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Production of nitric oxide from endothelial cells by 31-amino-acid-length endothelin-1, a novel vasoconstrictive product by human chymase

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Abstract

Human chymase selectively converts big endothelin (ET)-1 to 31-amino-acid-length ET-1 [ET-1(1-31)]. In this study we examined effect of ET-1(1-31) on endothelial function. ET-1(1-31) evoked contraction in a concentration-dependent manner at $>10^{-8}$ M, which was about 10 times weaker than that of conventional ET-1 [ET-1(1-21)]. BQ485, an ETA receptor antagonist, completely abolished ET-1(1-31)–induced contraction, but BQ788, an ETB receptor antagonist, slightly enhanced it, suggesting that ET-1(1-31) relaxes artery via endothelium. On endothelial cells, ET-1(1-21) and ET-1(1-31) increased $[Ca^{2+}]i$ and produced NO, both of which were significantly inhibited by BQ788 and not by BQ485. These results indicate that ET-1(1-31) increased $[Ca^{2+}]i$ and produced NO in endothelial cells through ETB receptor similarly with ET-1(1-21), although slight diference in effect on smooth muscle cells.

Keywords: Endothelin; Endothelium; Nitric oxide; Chymase

Introduction

Endothelin (ET) is a peptide which was purified from culture solution of the porcine coronary artery endothelial cells in 1988 (1), and it has strongly and continuously constrictive

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(1,2) and proliferative effects (3,4) to the various smooth muscles ETs take part in remodeling of vessel wall and endothelial thickness in arteriosclerosis. ET-1,2,3 make isopeptides family of ET, but mainly ET-1 appears in vessel. ET receptors have, at least, two types of receptors, i.e. ETA and ETB receptors (5,6). ETA receptor has affinity to ET-1,2 and ET-B has affinity to all three types of ET isopeptides equally. Although vascular endothelial cells express ETB receptor that mediate relaxation, via nitric oxide (NO) production, the effect of ET-1(1-21) on the vascular smooth muscles was mainly mediated by ETA receptor (5).

Endothelin converting enzyme produces ET-1 with 21 amino acids [ET-1(1-21)]. Recently, we found that human chymase selectively converts big endothelin (ET)-1 to 31-amino-acid-length ET-1 [ET-1(1-31)] (7). This novel compound exists in human tissues and its serum concentration elevated in the patients with acute myocardial infarction (manuscript submitted for publication). However, there have been no studies on NO production of this compound. In this study we examined NO production by this novel peptide in the porcine coronary artery, and the results were compared to those of ET-1(1-21).

Materials and methods

Vasoconstrictive response

Vasoconstrictive responses were measured by Magnus tubes (UFER, Kishimoto Ika, Kyoto, Japan). Coronary artery ring from the porcine heart was cut into 2 to 3 mm wide rings, and then hooked and bathed in organ bath which contained Tyrode's solution babbled by 95% O_2 and 5% CO_2 mixed gas. Tyrode's solution was exchanged every 20 minutes, tension of 1.5 g was loaded to measure developed tension.

When tension became stable, vasoconstrictive response was measured by 50 mM KCl solution. Once tension became into stable state, Magnus tube was washed out with Tyrode's solution. Vasoconstrictive response by ETs was expressed as relative contraction by 50 mM KCl solution. Tyrode's solution contains 118.4 mM NaCl, 4.9 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25.0 mM $NaHPO_4$, 1.2 mM KH_2PO_4, 11.1 mM glucose. ET-1(1-31) having two intramolecular disulfide bonds, was synthesized by Peptide Institute (Osaka, Japan) using solution synthesis procedure (8).

Cell culture and measurement of intracellular calcium concentration

Endothelium was scraped from the coronary artery of the pig obtained from the local slaughterhouse and suspended in Dulbecco's minimum essential medium (DMEM; Flow Laboratories, Puteaux, France) containing 20% fetal calf serum (Flow Laboratories). They were grown on the cover slips in the culture dish in a 5% CO_2 atmosphere at 37°C and subcultured.

Fura-2/acetoxymethyl ester (fura-2/AM) (Wako Pharmaceuticals, Tokyo, Japan) was used in measurement of intracellular calcium concentration. Four μM fura-2/AM were added, and incubated for 30 minutes at 37°C. Then cells were washed out with normal Tyrode's solution again, cover glass was transferred into circulated temperature controlled chamber of analysis system ARGUS-50 (Hamamatsu Photonics, Hamamatsu, Japan).

Measurement of NO production by the electron paramagnetic resonance spin trapping

The amount of NO produced by endothelium was measured with electron spin resonance method which was modified by us (9,10). But briefly, Fe and diethyl-dithiocarbamate (DETC) as the trapping agents were added to the unstable compound NO, then comparatively stable radical NO-Fe-DETC was made. The amount of NO was measured by detection of the resonant spectrum with microwave radiation at the lower temperature. Porcine aorta, which was removed of the adipose tissue around the vessel was cut into 5 mm wide rings. These rings were cleansed by normal Tyrode's solution, put into the plate with 24 holes, and in this well 1 ml of normal Tyrode's solution was added. ET-1s and their receptor antagonists were administered into the well. Two minutes later the reaction was ceased by the trapping agent $FeSO_4$, DETC and albumin. Solution was transferred into tube, and frozen immediately. After these procedures EPR signals were measured by spectrometer (Nihon Koden, Tokyo, Japan). Measurements were done at -163 ± 1°C, modulation was 6.3 gauss, and gain was 5000 with 20mW microwave. The concentration of NO trapped was semiquantitatively determined by comparing the intensities of the first low-field derivative EPR signal heights and their amplitudes.

Results

Vasoconstrictive response

Fig. 1 shows vasoconstrictive responses of porcine coronary artery by ET-1(1-21) and ET-1(1-31). ET-1(1-21) evoked constriction in a concentration-dependent manner at $>10^{-9}$ M, although that of ET-1(1-31) at $>10^{-8}$ M, i.e., about 10-fold weaker potencies. BQ485 (0.3

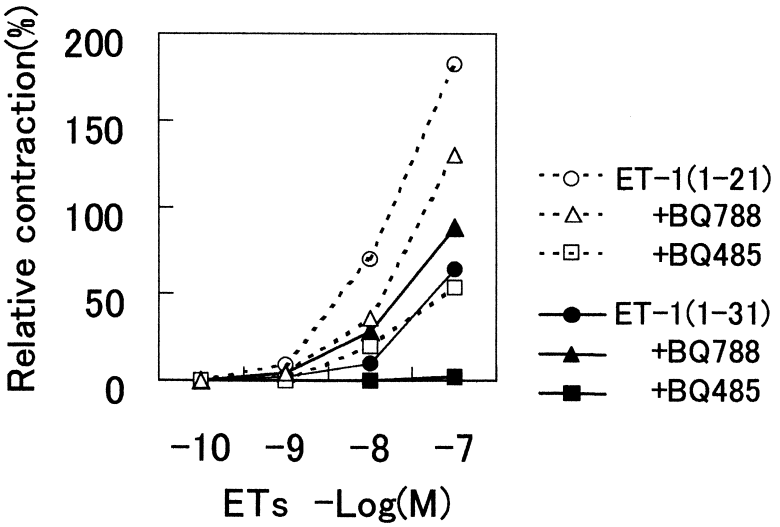

Fig. 1. Vasoconstriction evoked by ET-1(1-21) and (1-31) and effects of endothelin A and B receptor antagonists. ET-1(1-21) constriction in a concentration-dependent manner at ET-1(1-31) $>10^{-9}$ M, although evoked constriction in a concentration-dependent manner at $>10^{-8}$ M, and it's contraction was weaker than that of ET-1(1-21). The numbers of data were greater than 8 in each experiment, and SD was omitted to avoid confusion.

μM), an ETA receptor antagonist, almost completely suppressed the constriction caused by both ET-1(1-21) and ET-1(1-31). BQ788 (1 μM), an ETB receptor antagonist, slightly decreased contraction (not significant) by ET-1(1-21) in endothelium-intact and denuded arteries (data of denuded artery not shown). However, the contraction by ET-1(1-31) was slightly enhanced by 1 μM BQ788 in endothelium-intact artery (not significant). Enhancement of contraction was completely abolished in the endothelium-denuded rings or in the presence of 100 μM N^G-nitro-L-arginine methyl ester (L-NAME, data not shown). There exists endothelin converting enzyme (ECE) in the endothelium, which might convert ET-1(1-31) to ET-1(1-21), we also tested effect of ET-1(1-31) in the presence of phosphoramidon, an inhibitor of ECE. In the presence of 10 μM phosphoramidon, contracting effect of ET-1(1-31) was not altered.

Measurement of intracellular calcium concentration

Fig. 2 shows both ET-1(1-21) and ET-1(1-31) increased $[Ca^{2+}]i$ in a concentration-dependent manner in cultured endothelial cells. ET-1(1-21) produced slightly larger increase in $[Ca^{2+}]i$, although it did not reach the level of statistical significance. Fig. 3 shows that 1 μM BQ788 inhibited increase in $[Ca^{2+}]i$ by 10^{-7} M ET-1(1-31) although 0.3 μM BQ485 did not alter $[Ca^{2+}]i$ (n=7). Similar results were obtained by ET-1(1-21) (n=7). In the presence of 10 μM phosphoramidon, ET-1(1-31) could induce $[Ca^{2+}]i$ increase, which was similar in degrees with those without phosphoramidon (n=5).

Measurement of NO production

Fig. 4 summarizes the effect of 10^{-7} M ET-1(1-31) on NO production. NO production evoked by ET-1(1-31) was not inhibited by 0.3 μM BQ 485. However, 1 μM BQ788 significantly inhibited NO production. ET-1(1-21) at a concentration of 10^{-7} M produce slightly greater NO ($p<0.05$) than ET-1(1-31). This increase was also suppressed by 1 μM BQ788.

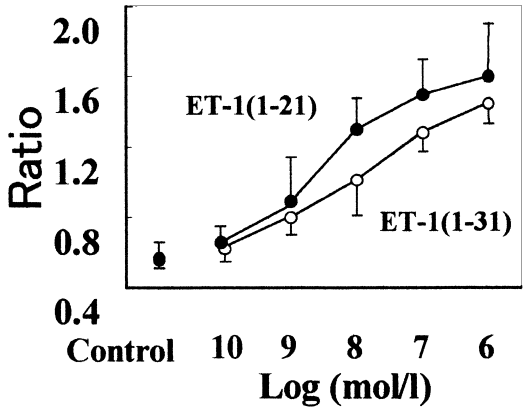

Fig. 2. Effects of ET-1(1-21) (n=8) and ET-1(1-31) on (n=11) in cultured vascular endothelial cells. Endothelial $[Ca^{2+}]i$ was expressed as ratio of F340/F380.

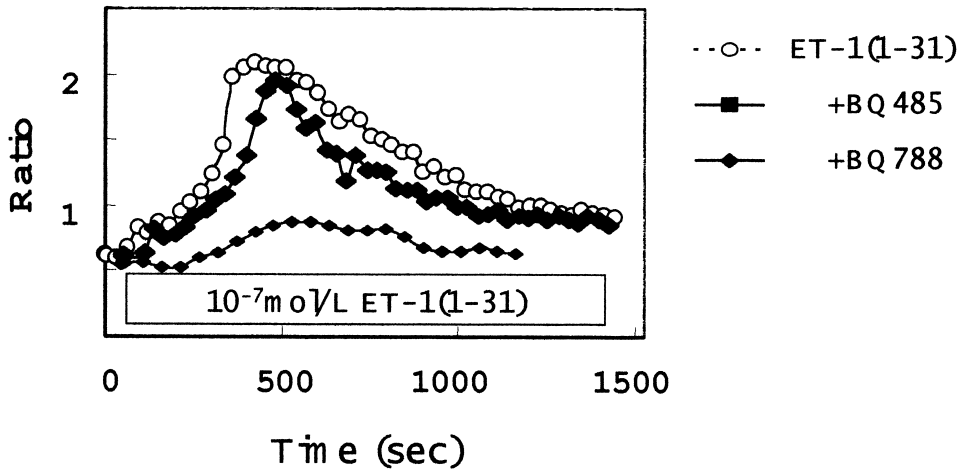

Fig. 3. Effect of BQ485 and BQ788 on ET-1(1-31)-induced increase in $[Ca^{2+}]i$ in endothelial cells.

Discussion

In this study, we examined the ET receptor subtypes of endothelium to a novel peptide, ET-1(1-31), by measuring $[Ca^{2+}]i$ and NO production. Specificity to receptor subtypes was similar between ET-1(1-21) and ET-1(1-31) both in mobilization of $[Ca^{2+}]i$ of endothelial cells and production of NO. Studies using an inhibitor of ECE, showed that these effects were direct effects of ET-1(1-31), and were not the result of conversion of ET-1(1-31) to ET-1(1-21).

ET receptors have at least two types of receptors, which are ETA that has an affinity to ET-1, 2 and ETB to all three types of ET isopeptides equally. In the present study, BQ788, an

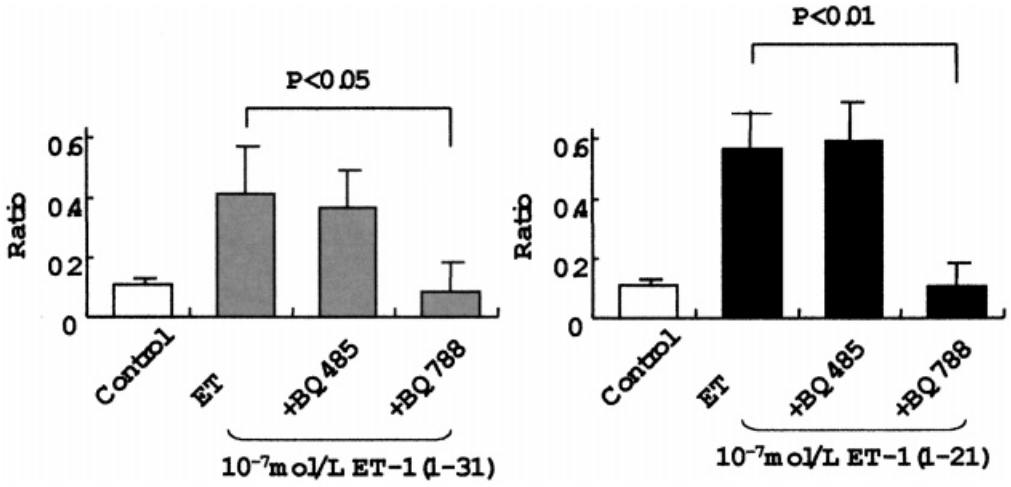

Fig. 4. Effect of 10^{-7} M ET-1(1-31) and ET-1(1-31) on NO production by the electron paramagnetic resonance spin trapping.

ETB receptor antagonist, did not suppress contraction by ET-1(1-31), or even enhanced it in the endothelium-intact artery, although BQ788 slightly but significantly suppressed ET-1(1-21)-induced contraction of artery with and without endothelium. This can be explained by that a portion of the ET-1(1-21)-induced contraction seems to be mediated by ETB receptors. In agreement with our study, some studies indicated that not only ETA but also ETB contributes to the constriction of smooth muscle in certain vessels (11–13). In addition, some studies reported that mRNA of both ETA and ETB receptors were detected by the reverse-transcription PCR in smooth muscle cells (14). These results indicated that in addition of ETA receptor ETB receptor also contributes contraction by ET-1(1-21) and that this effect is negligible in the case of ET-1(1-31).

Another explanation is that the contractile response to ETB stimulation is in part counteracted by release of nitric oxide. On the endothelium, mainly ETB receptor appears. When ET-1(1-21) binds ETB receptor, they increase $[Ca^{2+}]i$ in the endothelial cells. Subsequently, NO is derived from L-arginine by the NOS, which causes relaxation of the smooth muscle (15). In agreement with these findings, in the present study both ET-1(1-21) and ET-1(1-31) could increase $[Ca^{2+}]i$ of endothelium and produce NO. These effects were blocked by ETB antagonist but not by ETA antagonist. Although contraction of artery by ET-1(1-21) was about 10 times greater than that of ET-1(1-31), the potencies in the increase $[Ca^{2+}]i$ of in endothelial cells and production of NO between two peptides might be similar. ET-1(1-31), via ETB receptors, produced greater relaxation by endothelial-derived NO than contraction on smooth muscle. Thus, suppression of NO production by BQ788 induced further contraction in the endothelium-intact artery by ET-1(1-31).

These results indicate that the receptor subtypes are qualitatively similar between both peptides, although there seemed to be differences in balance between contraction and relaxing effects by ET-1(1-21) and ET-1(1-31).

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