of the chamber. During the experiment, water-saturated gas (90% O_2 and 10% CO_2) was gently blown over the surface of the bath to maintain the pH at 7.4.

The afferent arteriole was cannulated with a pipette system as illustrated (Fig. 1). The method used for cannulating the afferent arteriole into the micropipette system was similar to that reported by Osgood *et al.* (19) and by Ito and Carretero (20). The afferent arteriole was drawn into the holding pipette, which had a constriction. The tip of the perfusion pipette was advanced into the lumen of the afferent arteriole. A strong vacuum was then applied to the holding pipette to pull the afferent arteriole further toward the constriction in the holding pipette, and thereby seal it between the two pipettes. The pressure pipette, which was filled with 0.9% NaCl solution containing FD & C green and 4% KCl, was then advanced into the afferent arteriole through the opening of the perfusion pipette. The intraluminal pressure was measured by Landis' technique (19) using this pressure pipette. The afferent arteriole was microperfused with oxygenated medium 199 containing 5% bovine albumin fraction V (Fig. 2). After the completion of cannulation, the intraluminal pressure was set at 60 mmHg and maintained throughout the experiment. The intraluminal pressure was continuously monitored with a pressure transducer and monitor(Digic VPC, Valcom, Tokyo, Japan). Microdissection and cannulation of the afferent arteriole was completed within 90 minutes. The temperature of the bath was gradually raised to 37°C and monitored during the experiment (E5CS, Omron, Tokyo, Japan). A 30-minute equilibration period was allowed before the experiment. The image of the afferent arteriole during the experiment

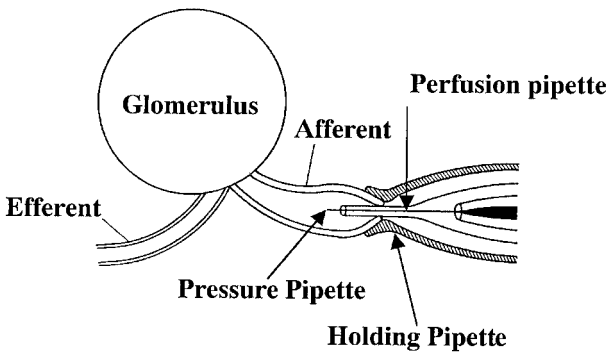

Fig. 1. Schematic illustration of the pipette system.

was recorded with a video system consisting of a CCD camera and control unit (CCD-10, Olympus, Tokyo, Japan), a monitor (NV-0930Z, Mitsubishi, Tokyo, Japan), and a video recorder (Timelapse BR-9000, JVC, Tokyo, Japan). The effect of ETs was evaluated on the basis of the change in the lumen diameter of the microperfused afferent or efferent arterioles. The lumen diameter of the arteriole was measured directly on the video monitor screen. At the end of the experiment, the viability of the vessel was assessed by the response to 10^{-5} M norepinephrine.

Experimental Protocols

Effect of ETs on the lumen diameter of microperfused afferent and efferent arteriole.

Following a 30-minutes equilibration, ET was applied to the bath in increasing concentrations, to determine its dose-response curve. The control measurements of the lumen diameter were made at 1-min intervals for 3 minutes, and the control value was the mean value of three measurements. During the control measurements, we confirmed that the lumen diameter was stable. The continuous bath exchange was stopped and the bath medium was rapidly exchanged for the medium containing the lowest concentration of ET. The bath exchange was resumed with medium containing the same concentration of ET, and the arteriole was observed for 5 minutes. Every 5 minutes, the concentration of ET was increased by one order of magnitude, up to 10^{-6}M. The effects of ET-1(1-31) on the lumen diameter of afferent and efferent arterioles were evaluated using different sets of microperfused glomeruli. The effects of ETs on the lumen diameter of arterioles were measured 5 minutes after the addition of ETs.

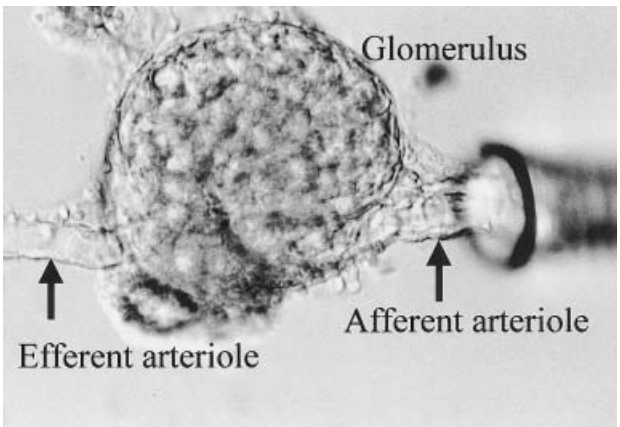

Fig. 2. A microperfused afferent arteriole with a glomerulus and an efferent arteriole.

Effect of phosphoramidon on the ET-1(1-31)-induced afferent arteriolar vasoconstriction.

We investigated the possibility that ET-1(1-31)-induced afferent arteriolar vasoconstriction may be due to further degradation of ET-1(1-31) to ET-1 by endothelin-converting enzyme in the medium or microdissected glomerulus with afferent and efferent arterioles. We examined the effect of an inhibitor of endothelin-converting enzyme, phosphoramidon (21), on the ET-1(1-31)-induced afferent arteriolar vasoconstriction.

After the microperfusion of the isolated afferent arteriole was completed, we added phosphoramidon to the perfusate and bath medium. After preincubation with 10^{-5}M phosphoramidon, ET-1(1-31) and 10^{-5}M phosphoramidon were added to the bath medium, and the effect of ET-1(1-31) was evaluated in the manner as described above.

Effects of endothelin receptor antagonists on the ET-1(1-31)-induced arteriolar vasoconstriction.

We examined the effects of endothelin receptor antagonists on the ET-1(1-31)-induced arteriolar vasoconstriction to determine whether the effect of the ET-1(1-31) is a receptor-mediated phenomenon. There are at least two subtypes of endothelin receptors, termed endothelin ET_A and ET_B (22). We examined the effects of a specific endothelin ET_A receptor antagonist, BQ123(15), and a specific endothelin ET_B receptor antagonist, BQ788(16), on the ET-1(1-31)-induced arteriolar vasoconstriction. After the microperfusion of the isolated afferent arteriole was completed, we added an endothelin receptor antagonist to the perfusate and bath medium. Following preincubation with an endothelin receptor antagonist, the effect of ET-1(1-31) was evaluated in the manner described above.

Data Analysis

Values are expressed as means±SEM. The data were analyzed by one-way analysis of variance, followed by a least significant different test. $P<0.05$ was considered to be a statistically significant difference.

RESULT

Effect of ET-1(1-31) on the lumen diameter of microperfused afferent and efferent arterioles.

The basal lumen diameter of microperfused afferent arterioles was 13.4±0.7 μm (n=7), and the

basal lumen diameter of microperfused efferent arterioles was 9.8±0.4 μm (n=7) (Fig. 3). ET-1(1-31) decreased the lumen diameter of afferent and efferent arterioles dose-dependently (Fig. 3). In some experiments, we examined the duration of the constrictor effect of ET-1(1-31). Five minutes after the addition of 10^{-6}M ET-1(1-31), 10 exchanges of the bath medium were made to remove residual ET-1(1-31). ET-1(1-31)-induced renal arteriolar vasoconstriction lasted for 60 minutes at least (data not shown).

Effect of phosphoramidon on the ET-1(1-31)-induced afferent arteriolar vasoconstriction.

As illustrated in Fig. 4, phosphpramidon (10^{-5}M), an inhibitor of endothelin-converting enzyme, did not affect on the ET-1(1-31)-induced afferent arteriolar vasoconstriction. These results suggest that the vasoconstrictor effect of ET-1(1-31) is not due to the conversion of ET-1(1-31) to ET-1.

Effects of endothelin receptor antagonists on the ET-1(1-31)-induced afferent arteriolar vasoconstriction.

BQ123(10^{-7}M), a specific endothelin ET_A receptor antagonist, completely abolished the ET-1(1-31)-induced afferent arteriolar vasoconstriction (Fig. 5). On the other hand, pretreatment with BQ788(10^{-7}M), a specific endothelin ET_B receptor antagonist, did not affect the ET-1(1-31)-induced vasoconstriction (Fig. 5). These results suggest that ET-1(1-31) decreased the lumen diameter of the isolated microperfused afferent arteriole via ET_A receptor or ET_A like receptor.

Effect of ET-1 on the lumen diameter of the microperfused afferent arteriole.

As shown in Fig. 6, ET-1 decreased the lumen

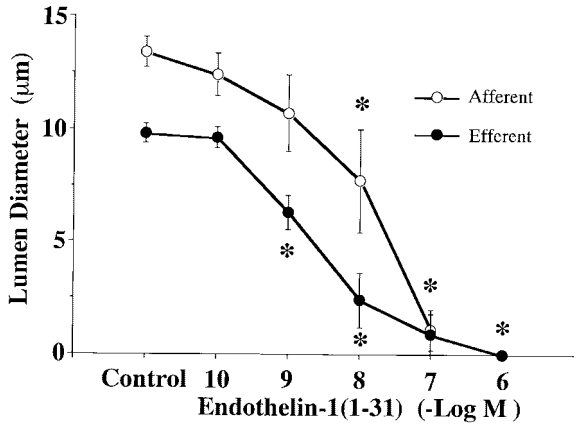

Fig. 3. Dose-response effect of endothelin-1(1-31) on the lumen diameter of microperfused afferent (n=7) and efferent arterioles (n=7). *p<0.05, compared with each control.

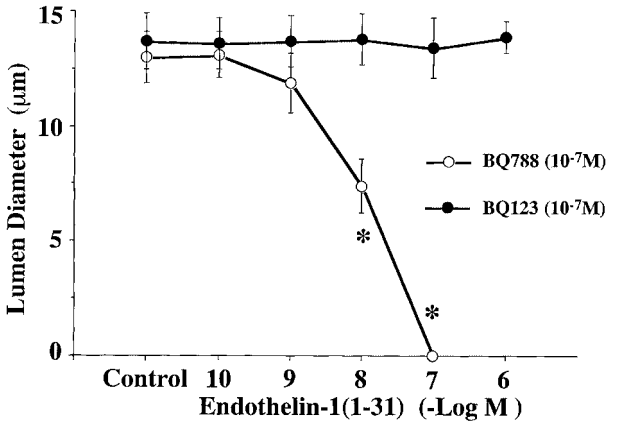

Fig. 5. Effect of endothelin-1(1-31) on the lumen diameter of microperfused afferent arterioles pretreated with BQ123(n=7), an ET_A receptor antagonist, or BQ788(n=4), an ET_B receptor antagonist. *p<0.05, compared with each control.

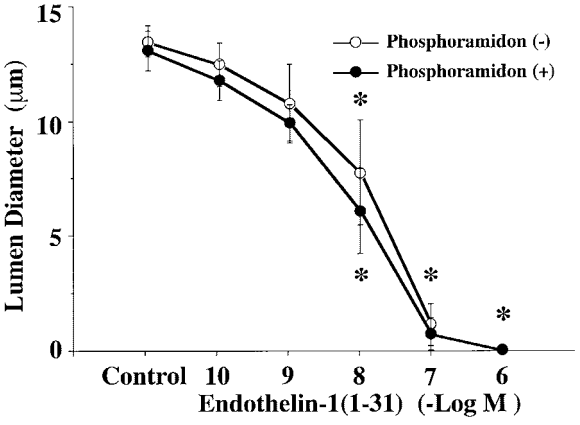

Fig. 4. Effect of endothelin-1(1-31) on the lumen diameter of microperfused afferent arterioles pretreated with (n=7) and without phosphoramidon (n=7). *p<0.05, compared with each control.

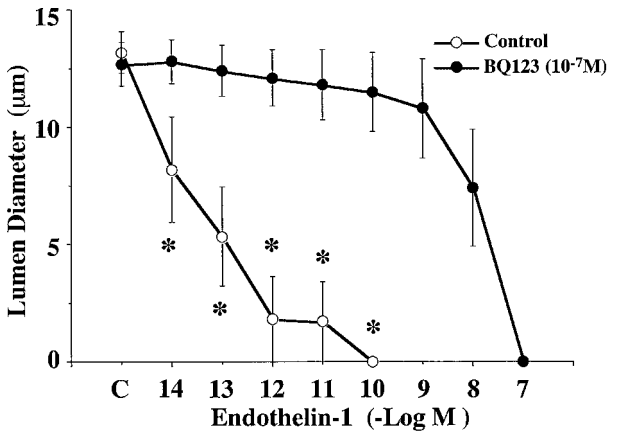

Fig. 6. Dose-response effect of endothelin-1 on the lumen diameter of microperfused afferent arterioles pretreated with (n=3) and without (n=7) BQ123, an ET_A receptor antagonist. *p<0.05, compared with each control.

diameter of isolated microperfused afferent arterioles dose-dependently. ET-1 was more potent than ET-1(1-31). BQ123(10^{-7}M), a specific endothelin ET_A receptor antagonist, inhibited ET-1-induced afferent arteriolar vasoconstriction.

DISCUSSION

ET-1 has been reported to induce powerful and long-lasting vasocontractile responses in various vessels with particularly potent actions on renal vascular beds (1, 3-6). Since the afferent arteriole is not only a resistance vessel in renal circulation, but also a major component which regulates kidney function, it would be important to understand the action of ETs in this vessel. In this study, we demonstrated that ET-1(1-31) decreased the lumen diameter of microperfused renal afferent and efferent arterioles dose-dependently. Phosphoramidon, an ET converting enzyme inhibitor, did not affect the ET-1(1-31)-induced afferent arteriolar vasoconstriction. ET-1(1-31)-induced renal arteriolar vasoconstrictin was inhibited by BQ123, an ET_A receptor inhibitor, but not by BQ788, an ET_B receptor.

We already reported that the plasma concentration of immunoreactive ET-1(1-31) was similar to that of ET-1 in young healthy volunteers (10), and that ETs(1-31) exist in human granulocytes and lungs at similar levels to those of ETs (11, 12). We also found that ET-1(1-31) increased [^{3}H]-thymidine incorporation into the cultured human coronary artery smooth muscle cells and cell numbers to a similar extent as ET-1 (13). Nakao *et al*. (9) reported that human mast cell chymase specifically converted big ETs to the 31-amino acid peptide ETs(1-31)s, which are different in amino acid length from the well-known 21-amino acid ETs. These findings suggest that ET-1(1-31) is a bioactive peptide in humans and is deeply involved in chymase-related pathophysiological processes in humans. It has been confirmed that human vascular tissue has a chymase-dependent angiotensin II(Ang II)-forming pathway. Human chymase is highly efficient in converting Ang I to Ang II (23). Miyazaki and Takai suggested that chymase plays a major role in the vascular Ang II-generating system, particularly in the case of vascular injuries (24). Their conclusion was as follows. In the normal state, a vascular angiotensin converting enzyme (ACE) regulates local Ang II formation and plays a crucial role in the regulation of blood pressure, whereas chymase is stored in mast cells and shows no Ang II-forming activity. On the other hand, chymase is activated immediately upon release into the extracellular matrix in vascular tissue after mast cells have been activated by a stimulus such as injury by a catheter or grafting of vessels (24). Increased expression of chymase in mast cells was related to the severity of interstitial fibrosis in human transplant rejected kidneys (25). We already comfirmed that ET-1(1-31) increased intracellular free Ca^{2+} concentration (26) and stimulated the proliferation of cultured human mesangial cells (10). Taken together, ET-1(1-31) may play an important role in chymase-related pathophysiological processes in humans.

In this study, we demonstrated that ET-1(1-31) decreased the lumen diameter of microperfused afferent and efferent arterioles via ET_A or ET_A -like receptors of the cells. Phosphoramidon, an inhibitor of endothelin converting enzyme (ECE), at a concentration of 10^{-5}M, had no effect on ET-1(1-31)-induced renal arteriolar vasoconstriction. These results suggest that renal arteriolar vasoconstriction caused by ET-1(1-31) is not the consequence of conversion to ET-1 by ECE. Our results are consistent with the finding that ECE requires the C-terminal structure of big ET-1 for enzyme recognition and is not able to cleave ET-1(1-31) (27). However, ET-1 was about 10^3～10^4-times more potent than ET-1(1-31) in our experimental condition. Although there is general agreement that the renal vasculature has enhanced sensitivity to ET-1, the constrictor potency relative to other physiological agonists remains controversial. Lanese *et al*. reported that EC_{50} of ET-1-induced afferent arteriolar vasoconstriction was $5.2\pm1.7\times10^{-11}$M, using isolated microperfused rat afferent arterioles (28). In the isolated perfused hydronephrotic rat kidney preparation, Loutzenhiser *et al.* (29) found that the lumen diameter of afferent arterioles decreased by 41% to 0.3×10^{-9}M ET-1. Edwards *et al.* (30) reported similar EC_{50} values in afferent arterioles to ET-1 in the order of 10^{-9}M using isolated rabbit afferent arterioles. Bloom *et al.* (31) found that 10^{-8}M ET-1 decreased the lumen diameter of afferent arterioles by 39±2%, using the split rat hydronephrotic kidney preparation. The marked differences in ET-1 responses in renal arterioles is difficult to explain. Although we found a large difference in constrictor potency between ET-1(1-31) and ET-1 in an *in vitro* isolated microperfused rabbit arteriole preparation, it should be noted that ET-1(1-31) itself has biological activity.

In previous studies, we demonstrated that ET-1(1-31)

stimulated human coronary smooth muscle cells and mesangial cell proliferation to a similar extent as that of ET-1 (10, 13). We also demonstrated that ET-1(1-31) caused a rapid and significant activation of mitogen-activated protein (MAP) kinases in a concentration-dependent manner in various cultured cells to a similar extent as that of ET-1 (10, 13, 32, 33). These effects of ET-1(1-31) were inhibited by BQ123, but not by BQ788. This suggests that the cell responses induced by ET-1(1-31) are mediated through ET_A or ET_A-like receptors. ET-1(1-31) increased the intracellular free Ca^{2+} in cultured smooth muscle cells and this activity of ET-1(1-31) was about 10-times less than that of ET-1 (34, 35). On the other hand, ET-1(1-31)-induced renal afferent arteriolar vasoconstriction was about 1000 to 10000-times less potent than ET-1 in our experimental condition. If the cell responses induced by ET-1(1-31) are mediated through an ET_A receptor, we can not explain the large potency difference. Although we have no evidence that the receptor of ET-1(1-31) is different from ET_A, the results suggest the existence of a different receptor(s) that mediates ET-1(1-31)-induced cell response.

In conclusion, ET-1(1-31) decreased the lumen diameter of afferent and efferent arterioles dose-dependently. ET-1(1-31)-induced renal arteriolar vasoconstriction may be mediated by ET_A -like receptors.

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