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Short communication

Effects of endothelin-1 on norepinephrine-induced vasoconstriction in deoxycorticosterone acetate–salt hypertensive rats

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Abstract

We investigated the effects of endothelin-1 on pressor responses to norepinephrine in perfused mesenteric arteries of deoxycorticosterone acetate (DOCA)–salt hypertensive rats. The response to norepinephrine (10^{-6} M) was significantly increased in DOCA–salt rats compared with that in uninephrectomized control rats. Perfusion of the arteries with subpressor dose of endothelin-1 (3×10^{-10} M) for 15 min markedly enhanced the pressor responses to norepinephrine (10^{-6} and 3×10^{-6} M) in control rats and this effect was significantly prevented by BQ788 [*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyl-tryptophanyl-D-norleucine] (10^{-6} M), but not by FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1H-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1H-indoyl)]propionyl]amino-3-(2-pyridyl)propionic acid) (10^{-6} M). In DOCA–salt hypertensive rats, increased pressor response to norepinephrine was declined to the level of control rats by BQ788, but not by FR139317. In contrast to the case seen with control rat, exogenous endothelin-1 had little effect on pressor responses to norepinephrine in the arteries of DOCA–salt hypertensive rats. Total immunoreactive endothelin content in the arteries of DOCA–salt hypertensive rats was significantly higher than that of uninephrectomized control rats. These results suggest that endothelin-1 enhances contractile responses to norepinephrine through endothelin ET_B receptor. Moreover, this phenomenon is stimulated tonically by endogenous endothelin-1 in DOCA–salt hypertensive rats and may contribute to the maintenance of hypertension in DOCA–salt rats. © 1998 Elsevier Science B.V.

Keywords: Endothelin-1; Endothelin ET_B receptor; DOCA (deoxycorticosterone acetate)–salt hypertension; Norepinephrine; Mesenteric artery

1. Introduction

Endothelin-1 is a potent vasoconstrictor peptide isolated from vascular endothelial cells (Yanagisawa et al., 1988). Its biological actions have been widely studied and this peptide is considered to participate in pathological states such as cerebral vasospasm after subarachnoid hemorrhage (Matsumura et al., 1991), atherosclerosis (Lerman et al., 1991) and hypertension (Vanhoutte, 1993; Lüscher et al., 1993).

Endothelin-1 provokes potent vasoconstriction which might play an important role in the development and/or maintenance of hypertension. However, the circulating levels of endothelin-1 are not sufficient to induce direct contraction in vasculature, although the levels of endothelin-1 are reported to be high during hypertension (Suzuki et al., 1990; Shichiri et al., 1990). Several papers have demonstrated that subthreshold concentrations of exogenous endothelin-1 enhance the contractile responses to norepinephrine (Henrion and Laher, 1993; Yang et al., 1990) and serotonin (Wong-Dusting et al., 1991; Yang et al., 1990) in various vascular tissues. Moreover, Dohi et al. (1992) noted that threshold concentrations of exogenous endothelin-1 potentiate contractions to norepinephrine in mesenteric arteries of spontaneously hypertensive rats, and suggested that such a phenomenon might play an important role in hypertension.

Larivière et al. (1993) have demonstrated that endothelin-1 mRNA levels in aorta and mesenteric artery were increased in deoxycorticosterone acetate (DOCA)–salt hypertensive rats. Recently, we reported that endothelin-1 content in vascular tissues was increased in DOCA–salt rats, compared with that in uninephrectomized control rats (Fujita et al., 1995). We also found that ET receptor antagonist produced a significant hypotensive effect in anesthetized DOCA–salt hypertensive rats. Taken together, it seems likely that stimulation of endothelin-1

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production in vascular tissues is one of the factors which contribute to the development and/or maintenance of DOCA–salt-induced hypertension.

In the present study, we examined the effects of endothelin-1 on contractility to norepinephrine in perfused mesenteric arteries of normotensive and DOCA–salt hypertensive rats. Moreover, we investigated the receptor subtype involved in endothelin-1-induced potentiation of contractility to norepinephrine, using endothelin receptor antagonists, FR139317 and BQ788. To date, several endothelin receptor antagonists have been used to characterize the physiological roles of endothelin-1 and its receptor subtypes. Among them, FR139317 ((*R*)2-[(*R*)-2-[(*S*)-2-[[1-(hexahydro-1H-azepinyl)]carbonyl]amino-4-methylpentanoyl]amino-3-[3-(1-methyl-1H-indoyl)]propionyl]amino-3-(2-pyridyl)propionic acid) is a selective endothelin ET_A receptor antagonist which inhibits endothelin-1-induced vasoconstrictor effects, in vitro and in vivo (Sogabe et al., 1993). BQ788 [*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine] is a selective endothelin ET_B receptor antagonist, which competitively antagonizes the vasoconstriction induced by a selective endothelin ET_B receptor agonist BQ3020 in isolated rabbit pulmonary arteries, and which abolishes the depressor response to endothelin-1 in rats (Ishikawa et al., 1994).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (SLC, Hamamatsu, Japan), weighing 160–180 g, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the right kidney was removed via a right flank incision. After a 1-week postsurgical recovery period, the rats were treated twice weekly with DOCA suspended in corn oil, which was administered subcutaneously (15 mg/kg), and 1% NaCl was added to their tap water for drinking. Control rats were uninephrectomized but not given DOCA or salt. Systolic blood pressure was monitored with a tail cuff and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan). After 4 weeks of the above treatment, the rats with a systolic blood pressure over 180 mmHg were used. In another experiment, the rats were exsanguinated 4 weeks after treatment and concentrations of ET-1 were measured.

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 95% O_2–5% CO_2, to adjust the pH at 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, Nihonkohden, Osaka, Japan) and recorded on a polygraph (RM 6000G, Nihonkohden, Osaka, Japan).

2.3. Experimental protocols

Following the equilibration period of 20 min, a cumulative dose-response experiment (10^{-6} M to 10^{-5} M 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 (3×10^{-10} M) for 15 min before the dose-response experiment. In some experiments, FR139317 (10^{-6} M) or BQ788 (10^{-6} M) 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.

2.4. Tissue extraction and endothelin-1 measurement

Endothelin-1 was extracted from the mesenteric artery, according to the method of Fujita et al. (1994). Briefly, mesenteric arteries were removed from animals, rapidly cleaned of fat and adherent connective tissue, weighed and homogenized for 2 min in 4-ml ice-cold organic solution (chloroform/methanol, 2:1, including 1 mM *N*-ethylmaleimide). The homogenates were left overnight at 4°, then 0.2 ml distilled water was added to the homogenates. Those arterial homogenates were then centrifuged at 3000 r.p.m. for 30 min and the supernatant was stored. Aliquots of the supernatant were diluted 1/10 with a 0.09% trifluoroacetic acid (TFA) solution and applied to Sep-Pak C_{18} cartridges. The sample was eluted with 3 ml of 63.6% acetonitrile and 0.1% TFA. Eluates were dried in a centrifugal concentrator and the dried residue was reconstituted in assay buffer for radioimmunoassay (RIA). The clear solution was subjected to RIA. Recoveries of endothelin-1 from mesenteric arteries in our extraction procedures were approximately 80%.

RIA for endothelin-1 was carried out as described elsewhere (Matsumura et al., 1990). The limit of detection of endothelin-1 in this assay was 3 pg/tube. Endothelin-1 antiserum (a generous gift from Dr. Marvin R. Brown,

Department of Medicine, University of California, San Diego, USA) did not cross-react with big ET-1, as described (Hexum et al., 1990).

2.5. 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 M NaOH and then diluted with saline. BQ788, a kind gift from Banyu Pharmaceutical (Tsukuba, Japan), was dissolved in dimethyl sulfoxide and then diluted with saline. Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.6. Statistical analysis

All values were expressed as mean ± S.E.M. 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

After 4 weeks of DOCA/salt treatment, systolic blood pressure was significantly higher in DOCA–salt rats than in uninephrectomized rats (191.2 ± 3.5 and 131.6 ± 2.5 mmHg, respectively). Wet weight of mesenteric arteries was significantly higher in DOCA–salt hypertensive rats than in uninephrectomized control rats (90.2 ± 11.1 and 59.8 ± 7.2 mg). Total immunoreactive (IR)-endothelin content in the arteries of DOCA–salt hypertensive rats was also significantly higher than that of control rats (0.48 ± 0.06 and 0.25 ± 0.03 ng).

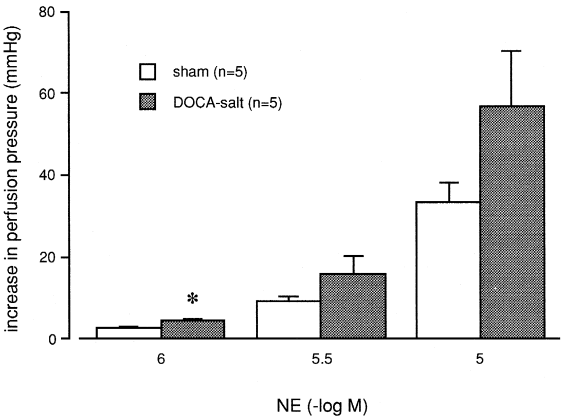

Fig. 1. Pressor responses to norepinephrine (10^{-6}–10^{-5} M) in perfused rat mesenteric arteries of DOCA–salt hypertensive and uninephrectomized control rats. Each column and bar represents the mean ± S.E.M. $* P < 0.05$, compared with the value in uninephrectomized control rat.

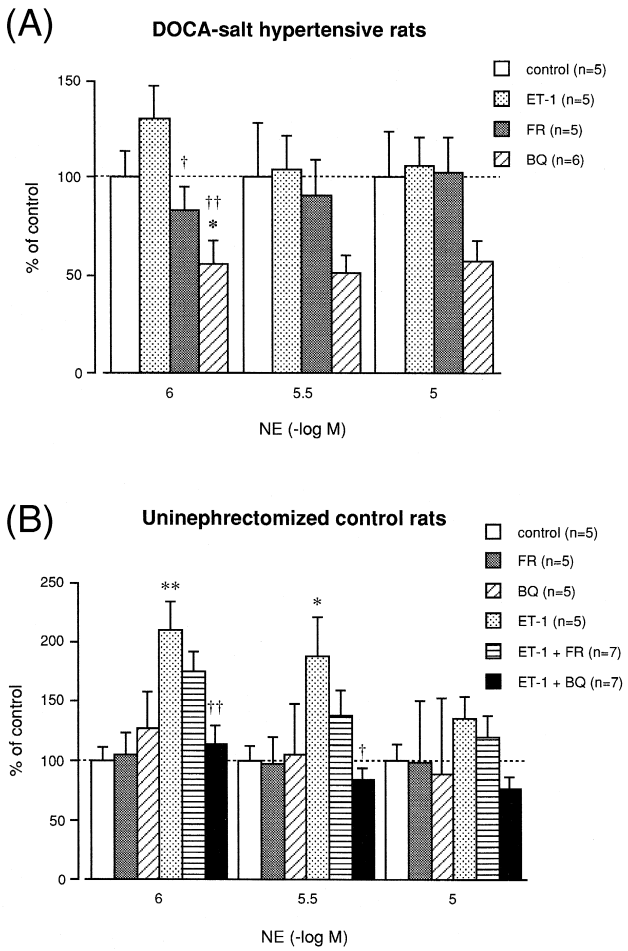

Fig. 2. Effects of endothelin-1 (3×10^{-10} M) and its receptor antagonists (10^{-6} M) on pressor responses to norepinephrine (10^{-6}–10^{-5} M) in perfused rat mesenteric arteries of DOCA–salt rats (A) and uninephrectomized control rats (B). Responses are expressed as a percent of control responses. Each column and bar represents the mean ± S.E.M. $* P < 0.05$, $** P < 0.01$, compared with each control value. $† P < 0.05$, $†† P < 0.01$, compared with values in the presence of endothelin-1.

Fig. 1 shows pressor responses to norepinephrine (10^{-6}–10^{-5} M) in perfused mesenteric arteries of uninephrectomized control and DOCA–salt hypertensive rats. Norepinephrine elicited a concentration-dependent contraction in perfused mesenteric arteries. Pressor responses to norepinephrine at a concentration of 10^{-6} M were significantly increased in DOCA–salt rats (4.5 ± 0.6 mmHg increase) compared with cases in uninephrectomized control rats (2.8 ± 0.3 mmHg increase). Pressor responses to 3×10^{-6} and 10^{-5} M of norepinephrine tended to increase in DOCA–salt rats (15.7 ± 4.4 and 56.8 ± 13.5 mmHg increase, respectively), but these changes were not statistically significant compared with those in control rats (9.3 ± 1.2 and 33.4 ± 4.6 mmHg increase, respectively).

Fig. 2 represents the effects of endothelin-1 on pressor responses to norepinephrine in perfused rat mesenteric arteries of DOCA–salt hypertensive and uninephrec-

tomized control rats. When the arteries from control rats were perfused with a subpressor dose of endothelin-1 (3×10^{-10} M) for 15 min, there were significant enhancements of contractions induced by norepinephrine at concentrations of 10^{-6} and 3×10^{-6} M (209.1 ± 24.3% and 187.8 ± 32.3% of each control value, respectively), but no significant alterations were observed at 10^{-5} M (134.8 ± 18.4% of the control value) (Fig. 2B). Endothelin-1 itself did not affect the basal perfusion pressure. Pretreatment of the arteries with BQ788 for 15 min prior to perfusion with endothelin-1 significantly attenuated the endothelin-1-induced enhancement of contractile responses to norepinephrine (113.6 ± 15.7% for 10^{-6} M and 84.9 ± 8.6% for 3×10^{-6} M of NE, respectively), but no significant alterations were seen in cases of FR139317 (173.7 ± 17.3% for 10^{-6} M and 138.0 ± 20.7% for 3×10^{-6} M norepinephrine, respectively). Both BQ788 and FR139317 had no apparent effects on pressor responses to norepinephrine. In DOCA–salt rats, when the arteries were perfused with endothelin-1, no significant alterations were observed in pressor responses to norepinephrine (130.6 ± 16.8, 104.0 ± 17.2 and 106.2 ± 14.9% for 10^{-6}, 3×10^{-6} and 10^{-5} M, respectively) (Fig. 2A). When BQ788 (10^{-6} M) was treated, increased pressor responses to norepinephrine (10^{-6} M) observed in DOCA–salt rats were significantly attenuated (55.6 ± 11.9% of the control value). FR139317 was without effect on the increased responses (83.3 ± 11.6% of the control value).

4. Discussion

The present study demonstrated that subpressor dose of endothelin-1 potentiates the pressor responses to norepinephrine in perfused rat mesenteric arteries mainly through endothelin ET_B receptor.

In perfused rat mesenteric arteries, norepinephrine elicited concentration-dependent contractions. When the arteries from uninephrectomized control rats were perfused with endothelin-1, the norepinephrine-induced contraction was markedly enhanced. These results are consistent with previous reports demonstrating that subthreshold concentrations of endothelin-1 potentiates the vasoconstrictor effect of norepinephrine in the rabbit aorta and ear artery and human left anterior descending coronary artery (Henrion and Laher, 1993; Yang et al., 1990).

The main purpose of the present study is to examine whether endothelin-1 also potentiates the contractility to norepinephrine in DOCA–salt hypertensive rats. Vascular reactivity to low dose of norepinephrine in DOCA–salt rats was about twofold over that in control rats. This finding is consistent with the previous report indicating that sensitivity to norepinephrine was increased in rat aorta (Katovich et al., 1984) and mesenteric arteries (Longhurst et al., 1988; Wu et al., 1996) of DOCA–salt hypertension compared with normotensive rats. When the arteries of the hypertensive rats were perfused with endothelin-1, pressor responses to the lower dose of norepinephrine was slightly potentiated, but no significant alterations were observed in contractility to norepinephrine. The question arises as to why endothelin-1-induced potentiation of contractility to norepinephrine was observed in normotensive rats, but not in DOCA–salt hypertensive rats. We and other investigators previously reported that endothelin-1 mRNA levels and its content in vascular tissues were increased in DOCA–salt rats, compared with uninephrectomized control rats (Larivière et al., 1993; Fujita et al., 1995). In the present study, we observed the increased content of endothelin-1 in mesenteric arteries of DOCA–salt hypertensive rats. Taken together, we assume 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 did not further enhance the contractility. To verify this hypothesis, we examined the effect of BQ788 on increased contraction to norepinephrine in DOCA–salt rats. As a result, increased pressor response to low dose of norepinephrine observed in DOCA–salt rats was declined to the level of control rats by BQ788. In contrast, BQ788 had no apparent effect on contractility to norepinephrine in control rats. These results suggest that vascular sensitivity to norepinephrine may be stimulated by endogenous endothelin-1 in DOCA–salt hypertensive rats.

Two distinct subtypes of endothelin receptors, ET_A and ET_B, have been characterized and cloned from bovine and rat lung, respectively (Arai et al., 1990; Sakurai et al., 1990). Endothelin ET_A receptors, which occur mainly on vascular smooth muscle cells, mediate vasoconstriction (Lüscher et al., 1993), and endothelin 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 endothelin ET_B receptor-mediated constrictor elements in vitro (Clozel et al., 1992; Cristol et al., 1993). In the present study, BQ788 effectively prevented endothelin-1-induced potentiation of contractile responses to norepinephrine in control rats. In DOCA–salt hypertensive rats, increased pressor responses to low dose of norepinephrine were attenuated by BQ788, but not by FR139317. These results suggest that endothelin-1-induced enhancement of contractility to norepinephrine is mediated by endothelin ET_B receptor in both groups of rats. Since the effect of endothelin-1 at lower dose of norepinephrine tended to be attenuated by FR139317, the possibility that the effects of endothelin-1 through endothelin ET_A receptor might partly contribute to endothelin-1-induced potentiation of contractile responses to norepinephrine cannot be ruled out. However, since the concentration (1 μM) of FR139317 used in this study is much higher than pA_2 value of 7.2 and since this concentration of

FRI39317 inhibits completely the contractile responses to endothelin-1 (Sogabe et al., 1993), it seems unlikely that endothelin-1 enhances the contractile responses to norepinephrine mainly via endothelin ET_A receptor.

In conclusion, the present study indicated that endothelin-1 enhances contractile responses to norepinephrine through endothelin ET_B receptor. Our results also suggest that this phenomenon is stimulated tonically by endogenous endothelin-1 in DOCA–salt hypertensive rats. Previous studies have suggested that enhanced vascular reactivity is responsible for the development of DOCA–salt-induced hypertension (Berecek et al., 1980; Ekas and Lokhandwala, 1980). Irrespective of the mechanisms, endothelin-1-induced enhancement of vascular contractility seems to play an important role in the pathogenesis of DOCA–salt-induced hypertension.

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