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Potentiation by endothelin-1 of vasoconstrictor response in stroke-prone spontaneously hypertensive rats

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Abstract

Norepinephrine-induced vasoconstriction was significantly greater in perfused mesenteric arteries of stroke-prone spontaneously hypertensive rats (SHRSPs) compared with cases of age-matched Wistar Kyoto rats (WKYs). Neither endothelin ET_A receptor antagonist FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methyl-pentanoyl] amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl) propionic acid) nor endothelin ET_B receptor antagonist BQ788 [*N-cis*-2,6-dimethylpiperidinocarbonyl-L-g-methylleucyl-D-1-methoxycarbonyl-tryptophanyl-D-norleucine] affected the increased responses observed in SHRSPs, suggesting that endogenous endothelin-1 is not involved in this phenomenon. Norepinephrine-induced vasoconstriction was significantly enhanced by subpressor dose of endothelin-1 (0.3 nM), both in SHRSPs and WKYs. In SHRSPs, endothelin-1-induced enhancement was abolished by FR139317, in contrast to the case with WKYs, in which BQ788 markedly suppressed endothelin-1-induced augmentation of norepinephrine responses. Our results indicate that exogenous endothelin-1 enhances contractile responses to norepinephrine in mesenteric arteries of WKYs and SHRSPs, through activation of different receptor subtypes. © 2001 Published by Elsevier Science B.V.

Keywords: Endothelin-1; Endothelin ET_A receptor; Endothelin ET_B receptor; Stroke-prone spontaneously hypertensive rat (SHRSP); Norepinephrine; Mesenteric artery, rat

1. Introduction

Endothelin-1 is a potent vasoconstrictor peptide isolated from vascular endothelial cells (Yanagisawa et al., 1988). This peptide possesses a wide variety of biological actions (Goto et al., 1996) and may play a crucial role in various cardiovascular disorders, including cerebral vasospasm after subarachnoid hemorrhage (Matsumura et al., 1991), hypertension (Lüscher et al., 1993) and chronic heart failure (Miyauchi and Masaki, 1999). In addition to its direct vasoconstrictor action, endothelin-1 at subthreshold concentrations has been known to augment the responses to vasoconstrictor substances such as norepinephrine (Henrion and Laher, 1993; Yang et al., 1990) and serotonin (Yang et al., 1990), in various vascular tissues. Recently, we noted that exogenously applied subpressor dose of endothelin-1 (0.3 nM) potentiated the norepinephrine-induced vasoconstriction in perfused mesenteric artery of normotensive rats, via the activation of endothelin ET_B receptors (Kita et al., 1997). In contrast, such a potentiating effect of exogenous endothelin-1 was not observed in the same preparation of deoxycorticosterone acetate (DOCA)-salt hypertensive rats (Kita et al., 1998), in which endothelin-1 plays an important role in the development and/or maintenance of the pathogenesis (Schiffrin, 1995; Matsumura et al., 1999). Interestingly, in these animals, a vasoconstrictor response induced by norepinephrine itself was increased, and this increment was selectively abolished by endothelin ET_B receptor antagonist. Based on evidences that endothelin-1 content and its mRNA expression are elevated in vascular tissues of DOCA-salt hypertensive rats (Larivière et al., 1993a; Fujita et al., 1995), we hypothesized that endothelin ET_B receptor-mediated enhancement of vasoconstrictor responses to norepinephrine is stimulated tonically by endogenous endothelin-1 in DOCA-salt hypertensive rats.

To explore whether the above alterations are specific for DOCA-salt hypertensive animals, we examined the vasoconstrictor responses to norepinephrine in perfused mesenteric arteries of stroke-prone spontaneously hyper-

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tensive rats (SHRSPs) and age-matched Wistar Kyoto rats (WKYs), in the absence or presence of subpressor dose of endothelin-1. Moreover, we checked the receptor subtype involved in endothelin-1-induced potentiation of contractility to norepinephrine, using endothelin ET_A receptor antagonist FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1*H*-azepinyl)]carbonyl]amino-4-methyl-pentanoyl] amino-3-[3-(1-methyl-1*H*-indoyl)]propionyl]amino-3-(2-pyridyl) propionic acid) and endothelin ET_B receptor antagonist BQ788 [*N-cis*-2,6-dimethylpiperidinocarbonyl-L-g-methyl-leucyl-D-1-methoxycarbonyl-tryptophanyl-D-norleucine].

2. Materials and methods

2.1. Animals

SHRSPs were kindly donated by the Kinki University School of Medicine (Osaka, Japan). Age-matched WKYs were obtained from Charles River, Japan (Yokohama). These animals were used at 15 weeks of age. Systolic blood pressure of SHRSPs was monitored with a tail cuff and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan), the rats with a systolic blood pressure over 190 mm Hg were used.

2.2. Isolated perfused rat mesenteric arteries

The rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and the abdomen was opened by a midline incision. The superior mesenteric artery was cannulated with a polyethylene catheter and perfused at a constant flow rate of 3 ml/min with Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 U/ml) and streptomycin (0.1 mg/ml). The perfusate was constantly bubbled with O_2–CO_2 (95%–5%), to adjust the pH to 7.4–7.6 and for oxygenation. The mesentery was placed in a siliconized 30-ml organ bath maintained at 37–38°C and perfused in the open system for 20 min to avoid contamination by plasma components, thereafter, the perfusion system was changed to the closed system. Changes in perfusion pressure were measured at a point closed to the mesentery by means of a pressure transducer (AP 601G, Nihon Kohden, Osaka, Japan) and recorded on a polygraph (RM 6000G, Nihon Kohden).

2.3. Experimental protocols

Following the equilibration period of 20 min, a cumulative dose–response experiment (1, 3, 10 μM of norepinephrine) was started. Although responses to norepinephrine were gradually increased by repeated addition, the dose–response was reproducible after the third trial. Thus, the third dose–response was used as a result. To study the effects of endothelin-1 on pressor responses to norepinephrine, the arteries were perfused with endothelin-1 (0.3 nM) for 15 min before the dose–response experiment. In some experiments, FR139317 (1 μM) and BQ788 (1 μM) were treated 15 min prior to the start of perfusion with endothelin-1. In another experiment, to study the effects of FR139317 or BQ788 on pressor responses to norepinephrine, the arteries were treated with these antagonists for 15 min before the dose–response experiment. Each dose–response to norepinephrine was obtained in the closed perfusion system; drugs were perfused continuously. One dose–response data was obtained from each animal.

2.4. Drugs

Endothelin-1 was purchased from Peptide Institute, (Osaka, Japan). Endothelin-1 was dissolved in saline solution containing 0.1% heat-inactivating bovine serum albumin. FR139317, a kind gift from Fujisawa Pharmaceutical, Osaka, Japan, was dissolved in 0.1 N NaOH and then diluted with saline. BQ788, a kind gift from Banyu Pharmaceutical, Tsukuba, Japan, was dissolved in dimethyl

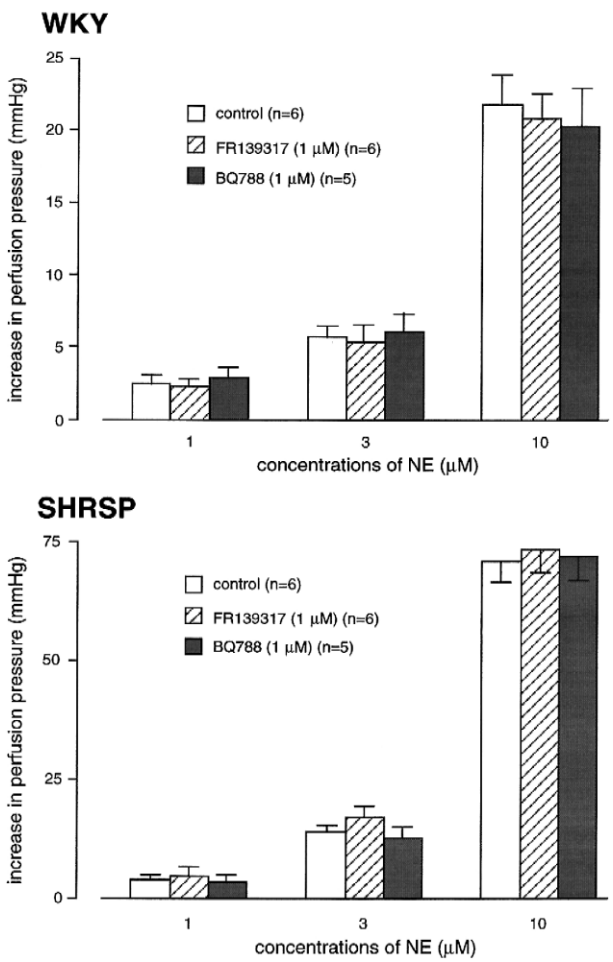

Fig. 1. Pressor responses to norepinephrine (NE) (1, 3, 10 μM) with or without FR139317 and BQ788, in perfused rat mesenteric arteries of WKYs and SHRSPs. Each column and bar represents the mean ± SEM.

sulfoxide and then diluted with saline. Other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.5. Statistical analysis

All values were expressed as mean ± SEM. For statistical analysis, we used the unpaired Student's *t*-test for two-sample comparisons and one-way analysis of variance combined with Duncan's new multiple range test for multiple comparisons. Differences were considered significant at $P < 0.05$.

3. Results

Fig. 1 shows pressor responses to norepinephrine (1, 3, 10 μM) in perfused mesenteric arteries of WKYs and SHRSPs. Norepinephrine elicited a concentration-dependent contraction in perfused mesenteric arteries. Pressor responses to norepinephrine at a concentration of 1 μM were significantly increased in SHRSPs (3.7 ± 0.2 mm Hg increase, $P < 0.05$ vs. WKY) compared with cases in age-matched WKYs (2.5 ± 0.4 mm Hg increase). Pressor responses to 3 and 10 μM of norepinephrine were also increased in SHRSPs (13.4 ± 0.8 and 72.3 ± 4.5 mm Hg

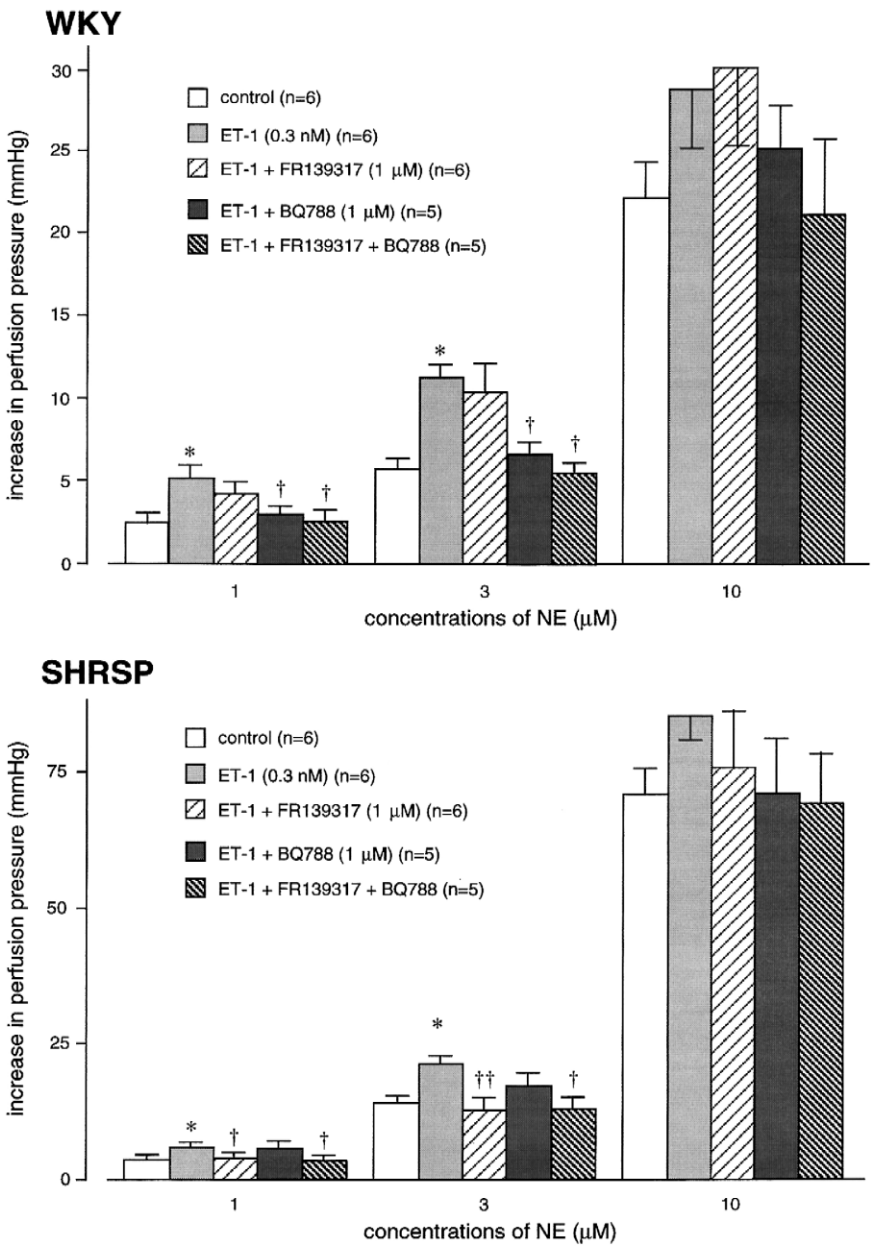

Fig. 2. Effects of endothelin-1 (ET-1) (0.3 nM) and its receptor antagonists (1 μM) on pressor responses to norepinephrine (NE) (1, 3, 10 μM) in perfused rat mesenteric arteries of WKYs and SHRSPs. Each column and bar represents the mean ± SEM, $^{*}P < 0.05$, compared with each control value. †$P < 0.05$, ††$P < 0.01$, compared with values in the presence of ET-1.

increase, respectively, $P < 0.01$ vs. WKYs), and these changes were statistically significant compared with those in WKYs (5.9 ± 0.5 and 22.1 ± 2.0 mm Hg increase, respectively). Neither FR139317 (1 μM) nor BQ788 (1 μM) affected vasoconstrictor responses to norepinephrine both in WKYs and SHRSPs.

Fig. 2 represents the effects of endothelin-1 on pressor responses to norepinephrine in perfused rat mesenteric arteries of SHRSPs and WKYs. When the arteries from WKYs were perfused with a subpressor dose of endothelin-1 (0.3 nM) for 15 min, there were significant enhancements of contractions induced by norepinephrine at concentrations of 1 and 3 μM (5.1 ± 0.9 and 11.4 ± 0.4 mm Hg increase, respectively). No significant enhancement by endothelin-1 was observed at 10 μM norepinephrine (28.9 ± 3.9 mm Hg increase). Endothelin-1 itself did not affect the basal perfusion pressure. Pretreatment of the arteries with BQ788 (1 μM) for 15 min prior to perfusion with endothelin-1 markedly attenuated the endothelin-1-induced enhancement of contractile responses to norepinephrine (3.0 ± 0.3 and 7.0 ± 1.0 mm Hg increase for 1 and 3 μM of norepinephrine, respectively), but no significant alterations were seen by the pretreatment with FR139317 at a concentration of 1 μM. Pretreatment with both BQ788 and FR139317 produced similar results to those seen with BQ788.

In SHRSPs, when the arteries were perfused with endothelin-1, contractions induced by norepinephrine at concentrations of 1 and 3 μM were significantly enhanced, although the magnitude was somewhat small (5.0 ± 0.3 and 18.9 ± 0.5 mm Hg increase, respectively) (Fig. 2, lower panel). No significant enhancement by endothelin-1 was observed at 10 μM norepinephrine. In contrast to cases of WKYs, the pretreatment with FR139317 abolished the endothelin-1-induced augmentation on pressor responses to norepinephrine, but BQ788 failed to significantly attenuate the peptide-induced actions. When the mixture of antagonists was applied, similar results to those seen with FR139317 were obtained.

4. Discussion

We observed increased contractile sensitivity to norepinephrine in the mesenteric artery of SHRSPs, when compared with that in age-matched WKYs. Similar findings have been demonstrated using femoral arteries prepared from WKYs and SHRs (Dohi et al., 1996), and using aortas and mesenteric arteries of DOCA-salt hypertensive rats (Wu et al., 1996). In perfused mesenteric arteries of DOCA-salt hypertensive rats, we recently noted that the increased pressor responses to norepinephrine was normalized to the level observed in normotensive control animals by the addition of ET_B-selective receptor antagonist (Kita et al., 1998). Taken together with evidences that endothelin-1 production is upregulated in vascular tissues of DOCA-salt hypertensive rats (Larivière et al., 1993a; Fujita et al., 1995), we suggested that endothelin ET_B receptor-mediated enhancement of vasoconstrictor responses to norepinephrine is stimulated tonically by endogenous endothelin-1 in DOCA-salt hypertensive rats. In the present study, neither ET_A receptor antagonist nor ET_B receptor antagonist affected the increased pressor responses to norepinephrine in SHRSPs, thereby suggesting that endogenous endothelin-1 does not contribute to the enhancement of contractile activity. Unlike in DOCA-salt hypertensive rats, vascular endothelin-1 production in SHRs is not upregulated (Larivière et al., 1993b). We also noted that vascular content of endothelin-1 in SHRSPs was similar to that of blood vessels in WKYs (unpublished observation). Thus, an enhancement of norepinephrine-induced vasoconstrictor response occurs through different mechanisms and mediators, between DOCA-salt hypertensive rats and SHRSPs. In the latter cases, other vasoactive substances (e.g., cyclooxygenase metabolites, etc.) (Zerrouk et al., 1998) may be contributive to the above enhancement, although precise mechanisms remain to be determined.

Subpressor doses of endothelin-1 are known to enhance the norepinephrine-induced vasoconstrictor responses in various vascular tissues (Henrion and Laher, 1993; Yang et al., 1990). Recently, we found that exogenously applied endothelin-1 or sarafotoxin S6c at subpressor dose (0.3 nM) potentiated the norepinephrine-induced vasoconstriction in perfused mesenteric artery of normotensive rats. In addition, the endothelin-1-induced potentiation was abolished by the pretreatment with BQ788, thereby suggesting the involvement of endothelin ET_B receptors (Kita et al., 1997). In the present study, similar findings were obtained in the same preparations of WKYs. On the other hand, when the arteries from SHRSPs were perfused with endothelin-1, the norepinephrine-induced contraction was significantly enhanced and this enhancement was abolished by ET_A receptor antagonist but not by ET_B receptor antagonist. The pathophysiological role of this phenomenon is unclear, since an acute treatment with ET_A receptor antagonist to SHRSPs produced a similar hypotensive effect to that seen in normotensive animals (Matsumura et al., 1995). Most recently, Iglarz et al. (1999) reported that prolonged ET_A receptor blockade decreased the responsiveness to phenylephrine in SHR aortas, without affecting blood pressure. In contrast, exogenous endothelin-1-induced potentiation of contractility to norepinephrine was not observed in DOCA-salt hypertensive rats. Based on evidences that endothelin-1 mRNA levels and its content in vascular tissues are increased in DOCA-salt rats, compared with sham-operated rats (Larivière et al., 1993a; Fujita et al., 1995), we suggested that endothelin-1 produced locally in mesenteric arteries is responsible for the potentiation of contractility to norepinephrine in DOCA-salt hypertensive rats, therefore exogenous endothelin-1 does not further enhance the contractility.

In our study, endothelin-1 failed to significantly potentiate vasoconstrictor responses to norepinephrine at high concentrations (10 μM), although norepinephrine-induced action tended to increase in the presence of endothelin-1. The most plausible explanation is that the vasoconstrictor response may be maximized by this concentration of norepinephrine.

Endothelin ET_A receptors, which occur mainly on vascular smooth muscle cells, mediate vasoconstriction (Lüscher et al., 1993), and ET_B receptors, which locate predominantly on endothelial cells, mediate vasodilation by generation of endothelium-derived relaxing factor and prostacyclin (Lüscher et al., 1993; Warner et al., 1989). However, it became apparent that non-ET_A receptors mediate some of the vasoconstrictor actions of endothelin-1. The renal vasculature and several other arterial and venous vascular beds appear to carry ET_B receptor-mediated constrictor elements in vitro (Clozel et al., 1992).

In conclusion, the present study indicated that exogenous endothelin-1 enhances contractile responses to norepinephrine in mesenteric arteries of WKYs and SHRSPs, through activation of different receptor subtypes. In addition, the role of endogenous endothelin-1 in vascular responsiveness to α-adrenoceptor stimulation seems to be different between SHRSPs and mineralocorticoid-dependent hypertensive animals. It remains to be seen whether the endothelin-1-induced enhancement of vasoconstrictor responses is involved in the pathogenesis of cardiovascular diseases.

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