Vasoconstriction by in situ formed angiotensin II: role of ACE and chymase

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Abstract

Objective: To assess the importance, for vasoconstriction, of in situ angiotensin (Ang) II generation, as opposed to Ang II delivery to AT receptors via the organ bath fluid. **Methods:** Ang I and II concentration–response curves in human and porcine coronary arteries (HCAs, PCAs) were constructed in relation to estimates of the clearances of Ang I and II (Cl_{AngI}, Cl_{AngII}) from the organ bath and the release of newly formed Ang II (R_{AngII}) into the bath fluid. HCAs were from 25 heart valve donors (age 5–54 years), and PCAs from 14 pigs (age 3 months). **Results:** Ang I- and II-evoked constrictions were inhibited by the AT_1 receptor antagonist, irbesartan, and were not influenced by the AT_2 receptor antagonist, PD123319. In HCAs Ang II was only three times more potent than Ang I, whereas, in the experiments with Ang I, comparison of Cl_{AngI} with Cl_{AngII} and R_{AngII} indicated that most of the arterially produced Ang II did not reach the bath fluid. Also in PCAs Ang I and II showed similar potency. In HCAs both the ACE inhibitor, captopril, and the chymase inhibitor, chymostatin, inhibited Ang I-evoked vasoconstriction, while only chymostatin had a significant effect on Cl_{AngI}. In PCAs Ang I-evoked vasoconstriction was almost completely ACE-dependent. **Conclusions:** This study points towards the functional importance of in situ ACE- and chymase-dependent Ang II generation, as opposed to Ang II delivery via the circulation. It also indicates that functionally relevant changes in local Ang I–II conversion are not necessarily reflected by detectable changes in circulating Ang II. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: ACE inhibitors; Angiotensin; Coronary circulation

1. Introduction

Since the classical experiments of Ng and Vane [1], it is known that the vasoconstrictor response to i.v. administration of angiotensin (Ang) I is mainly caused by its conversion in the pulmonary vascular bed. It is now established, however, that endogenous circulating Ang I is converted to Ang II not only in the lungs but also in other vascular beds [2,3]. It is therefore possible that, under natural circumstances, Ang II-dependent vasoconstriction is stimulated not so much by arterially delivered circulating Ang II as by Ang II that is formed in close proximity to the AT_1 receptors, as a result of local conversion of arterially delivered Ang I.

The enzyme responsible for Ang I–II conversion, ACE, is well characterized, but recently a serine protease, chymase, has been described that also converts Ang I to Ang II [4–9]. This enzyme is present in many tissues, including heart and blood vessels but, unlike ACE, not in blood plasma [4]. Chymase-like immunoreactivity has been demonstrated in the cardiac interstitium, and cardiac mast cells and endothelial cells are sites of chymase biosynthesis and storage [6]. Interstitial fluid contains an endogenous inhibitor of chymase, α_1-antitrypsin [9].

The physiological importance of chymase is presently unknown and may differ between species [8]. Most studies

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Time for primary review 38 days.

investigating this enzyme have determined its activity in tissue homogenates [4–9]. In vivo evidence supporting its role in Ang I–II conversion is still lacking but chymase has been implicated in the return of plasma Ang II to its normal level, after its initial suppression, in humans and animals on continuous ACE inhibitor treatment. Both in humans and pigs, however, ACE inhibitor treatment caused more than 90% inhibition of Ang I–II conversion in the coronary vascular bed [3,10]. Moreover Ang I–II conversion in freshly-obtained intact porcine coronary and carotid arteries appeared to be due to ACE only [11]. In contrast, the contractile response to Ang I in intact human coronary arteries, obtained from hearts that had been kept for 2–10 h in ice-cooled Krebs–Henseleit solution, was inhibited by the chymase inhibitor chymostatin and not by the ACE inhibitor cilazaprilat [7]. In the latter study only one (high) concentration of Ang I (1.0 μmol/l) was tested, and the possiblity of a cilazaprilat-induced shift in the Ang I concentration–response curve (CRC) was not investigated. It is also unknown to what degree mast cells are disrupted by the preparation of cardiac tissue and by its storage in organ-protecting solution.

In the experiments reported here we address these issues by constructing Ang I and Ang II CRCs in human coronary arteries (HCAs) and porcine coronary arteries (PCAs), in the presence and absence of inhibitors of ACE and chymase. Ang II is a well-known vasoconstrictor of coronary arteries in vivo [12,13]. We studied the angiotensin-induced responses in relation to measurements of Ang I and Ang II in the organ bath fluid, in order to assess the importance, for vasoconstriction, of in situ Ang II generation, as opposed to Ang II delivery to the vascular receptors via the organ bath. HCAs were obtained from organ donor hearts that had been kept in an organ-protecting solution. PCAs were obtained both from freshly explanted hearts and from hearts that had been stored in an organ-protecting solution.

2. Methods

2.1. Chemicals

[Ile[5]]-Angiotensin I (Ang I), [Ile[5]]-angiotensin II (Ang II), α_1-antitrypsin, captopril, chymostatin, L-NAME, prostaglandin $F_{2\alpha}$ and substance P were purchased from Sigma. The AT_1 receptor antagonist irbesartan was a gift of Bristol-Myers Squibb. The AT_2 receptor antagonist PD123319 was a gift of Parke Davis. All compounds were dissolved in distilled water, with the exception of chymostatin, which was dissolved in dimethylsulfoxide, and irbesartan, which was dissolved in ethanol. Stock solutions of chymostatin and irbesartan had concentrations of 10 and 1 mmol/l, respectively.

2.2. Collection of coronary arteries and tissue preparation

HCAs were obtained from 25 'heart beating' organ donors (13 male, 12 female; age 5–54 years, mean±SEM 37±3 years) who died of non-cardiac causes (fourteen cerebrovascular accident, seven head trauma, four hypoxia) less than 24 h before the heart was taken to the laboratory. Hearts were provided by the Rotterdam Heart Valve Bank (Bio Implant Services/Eurotransplant Foundation) after removal of the aortic and pulmonary valves for transplantation purposes. The study was approved by the joint Ethics Committee of the Erasmus University and the University Hospital, Rotterdam. Immediately after circulatory arrest, the hearts were stored in an ice-cooled sterile organ-protecting solution (University of Wisconsin, EuroCollins, HTK Bretschneider, or St. Thomas Hospital). After arrival in the laboratory, the HCA was removed and stored overnight in a cold, oxygenated Krebs bicarbonate solution of the following composition (mmol/l): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 8.3; pH 7.4. Vessels were then cut into segments of approximately 4-mm length (wet weight 25–50 mg), suspended on stainless steel hooks in 15-ml organ baths containing Krebs bicarbonate solution, aerated with 95% O_2–5% CO_2, and maintained at 37°C. Segments containing macroscopically visible atherosclerotic lesions were not used.

PCAs were obtained from 14 female 3-month-old pigs (Yorkshire×Landrace, weight 10–15 kg). The pigs had been used in in vivo studies of the hemodynamic effects of α-adrenoceptor and serotonin receptor agonists and antagonists under pentobarbital (600 mg, i.v.) anesthesia [14]. The protocol for this investigation had been approved by the Ethics Committee of the Erasmus University, Rotterdam, dealing with the use of animals for scientific experiments. The heart was explanted at the end of the experiment, and the PCA was either removed immediately ($n=7$) or after the heart had been stored for 24 h in an organ-protecting solution (HTK Bretschneider, $n=4$, or St. Thomas Hospital, $n=3$). The storage was done to mimic the situation for the human hearts. After their removal PCAs were handled in the same way as HCAs.

2.3. Experimental protocol

All vessel segments were allowed to equilibrate for at least 30 min and the organ bath fluid was refreshed every 15 min during this period. Changes in tissue contractile force were recorded with a Harvard isometric transducer. The vessel segments, stretched to a stable force of about 15 mN, were exposed to 30 mmol/l K^+ twice. The functional integrity of the endothelium was verified by observing relaxation to 1 nmol/l substance P after pre-contraction with 1 μmol/l prostaglandin $F_{2\alpha}$ [15]. Expressed as a

percentage of the prostaglandin $F_{2\alpha}$-mediated contraction, the relaxation induced by substance P was $59\pm8\%$ ($n=25$) in HCAs and 100% or more in PCAs. Subsequently, the tissue was exposed to 100 mmol/l K^+ to determine the maximal contractile response to K^+. The segments were then allowed to equilibrate in fresh organ bath fluid for 30 min. Thereafter, the vessel segments were pre-incubated with or without 100 μmol/l captopril, 100 μmol/l chymostatin, 100 μmol/l captopril plus 100 μmol/l chymostatin, 1.0 mg/ml α_1-antitrypsin, 1 μmol/l irbesartan or 1 μmol/l PD123319. Pre-incubation of PCAs was carried out in the presence of 100 μmol/l L-NAME. L-NAME caused an increase in basal tone, which indicates a high basal level of nitric oxide generating activity in these vessels. Without L-NAME, Ang I and Ang II were unable to contract PCAs. In HCAs, Ang I- and Ang II-induced contractions were similar with and without L-NAME ($n=3$; data not shown), and these vessels were therefore not pre-incubated in the presence of L-NAME. Following 30-min pre-incubation, Ang I and Ang II (0.1 nmol/l–1.0 μmol/l) CRCs were constructed. It took 5–10 min to reach a stable contraction plateau, and subsequent angiotensin doses were given rapidly after a stable plateau had been reached, in order to minimise desensitisation. To estimate the release of newly formed Ang II into the organ bath, bath fluid samples (50 μl) for Ang II measurements were taken at the time the Ang I CRCs were constructed, 15 min after the addition of 0.3 μmol/l Ang I and 15 min after the addition of 1.0 μmol/l Ang I, i.e. at the time a stable contraction had been reached. The elimination of Ang I and II was estimated from their measurement in samples taken just after the addition of 1.0 μmol/l Ang I and II, and 15, 30 and 60 min later. All samples were rapidly mixed with 5 μl angiotensinase inhibitor solution and stored at $-80°C$ [16].

2.4. Measurement of angiotensin I and II

Ang I and II concentrations in the organ bath fluid of HCAs were measured using sensitive radioimmunoassays [17]. Measurements were made in samples diluted 1:100 or 1:50 with 0.01 mol/l phosphate buffer, pH 7.4, containing 0.15 mol/l NaCl (total volume 100 μl). The Ang I antiserum crossreacted (100%) with Ang-(2–10) but not ($<0.1\%$) with Ang II, Ang-(2–8), Ang-(3–8) and Ang-(4–8). The Ang II antiserum crossreacted with Ang-(2–8), Ang-(3–8) and Ang-(4–8) (55%, 73% and 100%, respectively) but virtually not ($<0.2\%$) with Ang I and Ang-(2–10).

In blood plasma it is necessary to perform the radioimmunoassays after SepPak extraction and high-performance liquid chromatography (HPLC) separation of the angiotensins to remove interfering agents and crossreacting angiotensin peptides. The organ bath fluid is a buffered solution, and the assay samples are highly diluted and

therefore virtually free of degrading enzymes. In a previous study of the metabolism of Ang I by PCAs, in which ^{125}I-Ang I was added to the organ bath and its metabolites were identified in the bath fluid by HPLC, the main peptide peaks corresponded with intact ^{125}I-Ang I and intact ^{125}I-Ang II with very little ^{125}I-Ang-(2–10) and ^{125}I-Ang-(4–8) [11]. We therefore tested a simplified technique, i.e. radioimmunoassay without prior SepPak extraction and HPLC separation.

In two complete series of HCA experiments (i.e., control experiments and experiments in the presence of captopril, chymostatin, captopril plus chymostatin and α_1-antitrypsin), all bath fluid samples taken at 15 and 60 min after the addition of 1.0 μmol/l Ang I were assayed after SepPak extraction and HPLC separation as well as without extraction and separation. The simplified Ang II assays showed good agreement with the assays after extraction and HPLC separation (Fig. 1). The same was true for the Ang I assays (results not shown). Ang I and II concentrations presented under Results are based on measurements made using the simplified technique.

2.5. Data analysis

Data are given as mean$\pm$SEM and expressed as a percentage of the contraction in response to 100 mmol/l K^+ (41 ± 3 mN in HCAs, $n=25$; 33 ± 2 mN in PCAs, $n=14$). CRCs were analyzed using the logistic function described by de Lean et al. [18] to obtain pEC_{50} ($-^{10}$log EC_{50}) values. In the presence of enzyme inhibitors, Ang I CRCs in HCAs did not always reach a maximum (E_{max}) at the highest Ang I concentration of 1.0 μmol/l. In such cases we determined the Ang I concentration required to obtain 5% of the K^+-induced contraction ($E_{5\%K^+}$), in order to calculate the p$EC_{5\%K^+}$ value. $E_{5\%K^+}$ corresponded to ~25% of the E_{max} in the absence of enzyme inhibitors. The addition of L-NAME caused an increase in basal tone in PCAs. The responses to Ang I and Ang II were corrected for this increase in baseline. Statistical analysis was by one-way ANOVA, followed by post hoc evaluation (according to Tukey or Dunnett where appropriate). P values <0.05 were considered significant.

3. Results

3.1. Vasoconstrictor responses

3.1.1. Angiotensin I- vs. II-evoked responses
In HCAs (Fig. 2, left panel) Ang I and II displayed similar maximal effects (E_{max} $18\pm5\%$ and $17\pm3\%$, respectively, $n=13$). Ang I in HCAs was less potent than Ang II, but the difference was small (pEC_{50} 7.3 ± 0.1 and 7.8 ± 0.1, respectively, $P<0.001$). Also in PCAs Ang I and II displayed the same maximal effect (E_{max} $6.9\pm2.1\%$ and
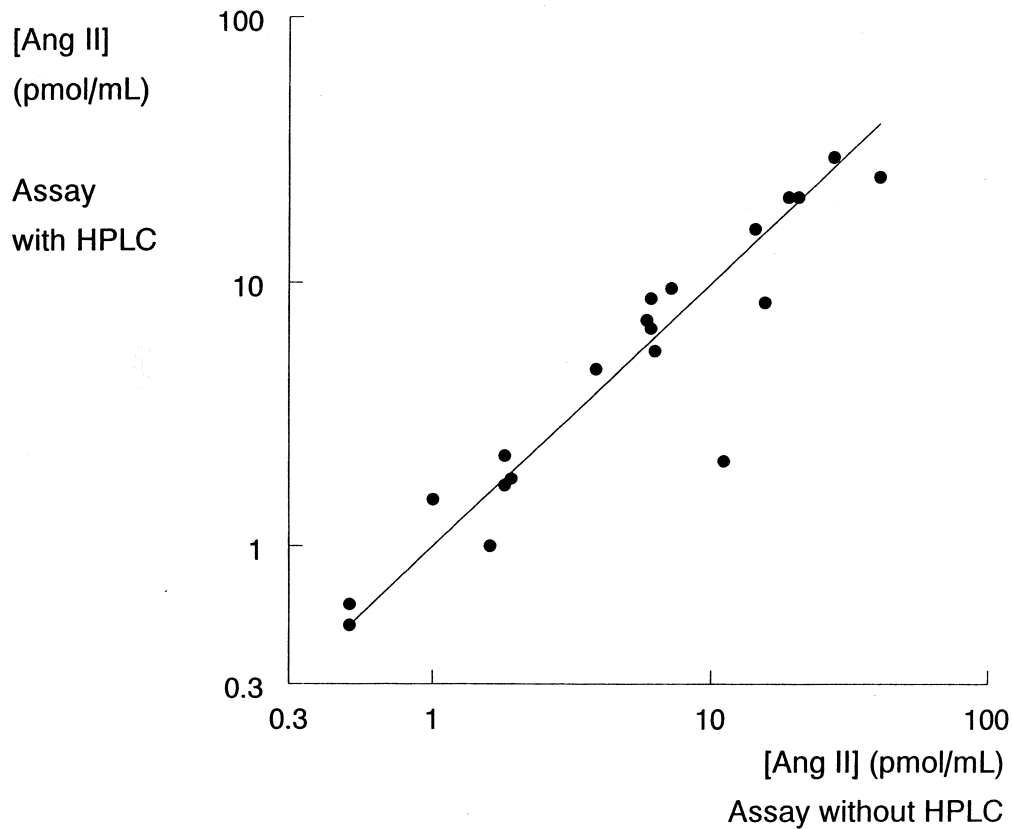
Fig. 1. Comparison between Ang II levels in organ bath fluid measured with and without prior HPLC separation. The line represents the line of identity.

5.4±2.0%, respectively, $n=14$), and Ang I was as potent as Ang II (pEC_{50} 8.3±0.2 and 8.7±0.2, respectively, $P=$NS). There was no significant difference in response between PCAs from fresh hearts and PCAs from hearts that had been stored for 24 h in an organ-protecting solution. Data of all PCAs were therefore combined (Fig.
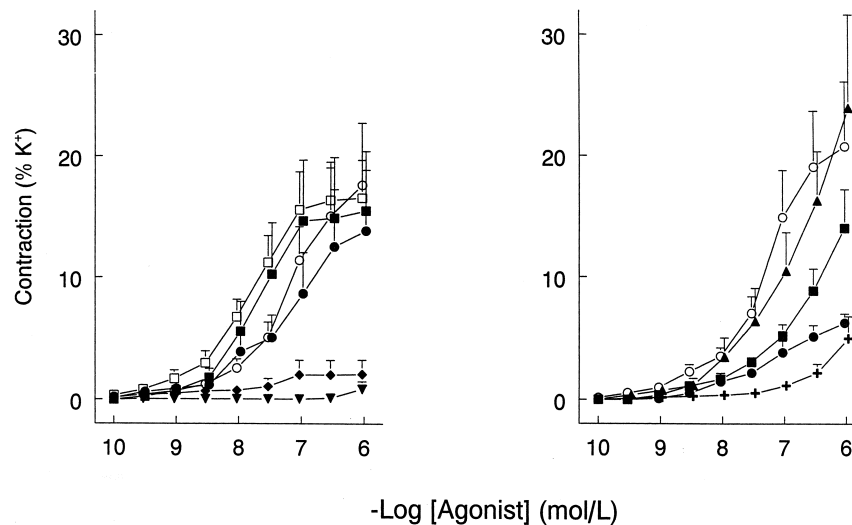

Fig. 2. Left panel. Contractions of HCAs to Ang I (open circles), Ang II (open squares), Ang I in the presence of the AT_1 receptor antagonist irbesartan (1 μmol/l; closed triangles), Ang II in the presence of irbesartan (1 μmol/l; closed diamonds), Ang I in the presence of the AT_2 receptor antagonist PD 123319 (1 μmol/l; closed circles), and Ang II in the presence of PD 123319 (1 μmol/l; closed squares). Right panel. Contractions of HCAs to Ang I in the absence (open circles) or presence of captopril (100 μmol/l; closed squares), chymostatin (100 μmol/l; closed circles), captopril plus chymostatin (both 100 μmol/l; +symbols) or α_1-antitrypsin (1.0 mg/ml; closed triangles). Data are expressed as a percentage (mean±SEM; $n=6$–13) of the response to 100 mmol/l K^+.
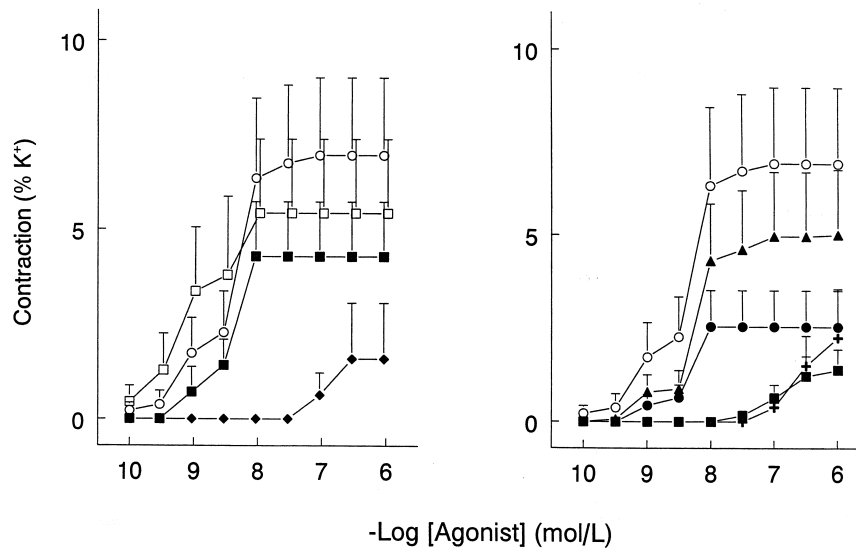
-Log [Agonist] (mol/L)

Fig. 3. Left panel. Contractions of PCAs to Ang I (open circles), Ang II (open squares), Ang II in the presence of the AT_1 receptor antagonist irbesartan (1 μmol/l; closed diamonds), and Ang II in the presence of the AT_2 receptor antagonist PD 123319 (1 μmol/l; closed squares). Right panel. Contractions of PCAs to Ang I in the absence (open circles) or presence of captopril (100 μmol/l; closed squares), chymostatin (100 μmol/l; closed circles), captopril plus chymostatin (both 100 μmol/l; +symbols) or α_1-antitrypsin (1.0 mg/ml; closed triangles). Data are expressed as a percentage (mean±SEM; $n = 7$ or 14) of the response to 100 mmol/l K^+.

3). Ang I and II were about one order of magnitude more potent in PCAs than in HCAs.

3.1.2. Effects of AT receptor antagonists

Both in HCAs (Fig. 2, left panel) and PCAs (Fig. 3, left panel), the AT_1 receptor antagonist irbesartan abolished the contractile response to Ang II. Irbesartan also abolished the contractile responses to Ang I in HCAs (Fig. 2, left panel). The AT_2 receptor antagonist PD123319 was without effect in HCAs and PCAs.

3.1.3. Effects of inhibitors of ACE and chymase

In HCAs, captopril, chymostatin, and captopril plus chymostatin reduced the contractions caused by Ang I (Fig. 2, right panel). As compared with the control $pEC_{5\% K^+}$ value (7.6±0.2, $n = 10$), the $pEC_{5\% K^+}$ values in the presence of the above inhibitors were significantly reduced (6.9±0.2, $P < 0.01$, $n = 10$; 6.7±0.2, $P < 0.01$, $n = 9$; and 6.2±0.1, $P < 0.01$, $n = 6$, respectively). In the presence of α_1-antitrypsin, the Ang I-induced contractions were not reduced and the $pEC_{5\% K^+}$ value (7.4±0.3, $n = 6$) was not different from control. The enzyme inhibitors did not affect the Ang II CRCs ($n = 5$, Fig. 4), indicating that their effect on Ang I-evoked contractions was caused by inhibition of Ang II generation and not by interference with the vasoconstrictor effect of Ang II.

Captopril, with or without chymostatin, virtually abolished the response to Ang I in PCAs from fresh hearts as well as in PCAs from stored hearts (Fig. 3, right panel, and Table 1). Chymostatin alone or α_1-antitrypsin did not cause a shift of the Ang I CRC in these vessels. The maximal contraction reached with Ang I was lower ($P < 0.01$) in the presence of chymostatin, which is most likely

related to the fact that the increase in baseline contractile force caused by L-NAME was smaller in the presence of chymostatin than in its absence (6.3±1.3% and 15.9±1.1%, respectively; $P < 0.05$). An increase in baseline contractile force is known to augment the contractile response [19].

3.2. Angiotensin I and II levels

Ang I and II were not detectable in HCA organ bath fluid ($n = 4$) prior to the addition of Ang I. The Ang II level in the organ bath at the time a stable contraction had
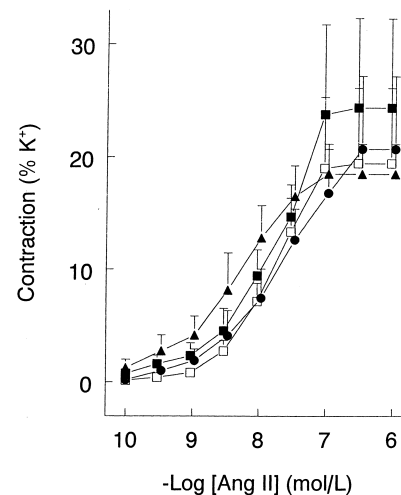

-Log [Ang II] (mol/L)

Fig. 4. Contractions of HCAs to Ang II in the absence (open squares) or presence of captopril (100 μmol/l; closed squares), chymostatin (100 μmol/l; closed circles), or α_1-antitrypsin (1.0 mg/ml; closed triangles). Data are expressed as a percentage (mean±SEM; $n = 5$) of the response to 100 mmol/l K^+.

Table 1
pEC_{50} values of angiotensin I in porcine coronary arteries obtained from fresh hearts and hearts that had been stored for 24 h in an organ-protecting solution[a]

| | Enzyme inhibitors | | | | |
	None	α_1-Antitrypsin	Captopril	Chymostatin	Captopril + chymostatin
Fresh hearts	8.3±0.2	8.2±0.1	<6.0	8.3±0.0	<6.0
Stored hearts	8.4±0.3	8.7±0.2	<6.0	8.9±0.3	<6.0

[a] Data are mean±SEM of seven experiments. pEC_{50} values <6.0 indicate that the contractions reached at the highest angiotensin concentration were below 50% of the maximum response that was observed without inhibitors or antagonists.

been obtained, 15 min after the addition of 0.3 μmol/l Ang I, was 2.4±1.1 nmol/l in the absence of enzyme inhibitors, 3.3±0.8 nmol/l in the presence of captopril, and 3.8±0.9 nmol/l in the presence of α_1-antitrypsin. It was close to or below the detection limit (0.5 nmol/l) in the presence of chymostatin or chymostatin plus captopril. The Ang II levels, 15 min after the addition of 1.0 μmol/l Ang I, were 5.6±2.6 nmol/l in the absence of enzyme inhibitors, 6.3±0.7 nmol/l in the presence of captopril, 8.4±2.7 nmol/l in the presence of α_1-antitrypsin and close to the detection limit in the presence of chymostatin or captopril plus chymostatin.

Captopril and α_1-antitrypsin did not alter the rate of disappearance of Ang I from the organ bath, whereas chymostatin and captopril plus chymostatin almost completely prevented the decrease in Ang I (Fig. 5, top panel). Ang II, after it had been added to the organ bath, remained

Fig. 5. Net decrease in Ang I (top panel) and increase in Ang II (bottom panel) in organ bath fluid of HCAs after the addition of 1.0 μmol/l Ang I in the absence (open circles) or presence of captopril (100 μmol/l; closed squares), chymostatin (100 μmol/l; closed circles), captopril plus chymostatin (both 100 μmol; +symbols), or α_1-antitrypsin (1.0 mg/ml; closed triangles). Data are expressed as a percentage (mean±SEM, $n=4$) of the Ang I levels (1.0±0.1, 1.2±0.2, 1.0±0.1, 0.8±0.1 and 1.1±0.2 μmol/l, respectively) measured immediately after the addition of Ang I ($t=0$).

virtually constant during the 60 min observation period, both in the absence and presence of captopril, α_1-antitrypsin or chymostatin (results not shown). The net vascular release of Ang II into the organ bath was almost completely blocked by chymostatin (Fig. 5, bottom panel). Thus, both the disappearance of Ang I from the organ bath and the vascular release of newly formed Ang II into the bath were largely determined by chymase-dependent Ang I–II conversion.

Fig. 6 compares the organ bath fluid levels of Ang II that were required for vasoconstriction when the artery was exposed to exogenous Ang II (i.e., after the addition of Ang II) with the levels of endogenous Ang II (i.e., after the addition of Ang I) that were associated with similar contractile responses. It appears that, under control conditions as well as in the presence of inhibitors, the bath fluid levels of Ang II required for the same contractile response were 1.5–2 orders of magnitude higher with exogenous Ang II than with endogenous Ang II.
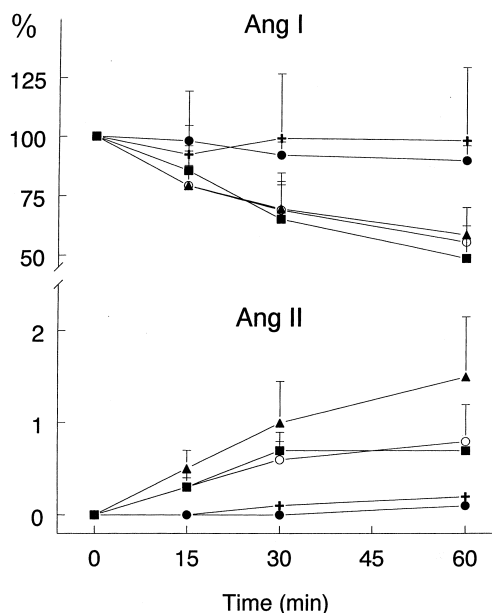
4. Discussion

This study addresses the functional significance of vascular in situ Ang I–II conversion as opposed to Ang II delivery via the circulation. We found that the difference in vasoconstrictor action between equimolar doses of Ang I and Ang II was small in HCAs. Similar results have been reported by others [20,21]. In PCAs Ang I and Ang II were equipotent, possibly because in this preparation the synthesis of NO, an endogenous inhibitor of ACE [22], was suppressed by L-NAME. Ang I and Ang II were more potent in PCAs than in HCAs, which may be related to the higher affinity of the porcine AT_1 receptors for Ang II [23–26].

ACE inhibition by captopril attenuated the vasoconstrictor response to Ang I in HCAs and PCAs. It has been reported that the ACE inhibitor, cilazaprilat, did not alter the response to 1.0 μmol/l Ang I in HCAs isolated from patients undergoing heart transplantation [7]. We also did not observe a clear inhibitory effect of captopril at this high dose of Ang I. Only by constructing a complete CRC did this effect become apparent.

Chymase is known to catalyze Ang I–II conversion in vitro and has been detected in human and porcine cardiac
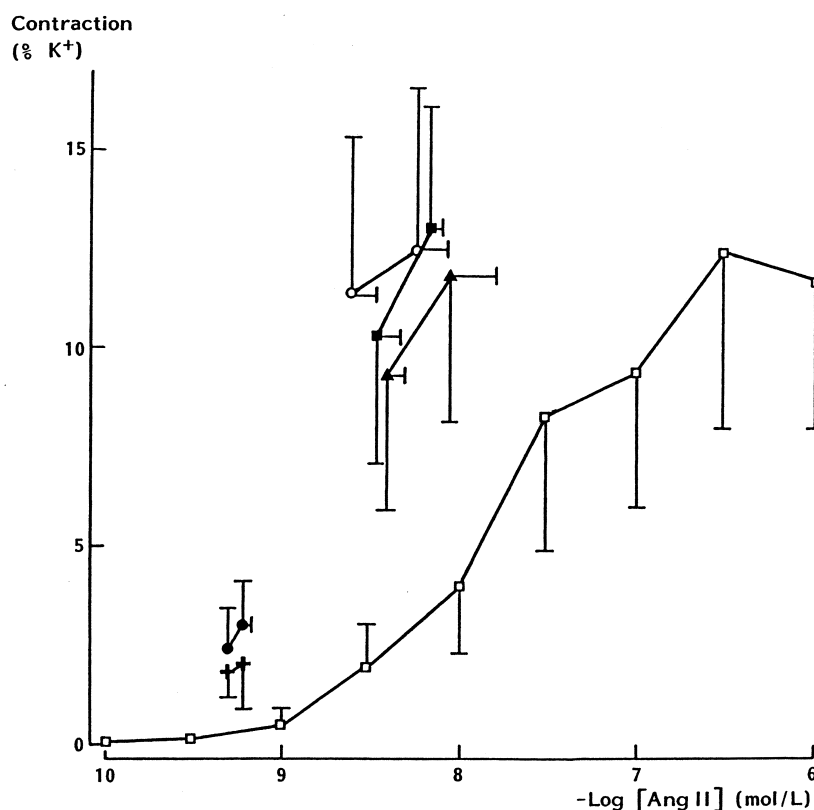
Fig. 6. Contractile responses of HCAs versus organ bath fluid levels of Ang II, measured 15 min after the addition of Ang I (0.3 and 1.0 μmol/l), in the absence (open circles) or presence of captopril (100 μmol/l; closed squares), chymostatin (100 μmol/l; closed circles), captopril plus chymostatin (both 100 μmol; +symbols), or α_1-antitrypsin (1.0 mg/ml; closed triangles). For comparison, the contractile responses of these vessel segments after the addition of Ang II (open squares) are also given. Contractile responses are expressed as a percentage (mean±SEM; $n=4$) of the response to 100 mmol/l K$^+$.

tissue homogenates [27]. Inhibition of this enzyme by chymostatin attenuated Ang I-evoked contractions in HCAs but not in PCAs. It is conceivable that this difference in response between HCAs and PCAs was caused by the release of chymase from disrupted mast cells during storage of the human hearts in organ-protecting solution. Chymostatin, however, was without effect, in PCAs prepared from hearts that had been stored in organ-protecting solution in the same way as the human hearts, as well as PCAs from fresh hearts.

The important contribution of chymase to Ang I-induced vasoconstriction in HCAs was also suggested by the effects of chymostatin on the Ang I elimination from the organ bath and the release of newly formed Ang II into the bath. Both were reduced to nearly zero by chymostatin, while captopril had little effect.

It is important to note that, in contrast with the bath fluid level of Ang I after the addition of Ang I, the level of Ang II remained nearly constant after the addition of Ang II. Since it is known that Ang I can serve as a substrate for enzymes that degrade Ang II, our findings indicate that the observed disappearance of Ang I from the organ bath was primarily caused by Ang I–II conversion and not by Ang I degradation without prior conversion.

In PCAs both the Ang I disappearance rate and the

release of newly formed Ang II were reduced by captopril to very low levels [11], and this is in accordance with the important contribution of ACE to Ang I-evoked contractions in PCAs, as opposed to the important role of chymase in HCAs.

In our experiments, α_1-antitrypsin, which is an inhibitor of chymase, did not inhibit Ang I-evoked contractions, in spite of the fact that we added this inhibitor in a concentration (1.0 mg/ml) that is about twice the concentration in interstitial fluid [9]. This concentration has been shown to cause complete inhibition of chymase in human cardiac tissue homogenates [9]. Possibly, α_1-antitrypsin is less effective as an inhibitor of chymase in intact tissue than in tissue homogenates [28].

In the present study of HCAs the level of newly formed Ang II in the organ bath 15 min after the addition of Ang I, i.e. at the time the vasoconstrictor response had reached a stable plateau, was <1% of the level of Ang I. We obtained comparable results in an earlier study of PCAs [11]. These findings contrast with the small difference in vasoconstrictor potency between Ang I and Ang II. It appears therefore that vasoconstriction following Ang I administration was caused by in situ generated Ang II that activates the vascular AT$_1$ receptors before it reaches the surrounding bath fluid. Vascular Ang I–II conversion in

close proximity to AT_1 receptors is an efficient mechanism to produce a high concentration of Ang II at the site of the receptors. A study of the local generation of Ang II and its vasoconstrictor effect in perfused rat hindquarters, comparing the venous Ang II levels after infusion of renin with those after infusion of Ang II, also indicated higher local Ang II levels than reflected by the Ang II concentrations in the effluent [29].

There is no agreement on whether or not the long-term blood pressure-lowering effect of ACE inhibitors in intact humans and animals is correlated with the effect of these drugs on the circulating level of Ang II. This lack of agreement is caused, at least partly, by the methodological difficulty in measuring low levels of Ang II in the presence of high levels of Ang I [30]. The assay has to be highly specific and sensitive, but not only that, any ex vivo Ang I–II conversion during blood sampling needs to be prevented. On top of this, our findings point towards a more fundamental problem, namely the possibility that physiologically relevant changes in local Ang I–II conversion after ACE inhibitor treatment is not reflected by detectable changes in circulating Ang II. In the present experiments with HCAs and in earlier experiments with PCAs [11] only a small fraction of the Ang I that had been converted by the arteries appeared as intact Ang II in the organ bath.

In humans the circulating level of Ang I is, under normal circumstances, higher than the circulating level of Ang II. In view of our results it is therefore possible that, for systemic vasoconstriction, arterially delivered Ang I is equally important as or even more important than arterially delivered Ang II.

Acknowledgements

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