ELSEVIER

European Journal of Pharmacology 303 (1996) 193–196

ejp

Short communication

Vasoconstriction by endothelin-1 in resistance and conduit portions of isolated human mesenteric arteries

Takashi Miyauchi [a,*], Yoko Tomobe [b], Tomohisa Ishikawa [b], Katsutoshi Goto [b], Yasuro Sugishita [a]

[a] *Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Ibaraki 305, Japan*
[b] *Department of Pharmacology, Institute of Basic Medical Sciences, University of Tsukuba, Ibaraki 305, Japan*

Received 4 January 1996; revised 19 February 1996; accepted 23 February 1996

Abstract

Although physiological processes related to vascular function differ greatly between resistance arteries and conduit arteries, it is not known whether the effects of endothelin-1 on these arteries differ in humans. In the present study, the conduit portion and the resistance portion of isolated human mesenteric arteries were suspended in a Krebs-Ringer solution. Norepinephrine and endothelin-1 produced concentration-dependent contractions in both portions. The EC_{50} value of norepinephrine in the resistance portion (3.7×10^{-7} M, $n = 8$) did not differ from that in the conduit portion (3.4×10^{-7} M, $n = 7$). However, the EC_{50} value of endothelin-1 in the resistance portion (3.0×10^{-9} M, $n = 8$) was significantly lower than that in the conduit portion (1.1×10^{-8} M, $n = 7$, $P < 0.05$). Although the maximum response to norepinephrine in the resistance portion (calculated as the percentage of 50 mM KCl-induced contraction) did not differ from that in the conduit portion, the maximum response to endothelin-1 in the resistance portion was significantly greater than that in the conduit portion. These results indicate that endothelin-1 induces more potent constriction in resistance portion than in conduit portion in isolated human mesenteric arteries.

Keywords: Endothelin-1; Norepinephrine; Resistance artery; Conduit artery; (Human)

1. Introduction

It is generally accepted that the physiological processes related to vascular function differ greatly between resistance arteries and conduit arteries. Resistance arteries, but not conduit arteries, are important for the regulation of both vascular resistance and regional blood flow (Folkow, 1982). When resistance arteries are constricted by neurohumoral factors such as norepinephrine and angiotensin II, vascular resistance increases and regional blood flow decreases.

Large arteries are thought of as passive conduits with the principal function of conducting and distributing cardiac output to various tissues. Although conduit arteries represent a low-resistance system that is not thought to be involved in blood flow regulation, they have an important buffering function that causes the phasic flow of cardiac output to be translated into a continuous flow at the peripheral level (Safar et al., 1984). Furthermore, the distensibility of these vessels affects the impedance to ventricular ejection, which is important in determining end-systolic ventricular wall stress, an important factor in the pathophysiology of cardiac hypertrophy and failure (Dzau and Safar, 1988).

Endothelin-1, an endothelium-derived peptide, induces potent vasoconstriction in various vessels in animals (Yanagisawa et al., 1988). In humans, we previously reported the potent and long-lasting vasoconstrictor effects of endothelin-1 and its presence in vascular endothelial cells, and suggested that endothelin-1 plays an important role in the regulation of vascular tonus in humans (Miyauchi et al., 1990). However, it is not known whether the effects of endothelin-1 on resistance arteries and on conduit arteries in humans differ. To study this question, isolated human mesenteric arteries were subjected to pharmacological analysis in the present study.

* Corresponding author. Tel.: (81) (298) 53-3210; fax: (81) (298) 53-3039.

PII S0014-2999(96)00160-4

2. Materials and methods

2.1. Subjects and tissues

Segments of mesenteric arteries were obtained from 8 male patients, aged 35–66 years (53.4 ± 3.2 years; mean ± S.E.M.), who were undergoing segmental resection of the intestine due to intestinal malignancy. None of the patients exhibited signs of vascular disease, metabolic disease, or connective tissue disorders. The patients were given diazepam, hydroxyzine hydrochloride, and/or atropine as premedication, and anesthesia was induced with nitrous oxide-oxygen. Supplementary doses of pancuronium bromide were given as needed. Immediately after removal, the vessels were transferred to an ice-cold Krebs-Ringer solution of the following composition (mM): NaCl, 113; KCl, 4.8; $CaCl_2$, 2.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; and glucose, 5.5. The arteries were divided into the following 2 groups: the conduit portion (6 ~ 8 mm in diameter) and the resistance portion (around 0.8 mm in diameter). The arteries had a macroscopically normal appearance. Written informed consent to participate in the study was obtained from each patient.

2.2. Pharmacological analysis

The following pharmacological analysis was performed as previously described (Miyauchi et al., 1990). After the artery was freed from the surrounding connective tissues, a 4-mm-long piece of the tissue was mounted in a silicon-coated 20-ml organ bath by means of 2 metal holders, one being anchored and the other being connected to a force displacement transducer for the measurement of isometric contractions. The solution was maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2. A resting tension of 1 g was applied to the tissue and an equilibration period of 2 h was allowed. During this period, the tissues were washed with fresh solution every 15 min. In our ring preparations, it was confirmed that 1 g was most appropriate for resting tension in the resistance portion of the isolated human mesenteric artery (Miyauchi et al., 1990). It was also confirmed that 1 g was appropriate for resting tension in the conduit portion of the isolated human mesenteric artery (our unpublished observation).

After equilibration, the maximum response to KCl (50 mM) was measured repeatedly at intervals of 30 min until steady responses were obtained (usually 3–4 times). Subsequently, the concentration-response relationship for endothelin-1 or norepinephrine was determined by means of a cumulative application. The endothelium was left intact and this finding was confirmed by the endothelium-dependent vasodilator response to 1 μM acetylcholine, which was defined as the vasodilator response being more than 30% of the pre-contraction with 1 μM methoxamine (α_1-adrenoceptor agonist).

2.3. Drugs and statistics

The drugs used were endothelin-1 (Peptide Institute, Osaka, Japan) and norepinephrine (Wako Pure Chemicals, Osaka, Japan).

Values are expressed as mean ± S.E.M. Statistical analyses were performed using Student's *t*-test for unpaired values. The *P* value less than 0.05 was accepted as the significance of the difference.

3. Results

3.1. The vasocontractile response to potassium

The maximum response to KCl (50 mM) in the resistance portion was 1.4 ± 0.3 ($n = 8$) g, whereas that in the conduit portion was 4.3 ± 0.5 ($n = 7$) g and these values were significantly ($P < 0.01$) different. Therefore, when we compare the responses to endothelin-1 or norepinephrine between in the conduit portion and the resistance portion, the responses to endothelin-1 or norepinephrine were represented as the percentage of maximum contraction to 50 mM KCl to normalize these responses.

3.2. Vasocontractile effects of norepinephrine

Norepinephrine produced concentration-dependent contractions in both the conduit and the resistance portion of the mesenteric artery (Fig. 1). The maximum response to

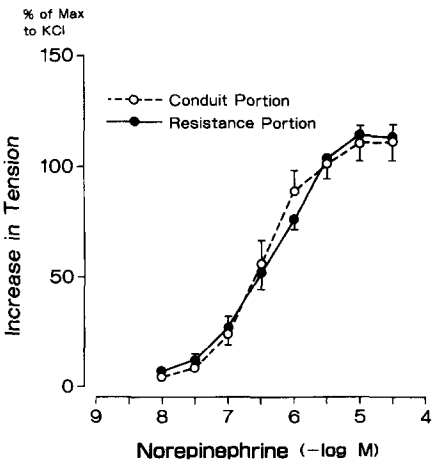

Fig. 1. The concentration-response curves of norepinephrine on the conduit portion ($n = 7$) and the resistance portion ($n = 8$) of isolated human mesenteric arteries. The abscissa indicates the anti-log molar concentration of norepinephrine. The ordinate indicates vasocontractile responses, which were represented as the percentage of maximum contraction to 50 mM KCl. Each point and bar represents the mean and S.E.M., respectively.

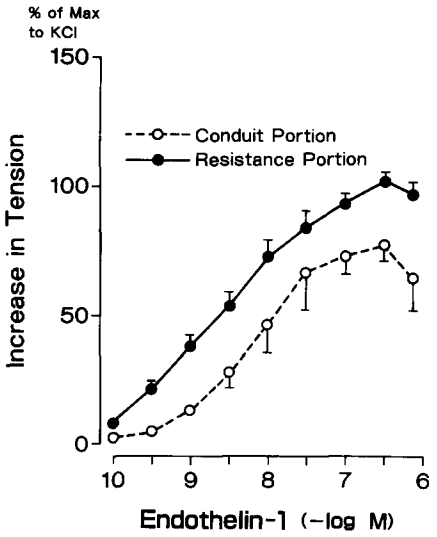

Fig. 2. The concentration-response curves of endothelin-1 on the conduit portion ($n = 7$) and the resistance portion ($n = 8$) of isolated human mesenteric arteries. The abscissa indicates the anti-log molar concentration of endothelin-1. The ordinate indicates vasocontractile responses, which were represented as the percentage of maximum contraction to 50 mM KCl. Each point and bar represents the mean and S.E.M., respectively.

norepinephrine in the resistance portion (115.2 ± 4.7% of 50 mM KCl-induced contraction, $n = 8$) did not differ from that in the conduit portion (111.3 ± 8.2% of 50 mM KCl-induced contraction, $n = 7$) (Fig. 1). When the responses were expressed as increase in g (grams), the maximum response to norepinephrine in the resistance portion was 1.6 ± 0.1 ($n = 8$) g whereas that in the conduit portion was 4.8 ± 0.4 ($n = 7$, $P < 0.01$) g. The EC_{50} value (the concentration eliciting a half-maximal response) of norepinephrine in the resistance portion (3.7×10^{-7} M, $n = 8$) did not differ from that in the conduit portion (3.4×10^{-7} M, $n = 7$).

3.3. Vasocontractile effects of endothelin-1

Endothelin-1 produced concentration-dependent contractions in both the conduit portion and the resistance portion (Fig. 2). The maximum response to endothelin-1 in the resistance portion (98.8 ± 3.1% of 50 mM KCl-induced contraction, $n = 8$) was significantly greater than that in the conduit portion (85.0 ± 5.1% of 50 mM KCl-induced contraction, $n = 7$, $P < 0.05$) (Fig. 2). When the responses were expressed as increase in g (grams), the maximum response to endothelin-1 in the resistance portion was 1.4 ± 0.1 ($n = 8$) g whereas that in the conduit portion was 3.7 ± 0.2 ($n = 7$, $P < 0.01$) g. The EC_{50} value of endothelin-1 in the resistance portion (3.0×10^{-9} M, $n = 8$) was significantly lower than that in the conduit portion (1.1×10^{-8} M, $n = 7$, $P < 0.05$) (Fig. 2), indicating that sensitivity to endothelin-1 was higher in the resistance portion than in the conduit portion.

4. Discussion

In the present study, the concentration-response curve for norepinephrine in the resistance portion of isolated human mesenteric arteries did not differ from that in the conduit portion. In contrast, endothelin-1 induced more potent constriction of the resistance portion than the conduit portion, since the maximum response to endothelin-1 (presented as the percentage of maximum contraction to 50 mM KCl) in the resistance portion was significantly greater than that in the conduit portion and the EC_{50} value of endothelin-1 in the resistance portion was significantly lower than that in the conduit portion. Therefore, it was suggested that the vasocontractile response to endothelin-1 is selectively augmented in resistance arteries, but not in conduit arteries, in the human mesenteric arteries.

Although the precise mechanisms for a difference in the response to endothelin-1 but not norepinephrine in various portions of the isolated human mesenteric vascular tree are unclear, the following explanations are possible. Since endothelin-1 exerts its vasocontractile action via the specific endothelin receptors (endothelin ET_A or ET_B receptors) (Davenport and Maguire, 1994), the difference in the density or subtypes of endothelin receptors on the vascular smooth muscles would produce a difference in the response to endothelin-1. Indeed, in human coronary artery tree, Godfraind (1993) reported that endothelin-1 is 10 times more potent in distal than in proximal segments and that endothelin ET_A receptors mediate the contractile response to endothelin-1 in distal, pre-resistant coronary arteries, but other endothelin receptors (probably endothelin ET_B receptors) are also involved in the contractile response of proximal segments. Alternatively, also in human coronary artery tree, Bax et al. (1994) suggested that the difference in the response to endothelin-1 between large proximal and small distal coronary artery segments could be due to facilitated diffusion of the large peptide molecules to the receptors in distal coronary artery segments. Therefore, it has a possibility that a difference in the response to endothelin-1 but not norepinephrine in various portions of the isolated human mesenteric vascular tree is due to differences in diffusion of the large endothelin-1 peptide molecules, but not of small norepinephrine molecules.

In the present study, it was shown that contraction to K^+ was far less in the resistance portion than in the conduit portion of the isolated human mesenteric arteries and that expression of contraction in g (grams) resulted in contraction being more potent in the conduit portions. Therefore, although the important observation of the present study was the difference in the normalized values between norepinephrine-induced contraction and endothelin-1-induced contraction in the resistance and conduit arteries, we cannot know whether the actual contraction induced by endothelin-1 at in vivo situation differs between in these 2 portions. Furthermore, the present paper

has the following study limitations. Since we confirmed that 1 g was most appropriate for resting tension in the resistance portion of the human mesenteric artery (Miyauchi et al., 1990) and that 1 g was also appropriate for resting tension in the conduit portion (our unpublished observation), a resting tension of 1 g was applied to both portions in the present study. However, since the resting tension may influence both EC_{50} values and the maximum responses to endothelin-1, we cannot know whether same findings are obtained under a different resting tension. Moreover, it should be emphasized that the present findings hold true for isolated arteries, which will tell us little about the in vivo situation. Indeed, it is difficult to translate tension obtained in this in vitro study to in vivo flow differences.

It is generally accepted that resistance arteries, but not conduit arteries, are important for the regulation of both vascular resistance and regional blood flow (Folkow, 1982). The fact that the EC_{50} values of endothelin-1 in the resistance portion of the isolated mesenteric artery (3.0×10^{-9} M) were far lower than those of norepinephrine (3.7×10^{-7} M) suggests that the vasoconstrictor action of endothelin-1 is about 100 times more potent than that of norepinephrine in the resistance portion. We previously reported that endothelin-1-like immunoreactivity is recognized in the vascular endothelium of the human mesenteric artery (Miyauchi et al., 1990). Recently, it was reported that the endothelin receptor antagonist TAK-044, which is a non-selective endothelin ET_A and ET_B receptor antagonist, has vasodilator effects in healthy humans (Haynes et al., 1995). These findings suggest that endogenously generated endothelin-1 contributes to the regulation of basal vascular tone in healthy humans. Furthermore, it has also been reported that bosentan, which is a non-selective endothelin ET_A and ET_B receptor antagonist, has vasodilator effects in patients with chronic heart failure (Kiowaki et al., 1995), suggesting that endogenously generated endothelin-1 also contributes to the regulation of basal vascular tone in patients with cardiovascular diseases.

The distensibility of conduit arteries affects the impedance to ventricular ejection, which is important in determining end-systolic ventricular wall stress, an important factor in the pathophysiology of cardiac hypertrophy and failure (Dzau and Safar, 1988). Furthermore, it is thought that large arteries are prone to injury because of continuous exposure to high pulsatile pressure and shear stress. Factors that decrease arterial compliance may increase the potential for vascular injury. We previously reported that a decrease in aortic compliance aggravates subendocardial ischemia in dogs with coronary artery stenosis (Watanabe et al., 1992), suggesting that changes in large vessel compliance affect peripheral blood flow in some conditions. In the present study, the EC_{50} values showed that the vasoconstrictor action of endothelin-1 was about 30 times more potent than that of norepinephrine in the conduit portion of isolated mesenteric arteries. However, since we have no knowledge of endothelin-1 concentrations next to vascular smooth muscle cells of the conduit artery, we cannot determine whether the local endothelin-1 in human conduit arteries contributes to changes in arterial compliance.

In summary, the present study showed that endothelin-1 induced more potent constriction of the resistance portion than the conduit portion in isolated human mesenteric arteries. These findings provide a possibility that endothelin-1 is important for the regulation of vascular function, particularly in the resistance portion of arteries in humans.

Acknowledgements

This study was supported by a grant from the Special Research Project on the Circulation Biosystem from University of Tsukuba, Uehara Memorial Foundation, Ciba-Geigy Foundation (Japan) for the Promotion of Science, Japan Research Foundation for Clinical Pharmacology, Kato Memorial Bioscience Foundation, and the Study Group of Molecular Cardiology, and by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan (7407019, 7557053, 7770493).

References

Bax, W.A., Z. Aghai, C.L.J. Tricht, C. Wassenaar and P.R. Saxena, 1994, Different endothelin receptors involved in endothelin-1- and sarafotoxin S6B-induced contractions of the human isolated coronary artery, Br. J. Pharmacol. 113, 1471.

Davenport, A.P. and J.J. Maguire, 1994, Is endothelin-induced vasoconstriction mediated only ETA receptors in humans?, Trends Pharmacol. Sci. 15, 9.

Dzau, V.J. and M.E. Safar, 1988, Large conduit arteries in hypertension: role of the vascular renin-angiotensin system, Circulation 77, 947.

Folkow, B, 1982, Physiological aspects of primary hypertension, Physiol. Rev. 62, 347.

Godfraind, T., 1993, Evidence for heterogeneity of endothelin receptor distribution in human coronary artery, Br. J. Pharmacol. 110, 1201.

Haynes, W.G., K.M. Skwarski, P.J. Wyld, H. Ripkeand and D.J. Webb, 1995, Vasodilator effects of the $ET_{A/B}$ antagonist, TAK-044, in man, Abstract Book of The Fourth International Conference on Endothelin, C24 (abstract).

Kiowaki, W., G. Sutsch, P. Hunziker, P. Muller, J. Kim, E. Oechslin, R. Schmitt, R. Jones and O. Bertel, 1995, Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure, Lancet 346, 732.

Miyauchi, T., Y. Tomobe, R. Shiba, T. Ishikawa, M. Yanagisawa, S. Kimura, Y. Sugishita, I. Ito, K. Goto and T. Masaki, 1990, Involvement of endothelin in the regulation of human vascular tonus; potent vasoconstrictor effect and existence in endothelial cells, Circulation 81, 1874.

Safar, M.E., A.C. Simon and J.A. Levenson, 1984, Structural changes of large arteries in sustained essential hypertension, Hypertension 6, 111.

Watanabe, H., S. Ohtsuka, M. Kakihana and Y. Sugishita, 1992, Decreased aortic compliance aggravates subendocardial ischemia in dogs with stenosed coronary artery, Cardiovasc. Res. 26, 1212.

Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto and T. Masaki, 1988, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332, 411.