Functional Importance of Angiotensin-Converting Enzyme–Dependent In Situ Angiotensin II Generation in the Human Forearm

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Hypertension 2000;35;764-768

Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514

 Print ISSN: 0194-911X. Online ISSN: 1524-4563

Functional Importance of Angiotensin-Converting Enzyme–Dependent In Situ Angiotensin II Generation in the Human Forearm

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Abstract—To assess the importance for vasoconstriction of in situ angiotensin (Ang) II generation, as opposed to Ang II delivery via the circulation, we determined forearm vasoconstriction in response to Ang I (0.1 to 10 ng · kg^{-1} · min^{-1}) and Ang II (0.1 to 5 ng · kg^{-1} · min^{-1}) in 14 normotensive male volunteers (age 18 to 67 years). Changes in forearm blood flow (FBF) were registered with venous occlusion plethysmography. Arterial and venous blood samples were collected under steady-state conditions to quantify forearm fractional Ang I-to-II conversion. Ang I and II exerted the same maximal effect (mean±SEM 71±4% and 75±4% decrease in FBF, respectively), with similar potencies (mean EC_{50} [range] 5.6 [0.30 to 12.0] nmol/L for Ang I and 3.6 [0.37 to 7.1] nmol/L for Ang II). Forearm fractional Ang I-to-II conversion was 36% (range 18% to 57%). The angiotensin-converting enzyme (ACE) inhibitor enalaprilat (80 ng · kg^{-1} · min^{-1}) inhibited the contractile effects of Ang I and reduced fractional conversion to 1% (0.1% to 8%), thereby excluding a role for Ang I-to-II converting enzymes other than ACE (eg, chymase). The Ang II type 1 receptor antagonist losartan (3 mg · kg^{-1} · min^{-1}) inhibited the vasoconstrictor effects of Ang II. In conclusion, the similar potencies of Ang I and II in the forearm, combined with the fact that only one third of arterially delivered Ang I is converted to Ang II, suggest that in situ–generated Ang II is more important for vasoconstriction than circulating Ang II. Local Ang II generation in the forearm depends on ACE exclusively and results in vasoconstriction via Ang II type 1 receptors. **(*Hypertension*. 2000;35:764-768.)**

Key Words: angiotensin ■ angiotensin-converting enzyme inhibitors ■ receptors, angiotensin II ■ blood flow

Circulating angiotensin (Ang) I is converted to Ang II in many vascular beds.[1–5] The functional importance of this locally generated Ang II compared with arterially delivered Ang II is currently unknown. Organ bath experiments in which the contractile responses of isolated human or porcine coronary arteries were recorded have shown that Ang I and Ang II display similar vasoconstrictor potencies,[6,7] despite the fact that the levels of Ang II in the bath fluid during exposure to Ang I are <1% of those during exposure to Ang II.[6] These in vitro experiments therefore suggest that locally generated rather than circulating Ang II mediates vasoconstriction. A study of the local generation of Ang II and its vasoconstrictor effects in perfused rat hindquarters, in which the venous Ang II levels after the infusion of renin were compared with those after the infusion of Ang I or II, also indicated that vasoconstriction was caused by local Ang II rather than by Ang II in the perfusion buffer.[8]

Two enzymes have been reported to contribute to Ang I-to-II conversion: ACE and chymase. ACE is present both in circulating blood plasma and on the membrane of vascular endothelial cells, whereas chymase is located in the adventitia, in the cytosol of mast cells.[9,10] Although the results of in vitro studies in isolated human vessels[6,7] and tissue homogenates[11,12] support the contribution of chymase to Ang I-to-II conversion, in vivo studies do not support this view, because ACE inhibition suppressed Ang I-to-II conversion in the human and porcine coronary vascular beds by >90%.[3,13] However, coronary Ang I-to-II conversion in these latter studies was quantified with systemic or intracoronary infusions of ^{125}I-labeled Ang I, an approach that does not allow the detection of Ang II generation with chymase in the adventitia if such generation does not result in Ang II overflow into the blood compartment. Moreover, contractile effects were not quantified in these studies.

It was the aim of the present study to compare the in vivo potencies of Ang I and II to assess the functional importance of locally generated Ang II. Ang I and Ang II were infused into the brachial artery, and forearm vasoconstriction was recorded under steady state conditions. Forearm Ang I-to-II conversion was quantified with measurment of the venous Ang I and II levels at steady state. Infusions were made in the presence and absence of the ACE inhibitor enalaprilat and the

Received September 13, 1999; first decision September 30, 1999; revision accepted November 1, 1999.

From the Departments of Pharmacology (J.J.S., A.H.J.D.) and Internal Medicine I (J.J.S., M.A.D.H.S.), Erasmus University Rotterdam, Rotterdam, the Netherlands; and Department of Nephrology (M.A. van D., I.K.), Leiden University Medical Centre, Leiden, the Netherlands.

Correspondence to A.H.J. Danser, PhD, Department of Pharmacology, Room EE1418b, Erasmus University Rotterdam, Dr Molewaterplein 50, 3015 GE Rotterdam, the Netherlands. E-mail danser@farma.fgg.eur.nl

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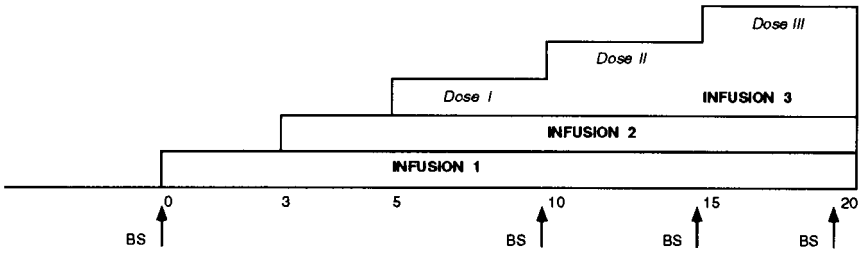

Experiment	INFUSION 1	INFUSION 2	INFUSION 3		
1	-	SNP	AngI 0.1	AngI 1	AngI 10
2	enal 80	SNP	AngI 0.5	AngI 5	AngI 50
3	-	SNP	AngII 0.1	AngII 0.5	AngII 5
4	los 3000	SNP	AngII 0.1	AngII 0.5	AngII 5

Figure 1. Schematic presentation of infusion experiments. Top, Intra-arterial infusion protocol. In experiments 2 and 4, continuous infusion 2 was started 3 minutes after start of continuous infusion 1. In all experiments, infusion 3 was started 5 minutes after start of infusion 1; infusion 3 consisted of 3 dose steps that lasted 5 minutes each. Blood sampling (BS) occurred under baseline conditions, before start of infusions, and at end of each dose step of infusion 3, when a steady state had been reached. Bottom, infusion rates in ng · kg^{-1} · min^{-1}. SNP indicates sodium nitroprusside; Ang, angiotensin; enal, enalaprilat; and los, losartan.

Ang II type 1 (AT_1) receptor antagonist losartan to investigate (1) whether enzymes other than ACE contribute to the local generation of Ang II and (2) whether Ang II mediates vascular effects through receptors other than the AT_1 receptor.

Methods

Subjects

Fourteen white male volunteers (mean age 39 years, range 18 to 67 years; mean weight 83 kg, range 64 to 107 kg) were recruited via advertisement after the Medical Ethics Committee of the Leiden University Medical Center approved the protocol of the study. All participants gave their informed consent. Medical history, physical examination, and routine laboratory tests did not reveal any abnormalities. All subjects were on a normal-sodium diet (≈180 mmol/d), and none of them received medication. Subjects did not smoke, and they refrained from the consumption of alcohol or caffeine-containing substances for ≥12 hours before the experiment.

Experimental Set-Up

Each experiment was performed with the subject in the supine position in a quiet room at a constant temperature of 22° to 24°C. Forearm and hand volumes were measured with water displacement. One-lead ECG was monitored continuously. After local anesthesia with 1% lidocaine, the brachial artery of the nondominant arm was cannulated. The cannula (1.0×45 mm) was connected to a Statham P23Id pressure transducer (Gould Inc). Drugs were infused into the brachial artery with Harvard Apparatus volumetric precision pumps (model 22). Both forearms were instrumented with mercury-in-Silastic strain gauges, which were connected to a Hokanson EC-2 plethysmograph. Both upper arms were connected to a Hokanson E-10 rapid cuff inflator. For the measurement of forearm blood flow (FBF), R wave–triggered cuff inflation (at 40 mm Hg) for venous occlusion plethysmography was controlled with a personal computer.[14] FBF was measured 4 times per minute, and the final 6 measurements at the end of each dose step, when a steady state had been reached,[15] were used for further analysis. During each infusion experiment, the hands were continuously excluded from the circulation with the inflation of small wrist cuffs to a minimum of 40 mm Hg above systolic blood pressure. Heart rate from the ECG, intra-arterial blood pressure, and left and right FBF values were recorded on a polygraph (Gould Inc) and on a personal computer with an analog-to-digital converter (model DT 2801; Data Translation Inc).

Study Protocol

The infusion studies were started ≥60 minutes after the cannulation of the brachial artery. Between the various infusion experiments, the wrist cuffs were deflated, and sufficient time (minimum of 45 minutes) was taken to allow the subjects to recover from hand ischemia and to allow FBF to return to baseline levels. The protocol is summarized in Figure 1. Baseline arterial and venous blood samples were taken before the start of the infusions. Steady-state venous blood samples were obtained at the end of each Ang infusion. Sodium nitroprusside was used to predilate the vascular bed of the forearm to ≈5 mL · 100 mL^{-1} · min^{-1} because measurements of vasoconstrictor effects are more accurate when flow levels remain at >1 mL · 100 mL^{-1} · min^{-1}.[16]

Blood Sampling

Blood for Ang measurements was rapidly drawn with a plastic syringe containing the following inhibitors (0.25 mL inhibitor solution in 5 mL blood): 6.25 mmol/L disodium EDTA, 1.25 mmol/L 1,10-phenanthroline, and 0.01 mmol/L concentration of the renin inhibitor remikiren (final concentrations in blood). The blood was transferred into prechilled polystyrene tubes and centrifuged at 3000*g* for 10 minutes at 4°C. Plasma was stored at −70°C.

Measurement of Ang I and II

Baseline arterial and venous Ang I and II concentrations were measured with radioimmunoassay, after SepPak extraction and high-performance liquid chromatographic separation, as described previously.[2,3] The high Ang concentrations in the venous samples collected under steady state conditions at the end of each infusion were measured without prior high-performance liquid chromatographic separation.[6]

Data Analysis

Data were normally distributed and are expressed as mean±SEM. The Ang-induced effects are expressed as percentage change in FBF of the infused forearm. The percentage change was calculated relative to the values measured at baseline (ie, at the beginning of infusion 3) (Figure 1). The steady state arterial Ang plasma concentrations (in pmol/L) during the infusions were calculated as follows: $[Ang]_{art,\ steady\ state} = IR \times BW \times 10^6 / [(1-Ht) \times FBF \times FAV \times MW] + [Ang]_{art,\ baseline}$, where IR is Ang I or II infusion rate (in ng · kg^{-1} · min^{-1}), BW is body weight (in kg), Ht is hematocrit, FAV is forearm volume, MW is molecular weight of Ang I or II, and $[Ang]_{art,\ baseline}$ is arterial Ang I or II concentration at baseline.

Fractional conversion and degradation of Ang I (ie, the percentage of arterially delivered Ang I that is converted to Ang II or degraded into other metabolites) and fractional degradation of Ang II (ie, the

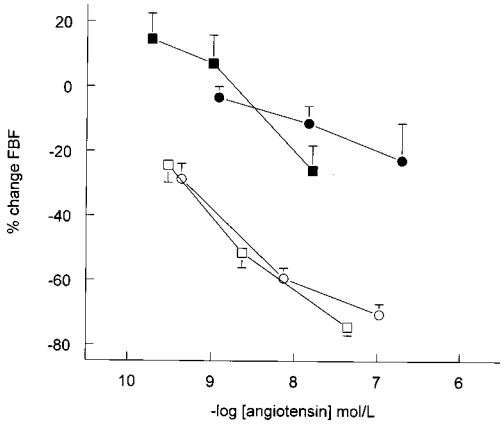

Figure 2. Percent change of forearm blood flow (FBF) in response to Ang I without (○) or with (●) enalaprilat and to Ang II without (□) or with (■) losartan. Data are mean±SEM of 14 experiments.

percentage of arterially delivered Ang II that is degraded) in the forearm were calculated as described previously.[2,3]

EC_{50} values (ie, the arterial Ang I or II concentration at which 50% of the maximal effect is achieved) were calculated from the arterial plasma concentrations and the corresponding FBF values with 4-parameter logistic regression analysis (InPlot 2.0; GraphPAD Software).[15,17]

Student's *t* test and ANOVA for repeated measures were used for statistical evaluation. Values of $P<0.05$ were considered statistically significant.

Results

Baseline arterial Ang I and II levels (6.9±0.6 and 2.7±0.2 fmol/mL, respectively) were comparable to baseline venous Ang I and II levels (10.8±2.3 and 3.0±0.2 fmol/mL, respectively). Baseline Ang levels were unrelated to age or weight.

FBF did not change in the noninfused control arm during the infusions, nor did the Ang infusions affect heart rate and blood pressure (data not shown).

Ang I and II reduced FBF by a maximum of 71±4% and 75±4%, respectively, with comparable potencies (EC_{50} 5.6±1.0 and 3.6±0.5 nmol/L, respectively; P=NS) (Figure 2). Enalaprilat virtually completely blocked the constrictor effects of Ang I. Losartan blocked the vasoconstrictor effects of Ang II; in fact, a tendency for a vasodilator effect (P=NS) was observed at the 2 lowest doses of Ang II in the presence of this drug.

Fractional Ang I conversion was similar at all Ang I doses and was reduced to very low values in the presence of enalaprilat (Table). Fractional Ang I and II degradations were higher at the high doses than at the low doses of these peptides, most likely because of the reduced FBF at these high doses. In support of this assumption, FBF correlated negatively with fractional Ang I and II degradation (fractional Ang I degradation$=-0.06\times$FBF$+0.69$ [$r=0.56$, $P<0.05$] and fractional Ang II degradation$=-0.06\times$FBF$+0.97$ [$r=0.76$, $P<0.01$]). The relationship between FBF and Ang degradation was unaltered with enalaprilat and losartan (data not shown).

Discussion

The results of the present study provide in vivo evidence in humans that in situ–generated Ang II is more important for vasoconstriction than circulating Ang II. This conclusion is based on 2 findings. First, Ang I and Ang II induced forearm vasoconstriction with similar potencies, despite the fact that only one third of Ang I was converted to Ang II in the forearm circulation. Second, the venous Ang II levels at the highest Ang I infusion rate in the presence of enalaprilat were comparable to the venous Ang II levels at a 50-fold lower

Venous Angiotensin Levels, Fractional Ang I Conversion, and Fractional Ang I and II Degradation During Infusion of Ang I or II With or Without Concomitant Infusion of ACE Inhibitor Enalaprilat (80 ng · kg^{-1} · min^{-1}) or AT$_1$ Receptor Antagonist Losartan (3000 ng · kg^{-1} · min^{-1})

	Infusion Rate, ng · kg^{-1} · min^{-1}					
	Ang I			Ang I+Enalaprilat		
Parameter	0.1	1.0	10	0.5	5.0	50
Ang I, pmol/L	43±6	304±56	1691±334	583±108	6583±1029	46 161±6243
Ang II, pmol/L	70±5	544±96	5307±1037	29±5	103±30	662±193
Fractional Ang I conversion, %	41±3	32±3	34±2	2±1†	1±1†	1±0†
Fractional Ang I degradation, %	48±2	62±2*	64±2*	38±7	48±8	68±5*
	Ang II			Ang II+Losartan		
	0.1	0.5	5.0	0.1	0.5	5.0
Ang II, pmol/L	97±10	458±66	4283±627	97±7	443±62	3743±498
Fractional Ang II degradation, %	64±4	78±3*	88±3*	48±4	58±4	76±3*

Data are mean±SEM (n=14).
*$P<0.01$ vs lowest infusion rate.
†$P<0.001$ vs without enalaprilat.

Ang I infusion rate without enalaprilat, yet vasoconstriction was observed only in the latter case. These results confirm previous in vitro observations of the importance of local Ang II in rats and humans.[6,8] They are also in agreement with recent studies that show that despite the fact that ACE is present in the plasma of tissue ACE knockout mice, these animals display the same hemodynamic and vascular abnormalities as mice that are completely ACE deficient.[18,19] The potencies obtained for Ang I and II in the present study were in the nanomolar range, which is in agreement with the known affinity of AT receptors for Ang II.[20,21] It is therefore unlikely that vasoconstriction during Ang I infusion was mediated by Ang I.

In contrast with findings in isolated human blood vessels,[6,7,22] we did not obtain evidence for chymase-dependent vasoconstriction in the human forearm. The ACE inhibitor enalaprilat not only blocked forearm Ang I-to-II conversion by >95% but also almost completely inhibited Ang I–induced vasoconstriction. It is unlikely that the absence of a chymase-mediated effect in the present study is due to the inability of arterially infused Ang I to reach vascular chymase (ie, to diffuse into the adventitia[10]). It has been previously demonstrated that circulating Ang I and Ang II both rapidly diffuse into the interstitial space.[23,24] Furthermore, studies in which the chymase-specific substrate [Pro^{11},D-Ala^{12}]Ang I was administered intravenously to marmosets or hamsters showed clear dose-dependent pressor effects of this peptide that could not be blocked with an ACE inhibitor.[25,26] The discrepancy between in vitro and in vivo studies with regard to the importance of chymase might be due to the presence of an endogenous chymase inhibitor, α_1-antitrypsin, in interstitial fluid.[27] However, such in vivo chymase inhibition is not in agreement with the vasoconstrictor effects obtained with [Pro^{11},D-Ala^{12}]Ang I.[25,26] Moreover, α_1-antitrypsin appeared to inhibit chymase in tissue homogenates only,[27,28] not in intact preparations.[6,28] A more likely explanation therefore is disruption of mast cells during tissue storage or preparation, which will result in chymase concentrations in vitro that are far above those in vivo.

The forearm Ang I-to-II conversion rate obtained here is in agreement with previous studies in which forearm conversion was calculated during the infusion of ^{125}I-labeled Ang I.[2] In those studies, the levels of ^{125}I-Ang II that were obtained at steady state were too low to induce vasoconstriction. Remarkably, despite the clear dose-dependent vasoconstriction that occurred in the present study, fractional forearm Ang I-to-II conversion remained constant at all FBF values. In contrast, fractional forearm Ang I and II degradation correlated inversely with FBF. This latter finding is not surprising, because at lower flow rates, more time is available for metabolism. The fact that Ang I-to-II conversion was not related to FBF suggests that (1) it is a highly efficient process with a maximal result even at high flow rates and (2) conversion most likely precedes degradation (ie, that ACE might be located predominantly in the arterioles).

In the present study, losartan, a competitive AT_1 receptor antagonist,[29] fully prevented vasoconstriction at the 2 lowest Ang II doses and in large part (>70%) inhibited vasoconstriction at the highest dose of Ang II. These data are in agreement with the contention that Ang II induces vasoconstriction in the human forearm through the activation of AT_1 receptors.

References

1. Ng KKF, Vane JR. Fate of angiotensin I in the circulation. *Nature*. 1968; 218:144–150.
2. Admiraal PJJ, Danser AHJ, Jong MS, Pieterman H, Derkx FHM, Schalekamp MADH. Regional angiotensin II production in essential hypertension and renal artery stenosis. *Hypertension*. 1993;21: 173–184.
3. Danser AHJ, Koning MM, Admiraal PJJ, Derkx FHM, Verdouw PD, Schalekamp MADH. Metabolism of angiotensin I by different tissues in the intact animal. *Am J Physiol*. 1992;263:H418–H428.
4. Danser AHJ, Admiraal PJJ, Derkx FHM, Schalekamp MADH. Angiotensin I-to-II conversion in the human renal vascular bed. *J Hypertens*. 1998;16:2051–2056.
5. Neri Serneri GG, Boddi M, Coppo M, Chechi T, Zarone N, Moira M, Poggessi L, Margheri M, Simonetti I. Evidence for the existence of a functional cardiac renin-angiotensin system in humans. *Circulation*. 1996;94:1886–1893.
6. Maassen Van Den Brink A, de Vries R, Saxena PR, Schalekamp MADH, Danser AHJ. Vasoconstriction by in-situ formed angiotensin II: role of ACE and chymase. *Cardiovasc Res*. 1999;44:407–415.
7. Voors AA, Pinto YM, Buikema Hurata H, Oosterga M, Rooks G, Grandjean JG, Ganten D, van Gilst WH. Dual pathways for angiotensin II formation in human internal mammary arteries. *Br J Pharmacol*. 1998;125:1028–1032.
8. Hilgers KF, Bingener E, Stumpf C, Müller DN, Schmieder RE, Veelken R. Angiotensinases restrict locally generated angiotensin II to the blood vessel wall. *Hypertension*. 1998;31:368–372.
9. Urata H, Strobel F, Ganten D. Widespread tissue distribution of human chymase. *J Hypertens*. 1994;12(suppl):S17–S22.
10. Ohishi M, Ueda M, Rakugi H, Naruko T, Kojima A, Okamura A, Higaki J, Ogihara T. Relative localization of angiotensin-converting enzyme, chymase and angiotensin II in human coronary atherosclerotic lesions. *J Hypertens*. 1999;17:547–553.
11. Urata H, Healy B, Stewart RW, Bumpus FM, Husain A. Angiotensin II-forming pathways in normal and failing human hearts. *Circ Res*. 1990;66:883–890.
12. Balcells E, Meng QC, Johnson WH Jr, Oparil S, Dell'Italia LJ. Angiotensin II formation from ACE and chymase in human and animal hearts: methods and species considerations. *Am J Physiol*. 1997;273:H1769–H1774.
13. Zisman LS, Abraham WT, Meixell GE, Vamvakias BN, Quaife RA, Lowes BD, Roden RL, Peacock SJ, Groves BM, Raynolds MV, Bristow MR, Perryman MB. Angiotensin II formation in the intact human heart: predominance of the angiotensin-converting enzyme pathway. *J Clin Invest*. 1995;96:1490–1498.
14. Chang PC, Verlinde R, Bruning TA, van Brummelen P. A microcomputer-based, R-wave triggered system for hemodynamic measurements in the forearm. *Comput Biol Med*. 1988;18:157–163.
15. Baan J Jr, Chang PC, Vermeij P, Pfaffendorf M, van Zwieten PA. Effects of losartan on vasoconstrictor responses to angiotensin II in the human forearm vascular bed of healthy volunteers. *Cardiovasc Res*. 1996;32:973–979.
16. van Veen S, Chang PC. Modulation of vasoconstriction by insulin. *J Hypertens*. 1998;16:1157–1164.
17. Bruning TA, Hendriks MGC, Chang PC, Kuypers EAP, van Zwieten PA. In vivo characterization of vasodilating muscarinic receptor subtypes in humans. *Circ Res*. 1994;74:912–919.
18. Esther CR, Marino EM, Howard TE, Machaud A, Corvol P, Capecchi MR, Bernstein KE. The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest*. 1997; 99:2375–2385.
19. Krege JH, John SWM, Langenbach LL, Hodgin JB, Hagaman JR, Bachman ES, Jennette JC, O'Brien DA, Smithies O. Male-female differences in fertility and blood pressure in ACE-deficient mice. *Nature*. 1995;375:146–148.
20. Regitz-Zagrosek V, Friedel N, Heymann A, Bauer P, Neuss M, Rolfs A, Steffen C, Hildebrandt A, Hetzer R, Fleck E. Regulation, chamber

localization, and subtype distribution of angiotensin II receptors in human hearts. *Circulation*. 1995;91:1461–1471.
21. Nozawa Y, Haruno A, Oda N, Yamasaki Y, Matsuura N, Yamada S, Inabe K, Kimura R, Suzuki H, Hoshino T. Angiotensin II receptor subtypes in bovine and human ventricular myocardium. *J Pharmacol Exp Ther*. 1994;270:566–571.
22. Borland JAA, Chester AH, Morrison KA, Yacoub MH. Alternative pathways of angiotensin II production in the human saphenous vein. *Br J Pharmacol*. 1998;125:423–428.
23. de Lannoy LM, Danser AHJ, van Kats JP, Schoemaker RG, Saxena PR, Schalekamp MADH. Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart: local production of angiotensin I. *Hypertension* 1997;29:1240–1251.
24. de Lannoy LM, Danser AHJ, Bouhuizen AMB, Saxena PR, Schalekamp MADH. Localization and production of angiotensin II in the isolated perfused rat heart. *Hypertension*. 1998;31:1111–1117.
25. Mangiapane ML, Rauch AL, MacAndrew JT, Ellery SS, Hoover KW, Knight DR, Johnson HA, Magee WP, Cushing DJ, Buchholz RA. Vasoconstrictor action of angiotensin I-convertase and the synthetic substrate (Pro^{11}-D-Ala^{12})-angiotensin I. *Hypertension*. 1994;23:857–860.
26. Nishimura H, Buikema H, Baltatu O, Ganten D, Urata H. Functional evidence for alternative Ang II-forming pathways in hamster cardiovascular system. *Am J Physiol*. 1998;275:H1307–H1312.
27. Kokkonen JO, Saarinen J, Kovanen PT. Regulation of local angiotensin II formation in the human heart in the presence of interstitial fluid: inhibition of chymase by protease inhibitors of interstitial fluid and of angiotensin-converting enzyme by Ang-(1-9) formed by heart carboxypeptidase A–like activity. *Circulation*. 1997;95:1455–1463.
28. Takai S, Shiota N, Jin D, Miyazaki M. Functional role of chymase in angiotensin II formation in human vascular tissue. *J Cardiovasc Pharmacol*. 1998;32:826–833.
29. Vanderheyden PML, Fierens FLP, De Backer JP, Fraeyman N, Vauquelin G. Distinction between surmountable and insurmountable selective AT_1 receptor antagonists by use of CHO-K1 cells expressing human angiotensin II AT_1 receptors. *Br J Pharmacol*. 1999;126:1057–1065.