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Angiotensin-(1-7): a novel vasodilator of the coronary circulation

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Angiotensin-(1-7) [Ang-(1-7)] possesses novel biological functions that are distinct from angiotensin II (Ang II). In coronary arteries, the octapeptide Ang II and the heptapeptide Ang-(1-7) exert opposing actions. Ang II elicits vasoconstriction and Ang-(1-7) is a vasodilator. Ang-(1-7) elicits vasodilation by an endothelium-dependent release of nitric oxide. Further, the vasorelaxant activity is markedly attenuated by the bradykinin (BK) B_2 receptor antagonist icatibant and does not appear to be associated with the synthesis and release of prostaglandins. Ang-(1-7) vasodilation is mediated by a non-AT_1/AT_2 receptor, since [Sar^1Thr^8]-Ang II, but neither candesartan, an AT_1 receptor antagonist, nor PD123319, an AT_2 receptor antagonist, blocked the response. Specific and high affinity binding of ^{125}I-Ang-(1-7) to the endothelial layer of canine coronary arteries was demonstrated using ***in vitro*** *emulsion autoradiography. Binding was effectively competed for by either unlabeled Ang-(1-7) or the specific Ang-(1-7) antagonist [D-Ala^7]-Ang-(1-7). Additionally, Ang-(1-7) potentiated synergistically BK-induced vasodilation. The EC_{50} of BK vasodilation (2.45 ± 0.51 nmol/L* ***vs*** *0.37 ± 0.08 nmol/L) was shifted 6.6-fold left-ward in the presence of 2 μmol/L concentration of Ang-(1-7). The potentiated response was specific for BK, since Ang-(1-7) did not augment the vasodilation produced by either acetylcholine or sodium nitroprusside; further, it was specific for Ang-(1-7), since neither Ang I nor Ang II augmented the BK response. In contrast to the vasodilator actions of Ang-(1-7), the potentiated response was not blocked by candesartan, PD123319 or [Sar^1Thr^8]-Ang II. Novel studies from our group demonstrate that Ang-(1-7) is both a substrate and inhibitor for angiotensin converting enzyme (ACE). Ang-(1-7) was shown to retard the degradation of ^{125}I-[Tyr^0]-BK in coronary rings. These studies describe novel actions of Ang-(1-7) as a vasodilator and a local synergistic modulator of kinin-induced vasodilation in coronary arteries.*

Key terms*: angiotensin converting enzyme, angiotensin peptides, angiotensin receptors, bradykinin, coronary circulation, nitric oxide, kinins, vasodilation*

INTRODUCTION

Removal of the carboxyl-terminal phenylalanine from angiotensin II (Ang II) imparts selective properties to the heptapeptide angiotensin-(1-7) [Ang-(1-7)] (17). Unlike Ang II, Ang-(1-7) has no dipsogenic (20) or aldosterone-stimulating

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(36) effects, but similar to Ang II, it releases vasopressin (41,42), prostaglandins (27) and nitric oxide (43). It also exhibits potent natriuretic and diuretic actions in the kidney (14,21,24). Recent studies showed that Ang-(1-7) opposes the actions of Ang II (5,26,32). Infusion of Ang-(1-7) in spontaneously hypertensive rats (SHR) produced a transient decrease in blood pressure associated with increased urinary excretion of the 6-keto-PGF1α (prostacyclin metabolite) (4,5). In transgenic (mRen-2)27 hypertensive rats, cerebroventricular administration of antibodies to Ang-(1-7) caused significant elevation of blood pressure, whereas administration of a monoclonal antibody to Ang II reduced blood pressure (32). Moreover, acute treatment with an Ang-(1-7) monoclonal antibody partially reversed the antihypertensive effects of combined lisinopril/losartan treatment in SHR, a finding that shows that Ang-(1-7) may contribute to the antihypertensive effects of ACE inhibitors (26). Thus, these studies - taken together- suggest that Ang-(1-7) may be a counter regulator of the cardiovascular effects of Ang II by acting as a local modulator of vascular tone.

A local regional role for Ang-(1-7) as a vasodilator was first shown in the pithed rat (3). In piglet pial arterioles (3,31), the vasodilator effect was blocked by the cyclo-oxygenase inhibitor indomethacin. In perfused mesenteric and hindquarter vascular beds, Osei *et al* (34) reported that Ang-(1-7) vasodilation was mediated by the release of nitric oxide. Local production of Ang-(1-7) in the vasculature has been demonstrated (7,9,18,28,38). Ang-(1-7) is generated from either Ang I or Ang II by specific peptidases (7,18). Angiotensin converting enzyme inhibition was associated with 5- 50-fold increase in Ang-(1-7) both in tissues and in the circulation (9,28,38). In bovine, porcine and human aortic endothelial cells, and human umbilical vein endothelial cells, Ang I is processed to Ang-(1-7) by both neprilysin (neutral endopeptidase 24.11, EC 3.4.24.11) (40-50%) and prolyl endopeptidase (25-40%) (38). In vascular smooth muscle cells from SHR and Wistar Kyoto rats, Ang-(1-7) was the major product generated from Ang I, and its generation was dependent upon metallo-endopeptidase 24.15 (11). Further metabolism of Ang-(1-7) or Ang II by aminopeptidases and dipeptidases leads to the formation of the smaller fragments, Ang-(3-7) and Ang IV, which may also have biological function (22,40).

Ang-(1-7) has been identified in the venous effluent of the coronary sinus before and after converting enzyme inhibition and acute myocardial ischemia (39). Similarly, cardiac tissue levels of Ang-(1-7) in the rat increased 3-fold after administration of an ACE inhibitor (8). The local formation of Ang-(1-7) in the vasculature and the heart may convey cardioprotective effects by opposing the actions of Ang II. We review in this article evidence for a role of Ang-(1-7) in the modulation of coronary vasomotion.

ANG-(1-7) IS A CORONARY ARTERY VASODILATOR

Isometric tension was measured in intact canine coronary artery rings suspended in organ chambers perfused with 95% O_2 and 5% CO_2 at 37°C to characterize the vasoactive properties of Ang-(1-7). Coronary artery rings were preconstricted with 10 nmol/L of the thromboxane A_2 analogue U46619. Addition of Ang-(1-7) in concentrations ranging from 10^{-7} to 10^{-4} mol/L caused relaxation in a dose-dependent manner with a calculated EC_{50} averaging 2.73 ± 0.58 μmol/L (Fig 1). Removal of the endothelium abolished the response to Ang-(1-7), indicating the dependency of the response on an intact endothelium. Rings with intact endothelium were preincubated with the nitric oxide synthase inhibitor Nω-Nitro-L-Arginine (L-NA, 10^{-3} mol/L) to evaluate whether nitric oxide mediated this vasodilation. Pretreatment with L-NA eliminated the endothelium-mediated vascular relaxation of coronary artery rings in response to Ang-(1-7). Administration of the bradykinin (BK) antagonist icatibant (Hoe-140, 2 nmol/L) markedly attenuated by 75% the endothelium-mediated relaxation

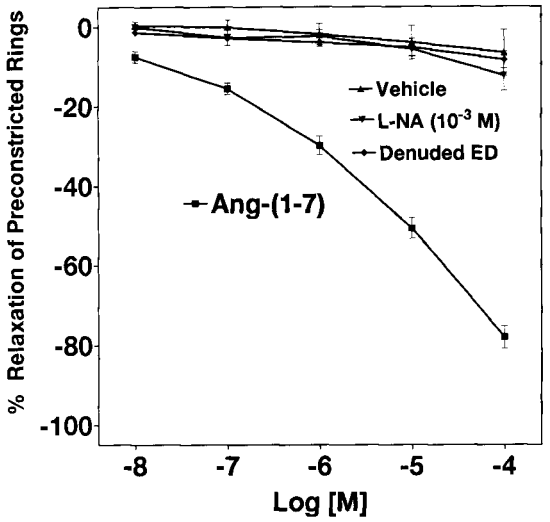

Fig 1. Average cumulative dose-response relaxation curves for Ang-(1-7) in precontracted left anterior descending (LAD) rings with intact (■) and denuded (◆) endothelium (ED). Effects of pretreatment of vessel with nitric oxide synthase inhibitor, 10-3 mol/L L-NA (▼), on the Ang-(1-7) dose-response curve also shown. Dose response curves to Ang-(1-7) generated in all vessels, except for time control experiments (VEHICLE, ▲), which were included to test for stability of LAD ring in absence of Ang-(1-7). Ordinate shows % relaxation of precontracted LAD vessels. In all experiments, 6-8 rings used in 2-4 separate experiments. Figure used with permission from *Hypertension* (6).

produced by Ang-(1-7). On the other hand, exposure of vascular rings to the cyclooxygenase inhibitor indomethacin (10 μmol/L) had no effect on the relaxation response produced by Ang-(1-7), suggesting that prostaglandins are not involved in the Ang-(1-7) mediated coronary response. Differences in the mechanism mediating Ang-(1-7) vasodilator responses in the heart and peripheral circulation suggest that Ang-(1-7) manifests heterogeneity or selective actions in alternate second messenger systems.

Coronary vessels were preincubated with either the AT_1, AT_2 receptor antagonists [CV11974, PD123319] or the non-selective angiotensin receptor antagonist [Sar^1 Thr^8]-Ang II, each at 1 μmol/L, to evaluate the angiotensin receptor subtype which may mediate the Ang-(1-7) response. The antagonists by themselves had no effect on isometric tension. Neither CV11974 (candesartan) nor PD123319 inhibited the Ang-(1-7) relaxation of coronary vascular rings (Fig 2). On the other hand, pretreatment of precontracted rings with the non-selective angiotensin receptor antagonist [Sar^1 Thr^8]-Ang II markedly attenuated the vasodilator response induced by administration of Ang-(1-7) (2,33).

Figure 3 shows that Ang-(1-7) binds specifically and with high affinity to the endothelial layer of canine coronary arteries (19,44). Binding to the canine coronary endothelium was effectively competed for by either unlabeled Ang-(1-7) or the selective Ang-(1-7) receptor antagonist [D-Ala^7]-Ang-(1-7). These binding results are in agreement with the further characterization of Ang-(1-7) receptor binding sites in bovine endothelial cells (45).

In summary, figure 4 illustrates the mechanism of Ang-(1-7) vasodilation in coronary vessels. Ang-(1-7) elicits coronary vasodilation that is specifically mediated by an endothelium-dependent release of nitric oxide. The attenuation of Ang-(1-7) vasodilator response in the presence of the B_2 receptor blocker further suggests that Ang-(1-7)-mediated release of nitric oxide may occur through the local

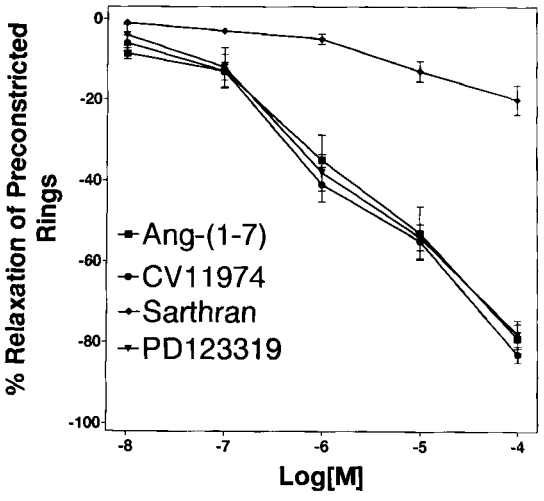

Fig 2. Effects of AT1 (●), AT2 (▼) and Sarthran (◆) Ang II receptor antagonists on cumulative dose-response relaxation curve for Ang-(1-7) (■) in precontracted LAD coronary artery with intact endothelium. Vessels preincubated for 30 min with selective antagonists [AT_1 (CV11974, 10^{-6} mol/L, ●), AT_2 (PD123319, 10^{-6} mol/L, ▼)] and non-selective antagonist (Sarthran, 10^{-6} mol/L, ◆) before Ang-(1-7) dose-response curve was done. Figure used with permission from *Hypertension* (6).

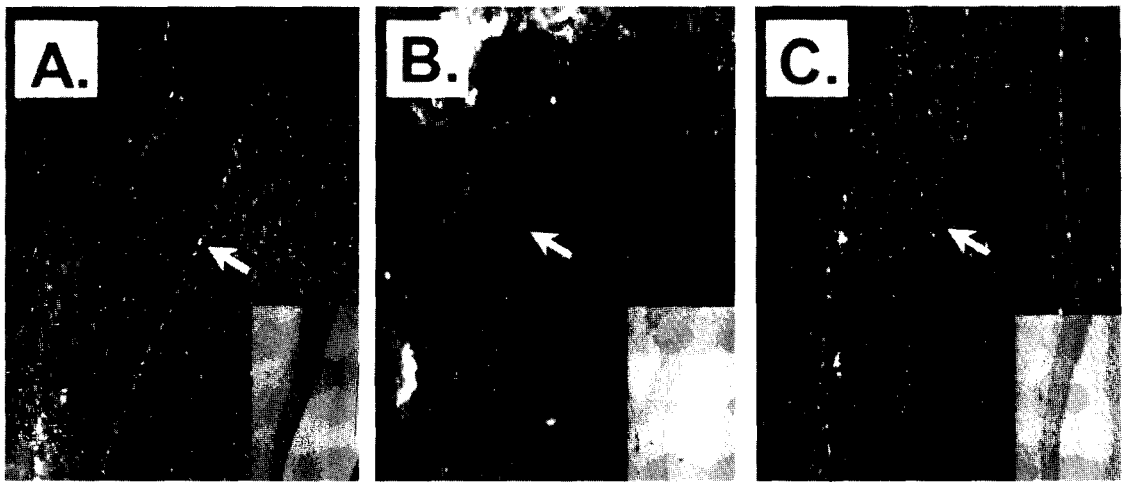

Fig 3. *In vitro* emulsion autoradiography showing specific binding of ^{125}I-Ang-(1-7) to endothelial cells on a canine coronary artery frozen-sectioned at 17 μm and incubated with 0.75 nmol/L ^{125}I-Ang-(1-7) in presence or absence of either 1 μmole/L Ang-(1-7) or D-Ala^7-Ang-(1-7). Sections processed for emulsion autoradiography as previously described (44). Arrows indicate endothelium lining the lumen. Abundant silver grains, indicative of specific binding of the radiolabeled peptide to the endothelial layer, are seen (A, Total). Fewer silver grains were observed over smooth muscle layer, and non-specific binding is apparent in surrounding fat. Endothelial ^{125}I-Ang-(1-7) binding effectively competed for by either unlabeled Ang-(1-7) (B) or [D-Ala7]-Ang-(1-7) (C). Inset shows hematoxylin and eosin-stained histological section. Figure used with permission from our review published in *Hypertension* (19).

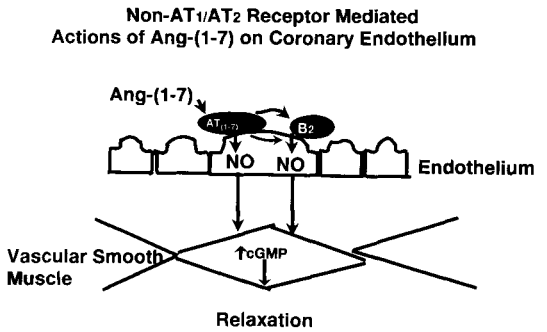

Fig 4. Mechanism of Ang-(1-7) vasodilation in coronary vessels. B_2 = bradykinin receptor; cGMP = guanosine 3'5' cyclic monophosphate; NO = nitric oxide; $AT_{(1-7)}$ = non-AT_1/AT_2 angiotensin receptor.

release of kinins. Finally, this vasodilator response to Ang-(1-7) is dependent upon a novel non-AT_1/AT_2 angiotensin $AT_{(1-7)}$ receptor.

SYNERGISTIC ACTIONS OF ANG-(1-7) AND BRADYKININ

Paula *et al* (35) showed that Ang-(1-7) may interact with kinins to augment bradykinin-induced vasodilator responses. They found that infusion of Ang-(1-7) potentiated BK-induced hypotensive responses in conscious rats. An angiotensin converting enzyme inhibitor (ACEI) quinaprilat enhanced Ang-(1-7)-induced vasodilation (37), a finding that may be interpreted as resulting from inhibition of BK degradation (15,25,30). Additionally, ACE inhibition was associated with significant elevations of Ang-(1-7), as blockade of ACE activity diverts the pathway of Ang II formation from Ang I into Ang-(1-7) (9,28,38). Both Porsti *et al* (37) and we (6) reported that the specific BK B_2 receptor antagonist icatibant inhibited the Ang-(1-7) evoked vasodilator response in coronary vessels. Abbas *et al* (1) showed that Ang-(1-7) can decrease blood pressure in anesthetized rabbits in the presence of BK by a mechanism dependent upon the B_2 receptor but not affected by angiotensin receptor antagonists (including an AT_1, AT_2 or non-selective angiotensin peptide antagonists). Furthermore, in rat kidney icatibant inhibited Ang-(1-7)-induced natriuresis and diuresis (23).

In order to explore the mechanisms of action of Ang-(1-7) in the bradykinin-potentiating response in coronary vessels, isometric tension was measured in intact

canine coronary artery rings suspended in organ chambers perfused with 95% O_2 and 5% CO_2 at 37°C. Coronary artery rings were preconstricted with 10 nmol/L of the thromboxane A_2 analogue U46619; one hour after equilibration, BK-induced relaxation (1 nmol/L) was produced. Ang-(1-7) at concentrations of 0.1~2 μmol/L was used to pretreat quiescent coronary artery rings for 10 min; then, 1 nmol/L BK-induced relaxation response was repeated in the pre-constricted rings. The BK-induced relaxation response was augmented by Ang-(1-7) in a dose-dependent manner (Fig 5).

The effect of Ang-(1-7) on the BK-induced relaxation response was specific for BK, since Ang-(1-7) did not augment relaxation responses induced by the endothelium-dependent vasodilator acetylcholine (0.05 μmol/L) (60 ± 5.5% *vs* 61 ± 6.9%; $p > 0.05$) or the endothelium-independent vasodilator sodium nitroprusside (0.1 μmol/L) (70 ± 8.2% *vs* 68 ± 8.5%; $p > 0.05$). Furthermore, the effect was specific for Ang-(1-7), since pretreatment with either Ang I or Ang II [EC_{50}: 2.45 ± 0.51 *vs* 2.33 ± 1.24 *vs* 2.09 ± 0.74; control *vs* Ang I *vs* Ang II; $p > 0.05$] had no effect on the BK-induced relaxation response.

Bradykinin (10^{-10}~10^{-6} mol/L) elicited concentration-dependent relaxation responses in submaximally pre-constricted rings with an EC_{50} of 2.45 ± 0.51 nmol/L. Ang-(1-7) at 2 μmol/L concentration elicited a significant 6.6-fold left-ward shift of the BK-induced relaxation response curve (0.37 ± 0.08 nmol/L) (Fig 6). Pre-incubation with indomethacin (10 μmol/L) had no significant effect on the BK-induced relaxation response nor on the potentiation response to BK produced by 2 μmol/l Ang-(1-7). In contrast, pretreatment with the nitric oxide synthase inhibitor L-NA (100 μmol/L) significantly shifted the BK-induced relaxation response curves to the right of both control and Ang-(1-7)-treated groups [control: 2.45 ± 0.51 *vs* 28.84 ± 9.68 nmol/L; $p < 0.01$; Ang-(1-7)-treated: 0.37 ± 0.08 *vs* 31.68 ± 9.59 nmol/L; $p < 0.01$; without *vs* with L-NA] and abolished the effect of Ang-(1-7) on the BK-induced relaxation response (EC_{50}: 28.84 ± 9.68 *vs* 31.68 ± 9.59 nmol/L; $p > 0.05$) (Fig 6).

Pretreatment with the bradykinin B_2 receptor antagonist icatibant at a 20 nmol/L concentration shifted the BK-induced relaxation response curves to the right of both control and Ang-(1-7)-treated group [control: 2.45 ± 0.51 *vs* 547.65 ± 19.63 nmol/L; $p < 0.01$; Ang-(1-7)-treated: 0.37 ± 0.08 *vs* 115.14 ± 23.96 nmol/L; $p < 0.01$;

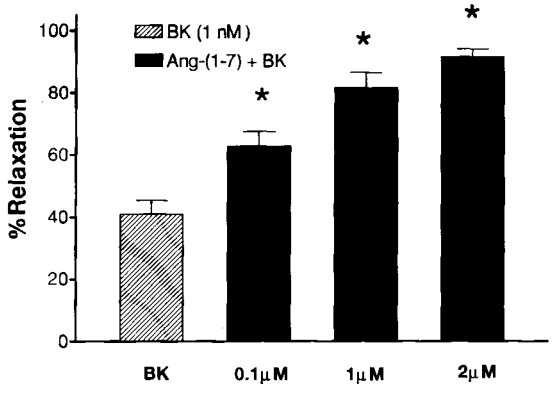

Fig 5. Average dose-response curves to 1 nmol/L bradykinin before (hatched bar) and after (black bars) 10 min pre-treatment with Ang-(1-7) (0.1~2 μmol/L). Values are means ± SEM's. BK responses are averages of 16 rings obtained from six dogs. All other groups contain 6~12 rings from 4~6 dogs. Figure used with permission from *Hypertension* (29).

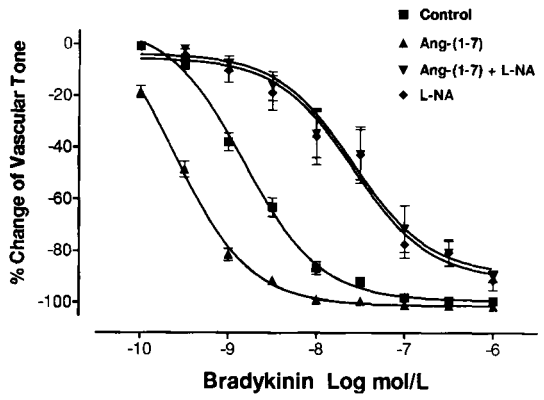

Fig 6. Cumulative dose response curves to bradykinin (10^{-10}~10^{-6} mol/L) alone (control, ■) and in presence of 2 μmol/L Ang-(1-7) (▲). Preincubation with nitric oxide synthase inhibitor L-NA (100 μmol/L, ◆) shifted BK-induced relaxation response to the right. Co-pretreatment with L-NA (100 μmol/L) and 2 μmol/L Ang-(1-7) (▼) showed no difference with L-NA treated alone. L-NA abolished Ang-(1-7) potentiating effect on BK-induced relaxation response. Values are means ± SEM's. Control group contains 33 rings from 12 dogs; and Ang-(1-7) treated group has 44 rings from 10 dogs. Other treatments include 6~10 rings from 3~4 dogs. Figure modified from a previous publication (29).

without *vs* with icatibant, respectively]. However, in the presence of 20 nmol/L icatibant, Ang-(1-7) still potentiated the BK-induced relaxation [EC_{50}: 547.65 ± 19.63 *vs* 115.14 ± 23.96 nmol/L; $p < 0.01$; control and Ang-(1-7) treated].

Preincubation with either the AT_1 or AT_2 receptor antagonists at a 10 times higher concentration (20 µmol/L CV11974 and 20 µmol/L PD123319) than Ang-(1-7) did not significantly inhibit the Ang-(1-7) potentiating response to BK (EC_{50} : 0.37 ± 0.08 *vs* 0.44 ± 0.06 *vs* 0.52 ± 0.16 nmol/L; Ang-(1-7) *vs* CV11974 *vs* PD123319). Similarly, pretreatment of pre-contracted rings with the non-selective angiotensin receptor antagonist Sar^1 Thr^8-Ang II (20 µmol/L) had no significant effect on the enhanced response to BK produced by Ang-(1-7) (EC_{50}: 0.37 ± 0.08 *vs* 0.41 ± 0.11 nmol/L, Ang-(1-7) *vs* Sar^1Thr^8 Ang II).

It is well known that ACE inhibitors prevent degradation of bradykinin. Thus, as expected, pretreatment with lisinopril (2 µmol/L) markedly shifted the BK-induced relaxation response curves to the left of both control and Ang-(1-7) treated group [control: 2450 ± 510 *vs* 0.25 ± 0.07 pmol/L; $p < 0.01$; Ang-(1-7) treated: 370 ± 80 *vs* 0.24 ± 0.05 pmol/L; $p < 0.01$; without and with lisinopril]. Pretreatment with lisinopril also abolished the potentiation response to BK produced by Ang-(1-7).

Recent experiments from our group (10,29) and from Deddish *et al* (12,13) provide an additional and novel explanation for the effects of ACE inhibition on the levels of Ang-(1-7) and its interaction with BK. These studies demonstrate that Ang-(1-7) is both a substrate and an inhibitor of ACE. We showed that Ang-(1-7) inhibited ACE activity purified from canine lungs with an IC_{50} of 0.65 µmol/L (29). Lisinopril, as expected, was a more potent inhibitor of canine ACE than Ang-(1-7), having an IC_{50} of 1.5 nmol/L. Pretreatment of coronary rings with 2 µmol /L Ang-(1-7) or 2 µmol/L lisinopril for 10 min significantly attenuated or blocked the rapid degradation of ^{125}I-[Tyr^0]-BK metabolism (Fig 7). Five minutes after the addition of radiolabeled BK, both Ang-(1-7) and lisinopril were shown to be equally effective in blocking its degradation. A more definitive study was done by Chappell *et al* (10) from our group, using ACE purified from canine lung. Their studies showed that ACE cleaved Ang-(1-7) into Ang-(1-5) with an affinity of 0.81 µM and a maximal velocity of 0.65 µmol/min/mg. The calculated turnover constant for the peptide was 1.8 s^{-1} with a catalytic efficiency (K_{cat}/K_m) of 2,200 s^{-1} mM^{-1}. In comparison, bradykinin exhibits a K_m of 0.5 to 1.0 µM and a K_{cat}/K_m of 3,900-6,000 s^{-1}. In keeping with these findings, the circulating half-life of Ang-(1-7) was markedly augmented in SHR treated with lisinopril (16; also Iyer *et al*, in press). These studies also showed that ACE is the primary mechanism for the inactivation of Ang-(1-7) in the rat lung (10,16). These findings provide a new and important insight into the mechanisms that may regulate the actions of Ang-(1-7) and its role in counteracting the effects of Ang II.

The interaction between Ang-(1-7) and ACE may extend beyond its participation in the metabolism of the peptide. According to Deddish *et al* (12,13), the inactivation of BK occurs via the C-domain of somatic ACE. While Ang-(1-7) metabolism is mediated by the N-domain of somatic ACE, Ang-(1-7) may also act as an endogenous inhibitor of the C-domain of human somatic ACE. Thus, changes in ACE activity may have an important effect on the mechanisms that regulate blood

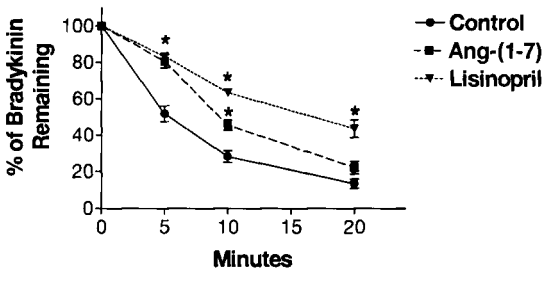

Fig 7. ^{125}I-[Tyr^0]bradykinin (final concentration 1 nmol/L) added to tubes containing three pre-incubated coronary rings with 1 ml Krebs buffer aerated with 95% O_2 and 5% CO_2 at 37°C. Lisinopril (2 µmol/L) (▼), Ang-(1-7) (2 µmol/L) (■) or Krebs buffer as control (●) added to the rings 10 min before addition of radiolabeled bradykinin. Aliquots of incubation medium removed at 5, 10 and 20 min. Results reported as means ± SEM's. n = 4 dogs. Figure used with permission from *Hypertension* (29).

pressure not simply by determining the rate of Ang II formation and bradykinin degradation, but also by influencing the fate and action of Ang-(1-7).

In summary, figure 8 illustrates both the direct vasodilator actions of Ang-(1-7) and its synergistic actions on bradykinin. The vasodilator actions of Ang-(1-7) are mediated by a non-AT_1/non-AT_2 angiotensin $AT_{(1-7)}$ receptor. Ang-(1-7) vasodilation is mediated by a kinin-mediated release of NO. On the other hand, the synergistic actions of Ang-(1-7) on the vasodilator response produced by BK is not mediated by a known angiotensin receptor since the effect persisted in the presence of AT_1, AT_2, and [Sar^{1}Thr^8]-Ang II receptor antagonists. Thus, the Ang-(1-7) effects mediated by the $AT_{(1-7)}$ receptor are not responsible for the potentiated response, since it would have been blocked by [Sar^{1}Thr^8]-Ang II. The specificity of the response for Ang-(1-7) was demonstrated by showing that neither acetylcholine, sodium nitroprusside nor prostaglandins potentiated the BK-induced relaxation. Furthermore, the specificity of the response was also shown when neither Ang I nor Ang II were effective in potentiating the BK response. Our studies indicate that the effect of Ang-(1-7) may be mediated by release of NO in conjunction with a B_2 receptor. Inhibition of endothelial ACE activity by Ang-(1-7) or competition of ACE by Ang-(1-7) with BK may in part contribute to the synergistic action of Ang-(1-7) on the vasodilator response to BK.

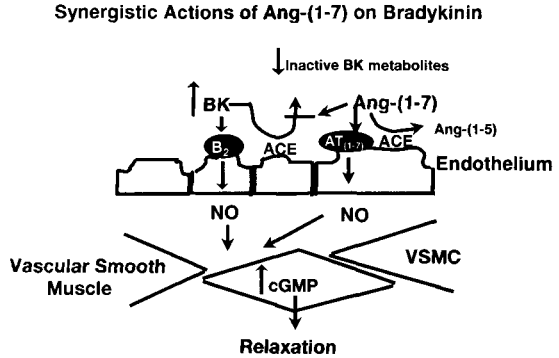

Fig 8. Vasodilator actions of Ang-(1-7) mediated by an $AT_{(1-7)}$ receptor and synergistic actions of Ang-(1-7) with bradykinin in coronary rings. B_2 = bradykinin receptor; cGMP = guanosine 3'5' cyclic monophosphate; NO = nitric oxide; $AT_{(1-7)}$ = non-AT_1/AT_2 angiotensin receptor; ACE = angiotensin converting enzyme.

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