RELAXING EFFECT OF ATRIAL NATRIURETIC FACTOR ON ENDOTHELIN-PRECONTRACTED VASCULAR STRIPS

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Endothelin (ET), a peptide recently isolated from the supernatant of cultured porcine aortic endothelial cells, is a potent vasoconstrictor. On the other hand, atrial natriuretic factor (ANF) is a powerful vasorelaxant found in cardiocytes. Its effect was investigated in ET-precontracted rabbit vascular strips. ANF-induced a dose-dependent relaxation of maximally-precontracted mesenteric, renal and aortic strips. Mesenteric artery strips were more sensitive to ANF than either renal or aortic strips. The relaxant effect of ANF on ET-precontracted arteries was more potent than that of other vasorelaxant agents, such as isoproterenol and sodium nitroprusside. Renal and aortic arteries were more sensitive to the vasoconstrictor effect of ET than mesenteric strips. From these results, we conclude that ANF may play a role as a physiological antagonist of ET. The different sensitivity of vascular segments to ET could be due to varying vascular ET receptor densities.

Atrial natriuretic factor (ANF), a peptide synthesized and secreted by mammalian cardiocytes, exerts extremely potent vasorelaxant and natriuretic/diuretic activities (1). Although its mechanism of action has not yet been totally elucidated, its endothelium-independent effect (2,3) has been established in several isolated arterial preparations contracted by various agonists (4,5).

Since the discovery of acetylcholine-induced endothelium-dependent vasodilatation by Furchgott and Zawadzki (6), the role of the endothelium in the regulation of vascular tone has stimulated profound interest. Recently, a novel vasoconstrictor peptide known as endothelin (ET) was isolated by Yanagisawa et al. (7) from the supernatant of cultured pig aortic endothelial cells, and its amino acid sequence was determined. Although its sequence shows local homologies to a certain group of peptide neurotoxins (7,8), ET does not belong to any well-recognized vasoconstrictor family, considering its distinct constrictive effect on vascular smooth muscle (7). The same group (7) has demonstrated that ET causes a slow-onset and long-

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lasting endothelium-independent contraction of several preparations of arterial strips that is characteristically difficult to wash out, (although it is completely reversed by the addition of isoproterenol and glyceryl trinitrate) (7). In addition to its vasoconstrictive effect, ET has recently been shown to be a potent secretagogue for ANF in cultured neonatal rat atrial myocytes (9). Because of the antagonistic effects of these two native peptides, we have investigated the possible direct impact of ANF on ET-precontracted mesenteric, renal and aortic strips for a better understanding of the modulation of vascular responsiveness.

MATERIALS AND METHODS

Male New Zealand rabbits (1.8-2.0 kg) were anesthetized with sodium pentobarbital (30 mg/kg iv). Their thoracic aorta and mesenteric and renal arteries were quickly excised, and excess fat and connective tissue were gently trimmed off and cut helically. Each vascular strip (2x20-25 mm of the mesenteric artery, 1x15-20 mm of the renal artery and 3x30-35 mm of the aorta) was suspended in a 5-ml tissue bath containing continuously-oxygenated ($95\%O_2$-$5\%CO_2$) Krebs solution at 37°C at pH 7.4. The strips were mounted between a fixed base and a force displacement transducer (GRASS, FT-03C). The contractions were registered on a Model 7 GRASS polygraph.

A tension of 1.000-1.500 mg was applied to each mesenteric arterial strip, 500-700 mg to each renal strip and 2.500-3.000 mg to each aortic strip. The tension was adjusted and bathing fluid changed every 15 min. The strips were allowed to equilibrate for two hours before the experimental procedure began.

The composition of the solution used in this study was (mmol/liter): NaCl, 119; KCl, 4.7; KH_2PO_4, 1.8; $MgSO_4.H_2O$, 1.17; $CaCl_2.6H_2O$, 2.5; $NaHCO_3$, 25.0; and dextrose, 5.5.

Cumulative dose-response (D-R) curves of synthetic porcine ET (Peptide Institute, Inc., Osaka, JAPAN) were built up in some strips, and 20 min after the maximal constrictive response was attained, a cumulative D-R curve of ANF (rANF 99-126; Bio-Mega, Montreal, Quebec) was obtained. Isoproterenol (ISO, Sigma Chemical, St. Louis, Missouri) and sodium nitroprusside (SNP, Fisher, Fair Lawn, New Jersey) were studied in the same way with ET-precontracted aortic strips. The cumulative D-R curve of ANF during the contraction induced by the ED_{50} dose of norepinephrine (NE; L-norepinephrine bitartrate, Sigma Chemical, St. Louis, Missouri) (4) in aortic strips was also studied.

Values of the half-maximum effective dose (ED_{50}) were calculated by computer-assisted non-linear regression analysis with the ALLFIT program (10), based on a four parameter logistic equation. The data from each individually-fitted curve were transformed into negative logarithmic values. One-way analysis of variance was used to compare statistical differences between groups. The statistical significance of each group of non-sigmoidal curves was assessed by the same test for each dose.

RESULTS

Fig. 1 shows the results of the D-R curves of ET on rabbit aortic, mesenteric and renal strips. The ED_{50} of ET was calculated for the three parameters. The renal and aortic strips tended to be more sensitive to the constrictive effect of this peptide, but no significant

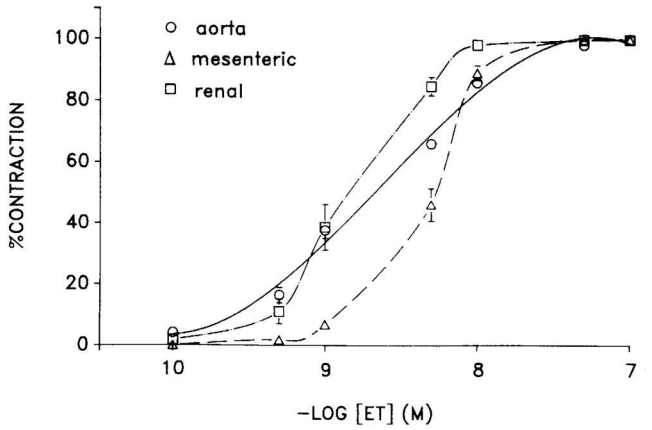

Figure 1. Dose-response curves of ET in rabbit aortic, mesenteric, and renal artery strips. Each point is the mean of the percentage of maximal responses to ET in each experiment; vertical lines indicate SE.

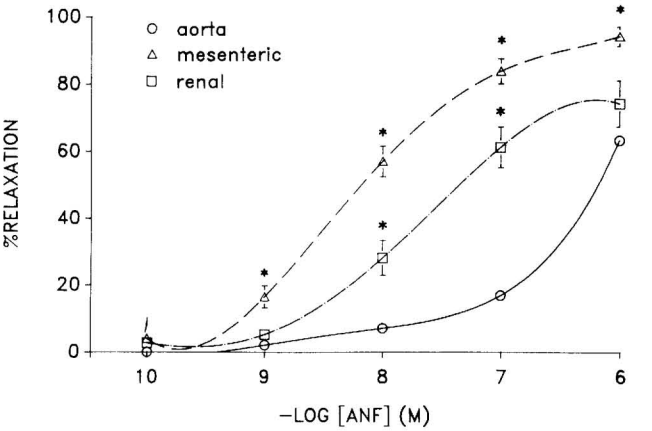

Figure 2. Dose-response curves of ANF on ET-precontracted rabbit aortic, mesenteric, and renal artery strips. Each point is the mean of the percentage of maximal responses to ANF in each experiment; vertical lines indicate SE, * $P \leq 0.001$ vs aorta.

Table I. Constrictive Effect of ET on Rabbit Aortic, Mesenteric, and Renal Artery Strips and the Relaxing Influence of ANF on These Same Strips

	ET		ANF	
	n	ED_{50}	*n*	ED_{50}
Aortic	8	2.02×10^{-9} *M*	16	5.77×10^{-7} *M*
Mesenteric	17	4.81×10^{-9} *M*	12	6.25×10^{-9} *M*
Renal	15	1.49×10^{-9} *M*	9	1.77×10^{-8} *M*

difference was found between them (Table 1). The D-R curve of the mesenteric strips was slightly displaced to the right.

Fig. 2 charts the relaxant effect of ANF on ET-precontracted strips. A significant difference ($p \leq 0.001$) was found for ED_{50} values between mesenteric and renal arteries (Table 1). A difference was also evident at several ANF doses between mesenteric and renal arteries and aortic strips (Fig. 2) The relaxant effect of ANF on ET-precontracted aortic strips was more potent than that of other vasorelaxants, such as isoproterenol (ED_{50} 1.06×10^{-6} M) and SNP (ED_{50} 2.59×10^{-6} M) (fig. 3). ANF produced a more sensitive dose-related relaxation (ED_{50} 1.95×10^{-8} M) in rabbit aortic strips that had been subjected to contraction with an ED_{50} dose of NE than in

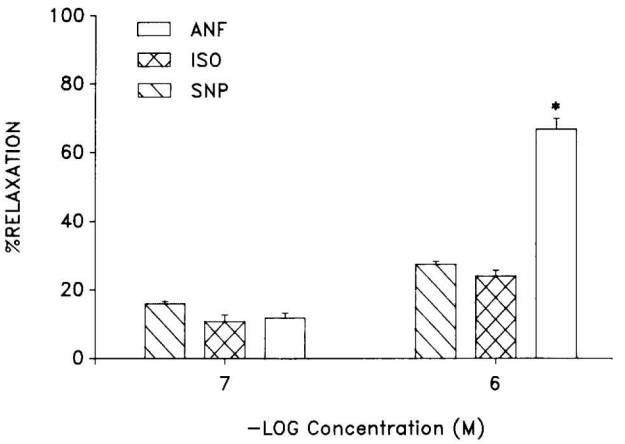

Figure 3. Relaxing effect of ANF ($n = 8$), ISO ($n = 8$), and SNP ($n = 8$) on ET-precontracted rabbit aortic strips. Each point is the mean of the percentage of maximal responses to vasorelaxant agents in each experiment; vertical line indicates SE. No statistical difference was found with doses lower than 10^{-7} *M*.

cumulative ET-precontracted aortic strips (data not shown).

DISCUSSION

It has been reported that porcine ET is one of the most potent vasoconstrictors known to date (7). Our results indicate that it has a constrictive effect on rabbit aortic, renal and mesenteric artery strips. This study is consistent with similar results obtained in a variety of blood vessels in many species (7,11). ET has a direct constrictive effect on smooth muscles, initiated by the mobilization of Ca^{2+} into cytosol

(7,12,13). Two differing lines of thought attempt to explain the actual mechanism of ET-induced contraction. The first suggests that ET-induced contraction is dependent on extracellular Ca^{2+} concentrations acting through potential-dependent channels (7,12), the other infers mobilization of Ca^{2+} from intracellular sites independently of extracellular Ca^{2+} levels (13). Finally, it has also been proposed that this process involves receptors that seem to be different from dihydropyridine recognition sites (14).

We now report that synthetic rat ANF exerts potent vasorelaxant activity in three different rabbit vascular preparations precontracted by synthetic porcine ET. It is known that ANF stimulates the production of cyclic guanosine 3', 5'-monophosphate (cGMP) (15). Consequently, the elevated levels of cGMP elicit a decrease of cytosolic Ca^{2+} needed for contraction. Three possible mechanisms have been proposed to explain this phenomenon: first, increased extrusion of Ca^{2+} through activation of membrane-associated Ca^{2+}-ATPase (5,16); second, inhibition of Ca^{2+} translocation through agonist- or receptor-operated Ca^{2+} channels (3,5); and third, sequestration of Ca^{2+} into the sarcoplasmic reticulum (16). Separate or together, these hypotheses suggest that ANF's mechanisms of relaxation antagonize those of ET-induced contraction (7,12-14).

The presence of specific affinity receptors for ANF in membranes isolated from several smooth muscle preparations is now well-established (17,18). Recently, Hirata et al. (12) demonstrated specific receptors for porcine ET in cultured rat aortic vascular smooth muscle cells, which suggests that the two peptides interact with the same target cells.

However, heterogeneity in the response of different vascular preparations to ANF has also been reported (4,19). This regional vasorelaxant diversity may be due to differences in the quantity or sensitivity of specific receptor sites for this peptide (2,4) and/or to an altered coupling of activated receptors to a second messenger system (i.e., particulate guanylate cyclase) (5). ET-induced vasoconstriction exhibits less variability among the three systems studied compared to the relaxation produced by ANF. Variability could also be due to a difference in distribution of high affinity ET receptors.

SNP mimicked the relaxation induced by ANF in ET-precontracted strips but was less sensitive than previously reported (19,20). Isoproterenol was also found to be less sensitive than ANF, since it did not completely reverse the ET-induced contractions, as observed by other groups (7) We did not, however, wash our strips before the addition of isoproterenol (7). ANF was more efficient in relaxing NE-precontracted strips than those precontracted with ET, but in the latter case the relaxation by ANF was performed after maximal doses of ET instead of the ED_{50}.

Yanagisawa et al. (11) have reported that the level of porcine preproendothelin mRNA in cultured endothelial cells and in the aortic endothelium in vivo is influenced by vasoactive agents (thrombin, adrenalin) known to produce endothelium-derived relaxing factor (EDRF). They suggest, based on these observations, that there is a distinct endothelium-mediated regulatory mechanism for blood pressure. In addition, the existence of binding sites for ANF on endothelial cells (21), coupled to the recent discovery that ET is

capable of inducing ANF secretion in cultured rat atrial myocytes (9), further supports the notion of a potential feedback regulatory loop involving both ET and ANF in arterial blood pressure.

At this stage, it is tempting to postulate that the antagonistic effects of these two native peptides on rabbit arteries (e.g., aorta, mesentery, renal) may participate in the modulation of systemic blood pressure and/or local blood flow. Meanwhile, further studies are required to elucidate their mechanisms of action and their potential interaction.

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