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Role of endothelium in angiotensin II formation by the rat aorta and mesenteric arterial bed

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Abstract

We investigated the angiotensin II (Ang II)-generating system by analyzing the vasoconstrictor effect of Ang II, angiotensin I (Ang I), and tetradecapeptide (TDP) renin substrate in the absence and presence of inhibitors of the renin-angiotensin system in isolated rat aortic rings and mesenteric arterial beds with and without functional endothelium. Ang II, Ang I, and TDP elicited a dose-dependent vasoconstrictor effect in both vascular preparations that was completely blocked by the Ang II receptor antagonist saralasin (50 nM). The angiotensin converting enzyme (ACE) inhibitor captopril (36 µM) completely inhibited the vasoconstrictor effect elicited by Ang I and TDP in aortic rings without affecting that of Ang II. In contrast, captopril (36 µM) significantly reduced (80-90%) the response to bolus injection of Ang I, without affecting those to Ang II and TDP in mesenteric arteries. Mechanical removal of the endothelium greatly potentiated (70-95%) the vasoconstrictor response to Ang II, Ang I, and TDP in aortic rings while these responses were unaffected by the removal of the endothelium of mesenteric arteries with sodium deoxycholate infusion. In addition, endothelium disruption did not change the pattern of response elicited by these peptides in the presence of captopril. These findings indicate that the endothelium may not be essential for Ang II formation in rat mesenteric arteries and aorta, but it may modulate the response to Ang II. Although Ang II formation from Ang I is essentially dependent on ACE in both vessels, our results suggest the existence of an alternative pathway in the mesenteric arterial bed that may play an important role in Ang II generation from TDP in resistance but not in large vessels during ACE inhibition.

Key words
- Angiotensin II
- Angiotensin converting enzyme
- Renin-angiotensin system
- Endothelium
- Blood vessels
- Captopril

Introduction

There is substantial evidence for the presence of essential components of the renin-angiotensin system (renin, angiotensinogen, angiotensin converting enzyme (ACE), angiotensin peptides and angiotensin receptors) in a variety of tissues, including blood vessels and heart, suggesting the existence of a local renin-angiotensin system (1). However, several studies using ACE inhibitors have raised questions as to whether ACE is the sole enzyme responsible for angiotensin II (Ang II) formation *in vivo*, since this pep-

tide is detected in plasma (2) and tissues (3) despite ACE inhibition. In addition, the existence of alternative Ang II-forming pathways in the rat vasculature (4-6) and human heart (7) has been reported.

Vascular endothelium participates actively in the control of vascular tone through the synthesis and metabolism of a number of vasoactive substances. In particular, vascular endothelium has been shown to be a major site of conversion of circulating angiotensin I (Ang I) to Ang II by ACE located on its luminal surface (8). Also, cultured bovine aortic endothelial cells have been shown to contain renin and angiotensinogen and to be capable of synthesizing and secreting angiotensins (9). Thus, endothelial cells seem to play an important role in the vascular formation of Ang II. However, in pharmacological studies in which large vessels were used, such as rabbit aorta, rat aorta, and rat femoral and caudal arteries, conversion of Ang I to Ang II occurred in the absence of the endothelial layer (10-13). Such apparently contradictory data may be due to the use of a variety of protocols applied to different studies or due to the diverse characteristics of distinct vessels from the same or from different animal species.

Given the above controversial data, we investigated the role of the endothelium in generating Ang II from Ang I and tetradecapeptide (TDP) renin substrate in both large and resistance vessels. The vasoconstrictor effect of Ang II, Ang I, and TDP on isolated rat mesenteric arteries and aortic rings with and without functional endothelium was analyzed in the presence and absence of an ACE inhibitor and Ang II receptor antagonist.

Material and Methods

Experiments were performed using 15-20-week old male Wistar rats (250-300 g) with free access to standard rat chow and tap water.

Perfused mesenteric arterial bed

The rats were anesthetized with ethyl ether and, after opening the abdominal cavity, the superior mesenteric artery was perfused through a polyethylene cannula (PE-50; Clay-Adams, Parsippany, NJ) inserted into the artery at its origin from the aorta with 10 ml of a modified Krebs solution (120.0 mM NaCl, 4.7 mM KCl, 3.0 mM $CaCl_2$, 1.43 mM $MgCl_2$, 25.0 mM $NaHCO_3$, 1.17 mM KH_2PO_4, 11.0 mM glucose and 0.03 mM EDTA) containing 500 IU heparin. The intestine was severed from the mesentery by cutting close to the intestinal border and the mesentery was removed from the rat and placed ready for perfusion in a water jacketed organ bath maintained at 37°C. The mesenteric arteries were perfused with Krebs solution equilibrated with 95% O_2 and 5% CO_2, pH 7.4, at a constant rate of 4 ml/min, using a peristaltic pump (LKB-2115 multiperpex pump, Bromma, Sweden). Perfusion pressure was recorded via a side arm of the mesenteric artery perfusion cannula with an HP-1280C pressure transducer and an HP-7754B recorder (Hewlett-Packard, Palo Alto, CA). After a 30-min stabilization period, phenylephrine was added to the perfusion solution at increasing concentrations (3 to 6 µM) until a stable perfusion pressure (100-120 mmHg) was achieved, in order to avoid Ang II tachyphylaxis (6).

Endothelium was disrupted by infusing sodium deoxycholate (1.0 mg/ml in saline) for 60 s at a perfusion rate of 4 ml/min (14). The successful disruption of the endothelium was confirmed in each preparation by the failure of acetylcholine (Ach; 11 pmol, bolus injection) to elicit relaxation in the mesenteric arteries precontracted with phenylephrine. This procedure did not affect the ability of the vascular smooth muscle of the mesenteric arteries to respond to sodium nitroprusside (67 pmol), which induces endothelium-independent vasodilatation.

Dose-response curves for Ang II (2.4-

38.4 pmol), Ang I (19.3-308.8 pmol), and TDP (142.1-2273.6 pmol) were obtained by bolus injection of 10-40 µl of the peptide solution (diluted in Krebs) into the perfusion stream before the pump. Injection of 40 µl of Krebs solution did not affect basal perfusion pressure. Single responses to Ang II (19 pmol), Ang I (116 pmol), and TDP (568 pmol) that produced an increase in perfusion pressure of approximately 40 mmHg, corresponding to 60% of the maximal response, were determined in the same preparation before and after: a) addition of the Ang II receptor antagonist [Sar^1-Ile^5-Ala^8]Ang II (saralasin; 50 nM), b) addition of the ACE inhibitor captopril (36 µM), c) perfusion of sodium deoxycholate (1 mg/ml during 60 s), and d) perfusion of sodium deoxycholate in the presence of captopril (36 µM). The interval between the injections was at least 15 min to avoid tachyphylactic responses. Saralasin and captopril were added to the perfusion solution and allowed to act for 30 min before testing the pressor response induced by the agonists.

Isolated aortic rings

The rats were killed by decapitation, and the thoracic aorta was removed and placed in an ice-cold oxygenated modified Krebs solution containing 135.0 mM NaCl, 5.0 mM KCl, 2.5 mM $CaCl_2$, 1.4 mM $MgSO_4$, 20.0 mM $NaHCO_3$, 1.17 mM KH_2PO_4, 11.0 mM glucose and 0.03 mM EDTA. The aorta was then carefully cleaned of adhering connective tissue and cut into rings (2-3 mm wide). The rings were suspended with two L-shaped stainless steel hooks under an initial tension of 1.0 g in a 10-ml jacketed tissue bath filled with a modified Krebs solution at 37°C gassed with 95% O_2 and 5% CO_2, pH 7.4. Contractile responses were measured with an isometric strain-gauge (UTC3, Gould-Statham, Cleveland, OH) coupled to a recording system (R-611, Beckman Inst. Inc., Schiller Park, IL). After a 30-min equilibration period with buffer replaced every 10 min, the rings were contracted with phenylephrine (50 nM) and treated with acetylcholine (28 nM) to confirm the integrity of the endothelium.

Endothelium was removed by gently scraping the endothelial cells away from the intima. The successful destruction of endothelial function was confirmed by the failure of acetylcholine (28 nM) to elicit relaxation in aortic rings half maximally precontracted with phenylephrine (50 nM). This procedure did not affect the relaxation induced by sodium nitroprusside (45 nM).

Dose-response curves for Ang II (2.4-19.0 nM), Ang I (2.0-16.0 nM), and TDP (7.0-114.0 nM) were obtained by adding the appropriate concentration to the bath. Each preparation received four or five different doses of just one of these peptides. Single responses to Ang II (5 nM), Ang I (5 nM), and TDP (19 nM) that produced an increase in tension of approximately 250 mg, corresponding to 75% of the maximal response, were determined in the same preparation before and after: a) addition of the Ang II receptor antagonist [Sar^1-Ile^8]Ang II (50 nM), b) addition of captopril (36 µM), c) mechanical disruption of the endothelium, and d) mechanical disruption of the endothelium in the presence of captopril (36 µM). The interval between the addition of the peptides was 20 min, with the buffer replaced every 5 min to avoid tachyphylaxis. [Sar^1-Ile^8]Ang II and captopril were added to the organ bath and allowed to act for 20 min before testing the contractile effect of the agonists.

Drugs and reagents

Captopril was obtained from Squibb (Princeton, NJ). Phenylephrine, sodium deoxycholate, Ach, sodium nitroprusside, [Sar^1-Ile^8]Ang II, saralasin, Ang I, Ang II and TDP (porcine) were purchased from Sigma Chemical Co. (St. Louis, MO). The concentration of each peptide stock solution

(1.0 mg/ml) was determined by amino acid analysis. The analysis of Ang I and TDP samples by reverse-phase high-pressure liquid chromatography revealed no contamination with Ang II.

Statistical analysis

Results are reported as the mean ± SEM. Statistical analysis was performed by the paired Student *t*-test. Differences were considered to be significant at $P<0.05$.

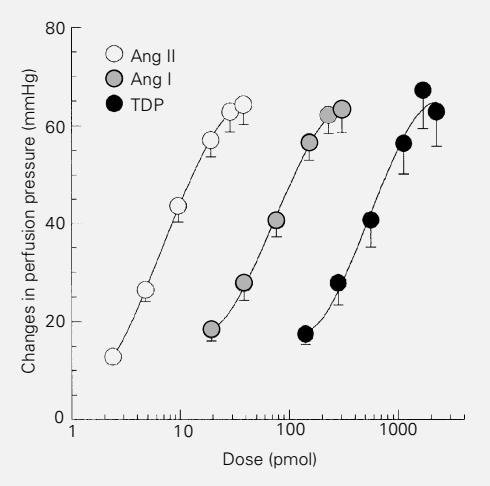

Figure 1 - Changes in perfusion pressure in the isolated rat mesenteric arterial bed induced by bolus injections of angiotensin II (Ang II; N = 7), angiotensin I (Ang I; N = 9), and tetradecapeptide (TDP; N = 7) renin substrate. Data are reported as mean ± SEM.

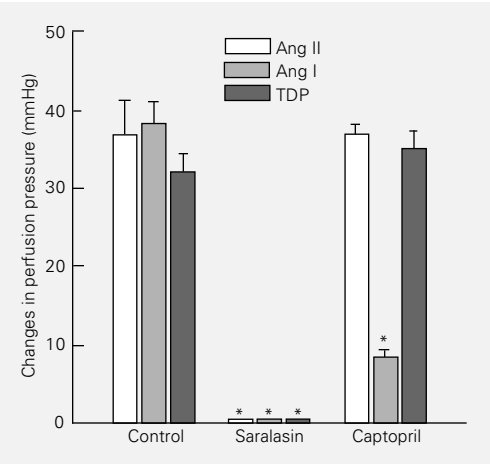

Figure 2 - Changes in perfusion pressure in the isolated rat mesenteric arterial bed induced by bolus injections of angiotensin II (Ang II; 19 pmol), angiotensin I (Ang I; 116 pmol), and tetradecapeptide (TDP; 568 pmol) renin substrate during a control period and in the presence of 50 nM saralasin or 36 µM captopril in the perfusion solution. Data are reported as mean ± SEM and correspond to 12, 4 and 8 observations for the control period, saralasin and captopril, respectively. *$P<0.001$ compared to the corresponding control value (paired *t*-test).

Results

Perfused mesenteric arterial bed

Ang II, Ang I and TDP produced a dose-dependent increase in perfusion pressure of the mesenteric vascular bed (intact preparations), with a similar maximal pressor response, as shown in Figure 1. When compared on an equipressor basis, from data presented in this figure (35 mmHg; 50% of the maximum response), Ang I and TDP had about 12.0% and 1.6%, respectively, of the pressor activity of Ang II on isolated mesenteric arteries.

The vasoconstrictor responses elicited by bolus injections of Ang II, Ang I and TDP were completely abolished by saralasin (50 nM) in the perfusion fluid. In the presence of captopril (36 µM), the pressor response elicited by Ang I was significantly reduced, whereas the responses to both Ang II and TDP were unaffected (Figure 2). Addition of saralasin or captopril to the perfusion solution did not affect basal perfusion pressure.

In intact preparations, Ach (11 pmol) caused a drop in perfusion pressure of the mesenteric arteries preconstricted with phenylephrine, indicating the presence of functional endothelium. After deoxycholate perfusion, the Ach response was abolished, whereas the pressor responses elicited by Ang II, Ang I, and TDP remained basically unchanged (Figure 3, upper panel). The pressor response caused by Ang I in the absence of functional endothelium, as indicated by the lack of response to Ach, was significantly reduced by captopril, while that caused by TDP was not significantly affected by the addition of the ACE inhibitor (Figure 3, lower panel).

Aortic rings

In intact preparations of aortic rings, Ang II, Ang I and TDP produced a dose-dependent increase in tension with a similar maxi-

mal pressor response, except for Ang I which exhibited a lower maximal contraction, as shown in Figure 4. When compared on an equipressor basis from data presented in this figure (150 mg), Ang II and Ang I were essentially equipotent while TDP had about 21.0% of the contractile activity of Ang II on isolated aortic rings.

In intact preparations, the contractions induced by Ang II, Ang I, and TDP were completely abolished in the presence of [Sar^1-Ile^8]Ang II (50 nM). Addition of captopril (36 µM) completely blocked the contraction elicited by both Ang I and TDP, whereas the response to Ang II was unaffected (Figure 5). Addition of [Sar^1-Ile^8]Ang II or captopril to the organ bath did not affect baseline tension.

After endothelium disruption, confirmed by the lack of a relaxant effect of Ach, the vasoconstrictor responses induced by Ang II, Ang I, and TDP were significantly potentiated. In this condition, the addition of captopril (36 µM) completely blocked the contraction elicited by Ang I and TDP, whereas the response to Ang II was unaffected (Figure 6).

Discussion

The present study shows that the Ang II-induced vasoconstrictor effect on the isolated rat mesenteric arteries was similar in the absence and presence of functional endothelium. This observation confirms previous studies performed on the rabbit aorta (10), canine carotid artery (15), and bovine intrapulmonary artery and vein (16). In contrast, the Ang II-induced contraction in the absence of a functional endothelium in rat aortic rings was approximately two-fold greater than that observed in intact preparations. This result supports previous experiments on the rat aorta (13) and bovine coronary artery (16) which showed that destruction of the endothelium significantly enhanced the magnitude of the Ang II-induced

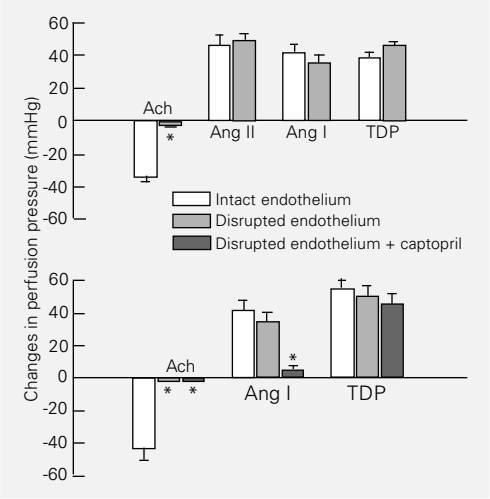

Figure 3 - Changes in perfusion pressure in the isolated rat mesenteric arterial bed induced by bolus injections of angiotensin II (Ang II; 19 pmol), angiotensin I (Ang I; 116 pmol), tetradecapeptide (TDP; 568 pmol) renin substrate and acetylcholine (Ach; 11 pmol) in intact and disrupted endothelium preparations in the absence (upper panel) and in the presence of 36 µM captopril in the perfusate (lower panel). Results are reported as mean ± SEM and correspond to 8 and 7 observations for the upper and lower panels, respectively. *P<0.001 compared to the intact endothelium value (paired *t*-test).

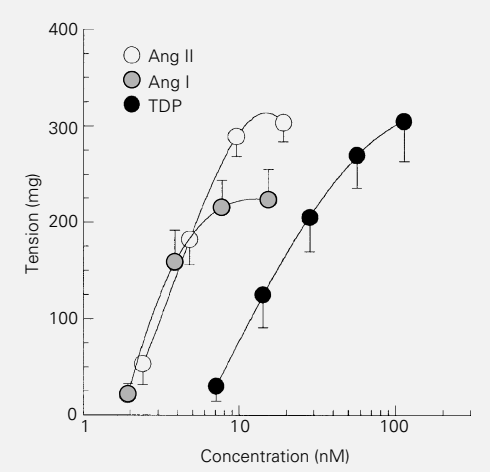

Figure 4 - Concentration-response curves for angiotensin II (Ang II; N = 7), angiotensin I (Ang I; N = 9), and tetradecapeptide (TDP; N = 5) renin substrate in intact isolated rat aortic rings. Data are reported as mean ± SEM.

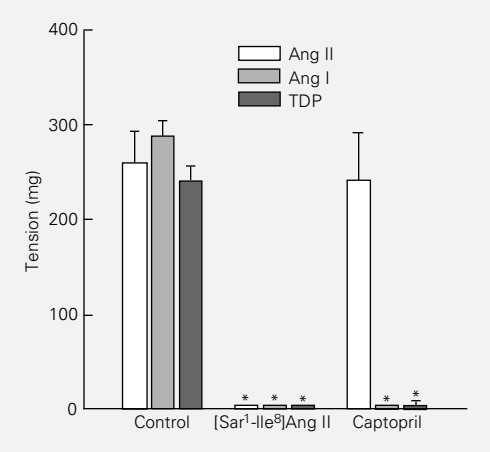

Figure 5 - Contraction induced by angiotensin II (Ang II; 5 nM), angiotensin I (Ang I; 5 nM), and tetradecapeptide (TDP; 19 nM) renin substrate in intact isolated rat aortic rings during a control period and in the presence of [Sar^1-Ile^8]Ang II (50 nM) or captopril (36 µM). Data are reported as mean ± SEM and correspond to 9, 4, and 5 observations for the control period, [Sar^1-Ile^8]Ang II, and captopril, respectively. *P<0.001 compared to the corresponding control value (paired *t*-test).

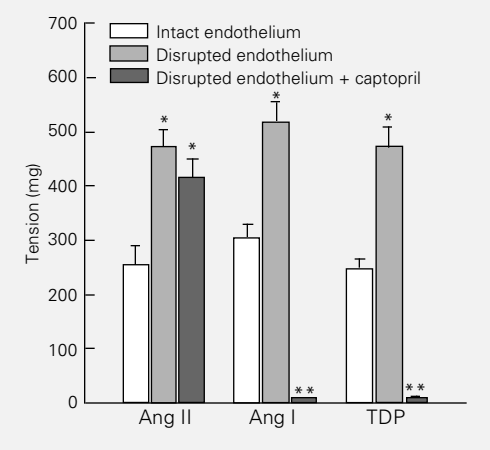

Figure 6 - Contraction induced by angiotensin II (Ang II; 5 nM), angiotensin I (Ang I; 5 nM), and tetradecapeptide (TDP; 19 nM) renin substrate in intact and disrupted endothelium preparations of aortic rings in the absence and in the presence of captopril (36 µM). Data are reported as mean ± SEM and correspond to 5 observations. *P<0.05 compared to the intact endothelium value; **P<0.001 compared to the disrupted endothelium value (paired *t*-test).

vasoconstrictor response. The inhibitory effect of the endothelium on the contraction induced by Ang II has been attributed to the release of relaxing factors such as nitric oxide (13,16-18).

Endothelium disruption did not prevent the local conversion of Ang I or TDP (a synthetic renin substrate) to Ang II since the vasoconstrictor effects of both peptides were unaffected or even potentiated by the perfusion of a detergent or by mechanical disruption of the endothelium in both mesenteric arteries and aortic rings. The demonstration that the Ang II receptor antagonist blocked the vasoconstrictor effect of Ang II, Ang I, and TDP in intact and disrupted endothelium vessels strongly suggests that the actions of both Ang I and TDP were preceded by their conversion to Ang II in isolated rat mesenteric arteries and aortic rings. These findings are in agreement with previous studies performed on the rat (13) and rabbit aorta (10).

Ang I is converted to Ang II essentially by ACE since captopril significantly reduced the pressor response induced by Ang I in rat mesenteric arteries and completely blocked the contraction in aortic rings in preparations with or without functional endothelium. This finding indicates that a substantial ACE activity is present in extra-endothelial layers of the vascular wall, and does not support previous data indicating that ACE is mainly localized in the vascular endothelium (8,19). However, our data are in agreement with the results of other pharmacological and biochemical studies. In different vessels such as rabbit aorta (10), and rat femoral (12) and caudal arteries (11), the absence of endothelium does not alter the functional response to Ang I. ACE activity, determined by using a synthetic substrate, was only partially reduced in aortic segments (20) and was found to be high in perfused mesenteric arterial bed after endothelium removal (14). In both cases this activity was sensitive to blockade by captopril. ACE activity was also found in rat aortic vascular smooth muscle cells (21), rat aorta adventitia (22), and vasa vasorum of the adventitia of large blood vessels (23). Furthermore, ACE activity and expression were detected in the media and the adventitia of the rat aorta (24,25).

Ang II formation from TDP in aortic rings with or without functional endothelium was found to be a two-step pathway formation through renin-like enzymes and ACE since captopril completely blocked the vasoconstrictor response elicited by TDP. On the other hand, Ang II formation from TDP in mesenteric arteries does not involve the classic renin/ACE pathway since captopril did not affect the pressor response induced by TDP in these vessels. These findings confirm a previous study from our laboratory (6) showing that the enhanced responses to TDP observed in arteries isolated from one-kidney, one clip hypertensive rats were not affected by ACE inhibition. Also, the present results are consistent with those reported for the isolated rat kidney (26) and isolated rat caudal artery (5), where the enhancement of noradrenergic transmission produced by TDP was unaffected by ACE inhibition. Furthermore, the contractile effect of TDP on rat femoral (27) resistance vessels and on the isolated rat hindlimb (28) was reported to be insensitive to ACE inhibition. The failure of an ACE inhibitor to block the response elicited by TDP strongly sug-

gests that Ang II generation by a non-ACE pathway may play an important role in the rat resistance vessels.

Several non-renin enzymes capable of generating Ang II from angiotensinogen or Ang I have been described (7,29). In addition, isolated rat mesenteric bed perfusate, obtained by recirculating the perfusion solution, contains a serine protease capable of releasing Ang II directly from both Ang I and TDP into the perfusion medium, as monitored by reverse-phase chromatography (30). In a recent study from our laboratory (31), we have shown that subpressor doses of Ang I that potentiate the sympathetic nerve stimulation-induced vasoconstriction in the isolated rat mesenteric bed were not blocked by captopril. This finding indicated that low (subpressor) concentrations of Ang I are converted to Ang II mainly by an ACE-independent mechanism, while in the present study, where high (pressor) concentrations were used, the conversion of Ang I to Ang II was mainly due to the action of ACE. The physiological significance of this alternative pathway of Ang II generation is not clear, since we do not know whether the TDP renin substrate behaves like the true renin substrate, angiotensinogen. Nevertheless, the increase in blood pressure induced by intravenous administration of TDP to conscious rats was not blocked by ACE inhibitor or pepstatin treatment (6).

The present findings indicate that the endothelium may not be essential for Ang II formation in rat mesenteric arteries and aorta, although it may modulate the response to Ang II. Although Ang II formation from Ang I is essentially dependent on ACE in both vessels, our results indicate the existence of an alternative pathway in Ang II generation from TDP in resistance but not in large vessels during ACE inhibition. Whether the contribution of this putative pathway plays a role in Ang II generation under normal or pathological condition and during chronic ACE inhibition remains to be determined.

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