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Activation of cardiac renin-angiotensin system in unstable angina / GG  Neri Serneri; M  Boddi; L  Poggesi; I Simonetti; M  Coppo; ML  Papa; GF Lisi; M Maccherini; R Becherini; A Boncompagni;T Toscano; PA Modesti. - In: JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY. - ISSN 0735-1097. - STAMPA. - 38:(2001), pp. 49-55. [10.1016/S0735-1097(01)01368-7]

*Availability:*

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*Published version:*

DOI: 10.1016/S0735-1097(01)01368-7

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02 May 2026

Journal of the American College of Cardiology
Vol. 38, No. 1, 2001
ISSN 0735-1097/01/$20.00
PII S0735-1097(01)01368-7
Published by Elsevier Science Inc.
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# Activation of Cardiac Renin-Angiotensin System in Unstable Angina

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**OBJECTIVES** The aim of this study was to investigate the activity of the cardiac renin-angiotensin system (RAS) in unstable angina (UA).

**BACKGROUND** Angiotensin (Ang) II locally produced by continuously operating cardiac RAS may affect the pathophysiology of UA.

**METHODS** In 35 patients with UA, 32 with stable effort angina (SA) and 21 with atypical chest pain (controls), cardiac RAS was investigated during coronary angiography after five days of Holter monitoring by combining the measurement of aorta-coronary sinus gradient for Ang I and Ang II with the kinetics study of $^{125}$I-Ang I. Messenger RNAs (mRNA) for all the components of RAS were also quantified with the reverse transcriptase-polymerase chain reaction (RT-PCR) and localized by in situ hybridization in myocardial biopsy specimens from patients who underwent aorta-coronary bypass surgery.

**RESULTS** Cardiac Ang II generation was higher in patients with UA than it was in patients with SA or in controls ($p < 0.001$) due to increased de novo cardiac Ang I formation and its enhanced fractional conversion rate to Ang II. Messenger RNA levels for angiotensinogen (AGTN), angiotensin-converting enzyme (ACE) and Ang II type 1 (AT1) subtype receptors were higher in patients with UA ($p < 0.01$) than they were in patients with SA or in control hearts. Messenger RNAs for AGTN and ACE were almost exclusively expressed on endothelial and interstitial cells. Angiotensin II formation was correlated with ischemia burden ($p < 0.001$). However, the amount of Ang II formed and the expression levels of mRNAs for AGTN, ACE and AT1 were not related to the time that had elapsed since the last anginal attack.

**CONCLUSIONS** In patients with UA, cardiac RAS is activated, resulting in increased Ang II formation. Myocardial ischemia is essential for RAS activation, but it is unlikely to be a direct and immediate cause of RAS activation. (J Am Coll Cardiol 2001;38:49–55) © 2001 by the American College of Cardiology

Components of the renin-angiotensin system (RAS) have been identified in the cardiac tissues of human and various animal species (1), and the existence of a functional cardiac RAS continuously forming low levels of angiotensin (Ang) II has been demonstrated in humans (2). Vascular formation of Ang II has also been demonstrated in experimental preparation of isolated vascular tissues (3) and in humans (4,5). Locally formed Ang II may directly or indirectly affect the pathophysiology of unstable angina (UA) either inducing coronary vasoconstriction or facilitating norepinephrine release from vascular nerve endings. In patients with coronary artery disease, angiotensin-converting enzyme (ACE) inhibition improves myocardial blood flow to ischemic regions (6). Moreover, clinical trials have shown that early-administered ACE inhibition improves the prognosis in patients with myocardial infarction and UA (7), indirectly suggesting a possible role of RAS in acute coronary syndromes. Increased ACE expression has, in fact, been found in hypercellular plaques and in areas of clustered macrophages and T-lymphocytes of patients with UA (8,9). Additionally, both Ang II and Ang II type 1 (AT1) receptors have been detected in close proximity to the presumed plaque rupture site in patients who died acutely after myocardial infarction (8). These findings prompted us to investigate whether cardiac RAS participates in the acute inflammatory response associated with UA (10–13). Therefore, we studied cardiac RAS in patients with UA and stable effort angina (SA) by combining the measurement of the aorta-coronary sinus gradient of endogenous Ang I and Ang II with the kinetics study of $^{125}$I-Ang I. We also quantified the messenger RNA (mRNA) levels of the cardiac RAS components with reverse-transcriptase polymerase chain reaction (RT-PCR) in myocardial biopsy specimens from patients with UA and SA who underwent aorta-coronary bypass surgery.

## METHODS

**Study population.** Thirty-five patients with UA in class IIB ($n = 12$) and IIIB ($n = 23$) of the Braunwald classification, and 32 patients with SA were investigated. The control group was made up of 21 normotensive patients

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Manuscript received December 6, 2000; revised manuscript received March 29, 2001, accepted April 5, 2001.

**Abbreviations and Acronyms**

ACE = angiotensin-converting enzyme
AGTN = angiotensinogen
Ang = angiotensin
AT1 = angiotensin II type 1 receptor
AT2 = angiotensin II type 2 receptor
CBF = coronary blood flow
CVR = coronary vascular resistance
GAPDH = glyceraldeyde-3-phosphate dehydrogenase
mRNA = messenger RNA
PRA = plasma renin activity
RAS = renin-angiotensin system
RT-PCR = reverse transcriptase-polymerase chain reaction
SA = stable effort angina
UA = unstable angina

who underwent coronary angiography for atypical chest pain. Angiography and routine diagnostic procedures did not reveal any abnormalities. The clinical characteristics of this study population are reported in Table 1. All the patients were on oral aspirin and nitrates. Twenty-one and nine patients in the UA group were on heparin and calcium-antagonists, respectively.

Patients with recent (within three months) myocardial infarction, conduction disturbances, heart failure or patients treated with ACE inhibitors or beta-blockers in the two weeks preceding the study were not admitted. Patients who had myocardial ischemia associated with pulmonary edema, hypotension or threatening arrhythmias at admission were also excluded.

Biopsy specimens (10 mg to 20 mg) were collected from the left ventricular free wall in the distribution territory of the culprit artery from the 10 patients with UA and the six patients with SA who underwent bypass surgery. Cardiac specimens were also obtained from the left ventricular free wall from six donors with no histories or signs of heart disease whose hearts could not be transplanted for surgical reasons or due to blood group incompatibility (control hearts).

**Table 1.** Characteristics of Controls and Patients With Angina

| Characteristics | Controls (n = 21) | Stable Angina (n = 32) | Unstable Angina (n = 35) |
|---|---|---|---|
| Men/Women | 15/6 | 23/9 | 28/7 |
| Age (yrs) | 55 ± 12 | 63 ± 7 | 59 ± 13 |
| Weight (kg) | 77.5 ± 7.2 | 79.9 ± 8.4 | 76.5 ± 8.6 |
| Smoking, n (%) | 13 (61) | 21 (64) | 23 (63) |
| Cholesterolemia (mmol/l) | 4.4 ± 0.8 | 5.3 ± 0.9 | 5.5 ± 0.6 |
| Diabetes, n (%) | 3 (14%) | 7 (22%) | 7 (21%)* |
| Hypertension, n (%) | 9 (43%) | 14 (44%) | 15 (42%) |
| Left ventricular mass (g/m$^2$) | 112 ± 14 | 110 ± 12 | 113 ± 14 |
| LVEF (%) | 70.2 ± 5.1 | 64.3 ± 3.5 | 65.9 ± 5.2 |
| Mean aortic pressure (mm Hg) | 95.1 ± 11.9 | 97.2 ± 12.8 | 101.4 ± 12.8 |
| Mean angiographic score† | — | 15.8 ± 3.6 | 16.2 ± 4.1 |
| Coronary blood flow (ml/min) | 85.1 ± 8.4 | 80.4 ± 4.1 | 82.5 ± 12.1 |
| CVR (mm Hg/ml/min) | 1.26 ± 0.34 | 1.51 ± 0.42 | 1.57 ± 0.42 |

*$p < 0.01$; †According to the American Heart Association.
CVR = coronary vascular resistance; LVEF = left ventricular ejection fraction.

The ethical committee of our institution approved the study protocol, and all patients gave their informed consent to participate in the study.

**Study design and experimental procedure.** After admission, patients received conventional noninvasive antianginal treatment and underwent a five-day period of Holter monitoring and recording of symptoms (anginal attacks). Coronary angiography was then performed with a measurement of coronary blood flow (CBF) and blood sampling for the determination of cardiac oxygen extraction and Ang I and Ang II assay according to the procedures previously described (2). Patients who underwent pulmonary edema, hypotension or myocardial ischemia and were not responsive to aggressive therapy were excluded from the study.

**Measurement of cardiac Ang formation.** Cardiac formation of Ang I and Ang II was expressed as the aorta-coronary sinus concentration gradient indexed by coronary flow. In a subgroup of subjects (nine UA, six SA and five controls), cardiac degradation, conversion and formation of Ang I and Ang II and the other parameters shown in Table 2 were calculated by studying the kinetics of $^{125}$I-Ang I infusion ($3.5 \times 10^6$ cpm/min, specific radioactivity of 81.4 TBq/mmol and calculated exposure to radioactivity of 0.6 mrad) as previously described (2,5).

Angiotensin I and Ang II plasma concentrations were measured by radioimmunoassay as previously described (5).

**Quantification of renin, AGTN, ACE, AT1 receptor, Ang II type 2 (AT2) receptor and chymase mRNA levels.** Total mRNA was isolated from homogenized frozen samples using TRIzol reagent (BRL-Life Technologies, Milano, Italy), as outlined by the manufacturer, and reverse transcribed using oligo dT-20. The RT-PCR studies were performed using specific primers for glyceraldeyde-3-phosphate dehydrogenase (GAPDH) (14), renin (15), angiotensinogen (AGTN) (14), ACE (16), chimase (17) and AT1 (18) and AT2 (18) receptors, as previously described (14).

**Localization of AGTN and ACE mRNAs in the myocardium.** The in situ hybridization procedure was performed using complementary DNA photobiotin-labelled probes for GAPDH (pHcGAP, ATCC n.57090), AGTN (HFBDR96, ATCC n.82996) and ACE (donated by Professor Soubrier, INSERM, Hopital Saint-Louis, Paris, France) as previously described (14). Each stained histological section was examined at 400× magnification.

**Statistical analysis.** Data are expressed as mean ± SD. Comparison between groups was performed using Student *t* test for paired and unpaired data or one-way analysis of variance followed by Tukey's multiple-range comparison test, as appropriate. The relation between cardiac Ang II formation measured by $^{125}$I-Ang I kinetics study and that evaluated by Ang II aorta-coronary sinus gradient and the relation between the amount of Ang II formed and the time elapsed since the last ischemic episode were tested by linear

**Table 2.** Kinetics Parameters During $^{125}$I-Ang I Infusion and Arterial-Venous Concentrations of Endogenous Ang I and II

| | Cardiac Tissues | | | Forearm Vascular Tissues | | |
|---|---|---|---|---|---|---|
| | Controls (n = 5) | Stable Angina (n = 6) | Unstable Angina (n = 9) | Controls (n = 5) | Stable Angina (n = 6) | Unstable Angina (n = 9) |
| $^{125}$I-Ang I extraction (%) | 35.2 ± 3.8 | 33.2 ± 4.1 | 38.3 ± 5.1 | 35.7 ± 5.8 | 34.9 ± 4.2 | 35.3 ± 5.1 |
| $^{125}$I-Ang I to $^{125}$I-Ang II conversion rate (%) | 24.3 ± 4.7 | 25.1 ± 4.9 | 39.6 ± 6.8* | 12.1 ± 4.3 | 12.5 ± 3.8 | 12.6 ± 5.2 |
| Ang I by PRA (pg/ml) | 1.6 ± 0.4 | 1.8 ± 0.6 | 1.7 ± 0.6 | 1.2 ± 0.4 | 1.2 ± 0.6 | 1.2 ± 0.5 |
| Ang I by cardiac tissues (pg/ml) | 1.9 ± 1.5 | 3.2 ± 0.9 | 13.9 ± 3.8† | 5.5 ± 2.1 | 5.2 ± 1.8 | 5.6 ± 1.8 |
| Total amount of Ang I formed (pg/ml) | 3.6 ± 1.5 | 4.9 ± 1.2 | 15.6 ± 4.3 | 6.8 ± 2.1 | 6.5 ± 2.7 | 6.9 ± 2.5 |
| $^{125}$I-Ang II extraction (%) | 26.8 ± 2.8 | 27.9 ± 1.9 | 27.5 ± 2.3 | 29.4 ± 3.7 | 29.2 ± 2.9 | 29.5 ± 2.4 |
| Ang II by arterially delivered Ang I (pg/ml) | 1.1 ± 0.6 | 0.9 ± 0.2 | 2.3 ± 1.1* | 0.6 ± 0.5 | 0.7 ± 0.3 | 0.6 ± 0.4 |
| Ang II by cardiac tissues (pg/ml) | 0.9 ± 0.3 | 1.5 ± 0.6 | 6.7 ± 5.1† | 2.3 ± 0.9 | 2.5 ± 0.6 | 2.7 ± 1.1 |
| Total amount of Ang II formed (pg/ml) | 2.1 ± 0.8 | 2.3 ± 0.7 | 9.2 ± 4.8† | 2.9 ± 1.4 | 3.1 ± 1.6 | 3.3 ± 1.9 |
| Ang I A-V gradient (pg/ml) | −1.8 ± 1.6 | −1.8 ± 0.9 | 8.9 ± 3.9* | 1.6 ± 0.8 | 1.7 ± 1.1 | 1.6 ± 0.8 |
| Ang II A-V gradient (pg/ml) | −0.1 ± 0.4 | 0.7 ± 0.6 | 6.9 ± 4.8† | 0.8 ± 1.5 | 0.9 ± 1.2 | 0.9 ± 1.3 |

*p < 0.05 vs. controls and stable angina; †p < 0.001 vs. controls and stable angina.
Ang = angiotensin; A-V = atrioventricular; PRA = plasma renin activity.

regression analysis. Statistical significance was taken as $p < 0.05$.

## RESULTS

**Clinical course.** All the patients with UA and 10 of 32 (33%) patients with SA suffered from ischemic episodes during the five days of the study period. The total number of ischemic episodes per patient during the five-day Holter monitoring were significantly higher in the patients with UA than they were in the patients with SA for anginal attacks (7.1 ± 0.5 vs. no attack), silent ischemic episodes (24.7 ± 3.8 vs. 2.6 ± 0.4, $p < 0.01$) and overall duration of ischemia (126 min ± 41 min vs. 5.6 min ± 1.9 min, $p < 0.01$). No patient had heart failure, myocardial infarction or died during the study period. Coronary blood flow and coronary vascular resistance (CVR) were not significantly different among the three groups (Table 1).

**Cardiac Ang formation.** Angiotensin I and Ang II concentrations and plasma renin activity (PRA) in peripheral venous blood did not differ among the various groups investigated (data not shown). Aorta-coronary sinus gradient values of both angiotensins were higher in patients with UA than in patients with SA or in controls (Fig. 1A) ($p < 0.01$ for both), without any significant difference between patients with SA and controls. The values of the aorta-coronary sinus Ang II concentration in patients with UA were above the 99% confidence limits ($p < 0.001$) of the values found in patients with SA and controls (Fig. 1A).

In all patients with UA, $^{125}$I-Ang I kinetics revealed a significant increase in cardiac Ang I and Ang II formation and in fractional conversion of $^{125}$I-Ang I to $^{125}$I-Ang II ($p < 0.05$ vs. SA and controls) (Table 2). Angiotensin I formed by PRA during the transcardiac passage of blood was low and not significantly different among the three groups (Table 2).

The majority of the de novo formed Ang II derived from the conversion of cardiac Ang I, and only 25% was formed by the conversion of the arterially delivered Ang I (Table 2). The cardiac Ang II formation indexed by CBF was 1,294 pg/min ± 283 pg/min in patients with UA and 5.6 pg/min ± 1.9 pg/min for patients with SA ($p < 0.01$).

A very good correlation was found between cardiac Ang II formation at kinetics study and Ang II aorta-coronary sinus gradient ($r = 0.97$, $p < 0.001$).

In contrast with the increased cardiac Ang II generation, the formation of both angiotensins and the Ang I to Ang II fractional conversion rate by forearm vascular tissues in patients with UA were not significantly different from that in patients with SA or in controls (Table 2).

**Measurement of mRNA levels for the cardiac RAS components.** The RT-PCR revealed that AGTN, ACE, AT1, AT2 and chymase genes were expressed in all myocardial ventricular biopsy specimens from patients with angina and from control hearts (Fig. 2). Conversely, the expression of mRNA for renin was not detectable. Densitometric ratios revealed overexpression of AGTN, ACE and AT1 genes in UA versus both SA specimens and control hearts ($p < 0.01$) (Fig. 2B). In patients with SA, the expression of mRNA for AGTN, ACE and AT1 did not differ from that of controls (Fig. 2). In contrast, AT2 mRNA levels did not significantly differ among the three groups of patients (Fig. 2). Finally, no significant differences were detectable in the expression levels of chymase mRNA among patients with UA, patients with SA and controls (Fig 2).

Negative and positive controls for in situ hybridization studies showed that the signal was specific for mRNA and that the mRNA in the biopsies was intact (Fig. 3C and I). In biopsy specimens from control hearts (Fig. 3A and B) and in ventricular myocardium from patients with SA (Fig. 3G and H), mRNA for AGTN and ACE was expressed only in trace amounts, whereas AGTN and ACE genes were overexpressed in the myocardium specimens from patients with UA and were almost exclusively expressed on the microvessel endothelial and interstitial cells (Fig. 3D and E).

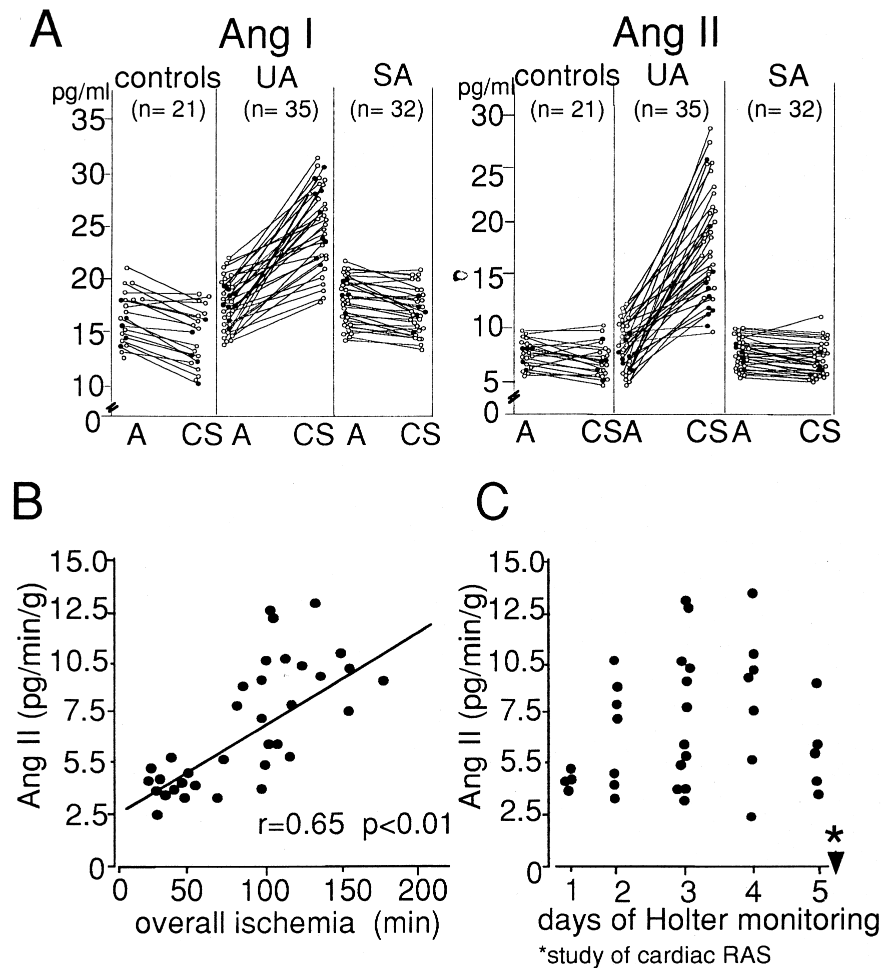


**Figure 1.** **Black circles** indicate the patients and the controls who performed $^{125}$I-Ang I kinetics study. **(A)** Aortic (A) and coronary sinus (CS) plasma concentrations of Angiotensin (Ang) I and II in anginal patients and in controls. **(B)** Relationship between cardiac Ang II formation and overall duration of ischemia in the five days of Holter monitoring preceding the study of cardiac RAS. **(C)** The amount of Ang II formed was not related to the time elapsed since the last ischemic episode or anginal attack ($F = 1.8$). Ang = angiotensin; RAS = renin-angiotensin system; SA = stable effort angina; UA = unstable angina.

**Myocardial ischemia and RAS activation.** Cardiac Ang II formation was positively related to the overall duration of ischemia ($r = 0.65$, $p < 0.01$) (Fig. 1B), but the amount of Ang II formed was not related to the time that had elapsed since the last ischemic episode or anginal attack (Fig. 1C), nor were the expression levels of mRNAs for ACE, AGTN and AT1 in myocardial biopsies related to the time that had elapsed since the last ischemic episode or anginal attack ($F = 1.7$).

## DISCUSSION

**Cardiac RAS activation and increased Ang II formation.** The aim of this study was to investigate the activity of cardiac RAS in UA. The results demonstrate that cardiac RAS is activated, resulting in an increased Ang II formation. This finding is supported by the increased aorta-coronary sinus concentration gradients for both the endogenous angiotensins, the $^{125}$I-Ang I kinetic study and the overexpression of mRNAs for ACE, AGTN and AT1 receptors in ventricular myocardium. The $^{125}$I-Ang I kinetics study reveals that the increased Ang II generation is almost completely attributable to the increased Ang I formation by cardiac tissues and to its conversion to Ang II via a significant increase in ACE activity. In contrast with the activation of cardiac RAS, functional activity of forearm vascular RAS in patients with UA was not significantly different from that in patients with SA or controls, thus indicating that the activation of cardiac RAS is essentially due to local factors that are not related to the systemic inflammatory reaction commonly associated with UA (10,11,13).

The increase in cardiac Ang I formation occurred despite the fact that RT-PCR did not reveal any expression of renin mRNA in myocardial ventricular biopsy specimens from patients with UA. This finding does not contradict the increase in cardiac Ang I formation because renin may enter the heart either through diffusion in the interstitial space (19) or through binding to renin binding proteins (20). Renin is bound to endothelial and subendothelial cells (21). Although AGTN may diffuse from the blood compartment

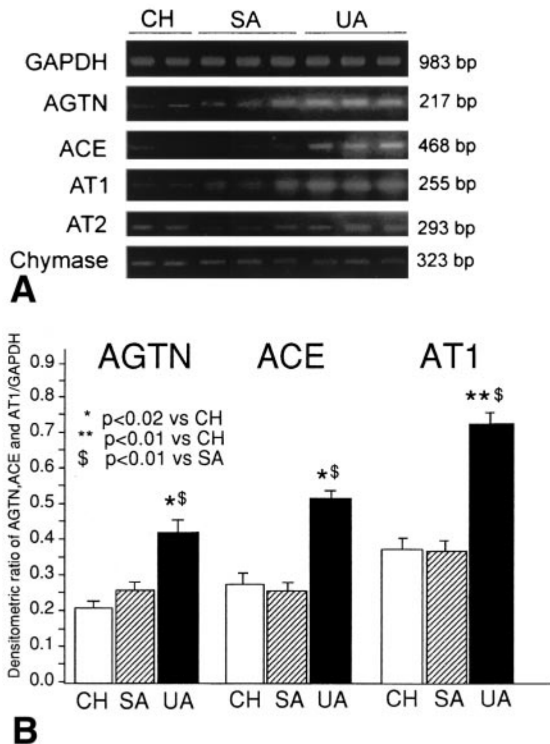


**Figure 2.** **(A)** Reverse-transcriptase polymerase chain reaction (RT-PCR) products of angiotensinogen (AGTN), angiotensin-converting enzyme (ACE), AT1, AT2 and chymase from the myocardium of the control hearts (CH) and from the hearts of patients with stable effort angina (SA) and unstable angina (UA). **(B)** Densitometric quantification of RT-PCR products of AGTN, ACE and AT1 from human myocardium in a nonfailing heart, SA and UA hearts. In UA hearts, the levels for AGTN, ACE and AT1 messenger RNA were significantly increased versus control hearts and SA hearts (for both $p < 0.01$). Data are reported as mean ± SD.

into interstitial space (20,21), RT-PCR showed that AGTN mRNA levels were notably increased in UA patients, indicating a selective activation of cardiac AGTN gene expression. Several studies (17,22) have suggested that, in cardiac tissues, Ang II may be generated by Ang II-forming pathways other than ACE, in particular by chymase, a serine-proteinase chymostatin-sensitive enzyme mainly contained in the mast cells (17). In this study, ACE mRNA levels in myocardial specimens from patients with UA and myocardial ACE activity were increased in contrast with the lack of significant changes in mRNA levels for chymase. Thus, although the contribution of chymase in cardiac Ang II formation remains to be further investigated, the increased Ang II formation in UA patients appears mainly to occur through the ACE-dependent pathway.

**Myocardial cells involved in the RAS activation.** All myocardial cells are provided with AGTN and ACE mRNAs (1) and are, thus, able to form Ang I and Ang II in the presence of renin or other AGTN-activating enzymes. However, in this study, in situ hybridization studies showed that ACE and AGTN mRNAs were overexpressed almost exclusively on the endothelial cells of coronary microvessels and on the interstitial cells, whereas myocytes did not express any increased levels of these genes. Although the lower sensitivity of in situ hybridization in comparison with quantitative analysis does not rule out the possibility that AGTN and ACE genes may be increased also in myocytes, our present findings seem to suggest that myocytes are not directly involved in the activation of myocardial RAS in patients with UA. Thus, RAS activation in patients with UA seems confined to particular cellular types of the myocardium, such as the interstitial and coronary microvessel endothelial cells frequently involved in inflammatory reactions.

**Increased expression of mRNA for AT1 receptors.** Besides the enhanced mRNA for AGTN and ACE, we found a selective increase in AT1 mRNA levels in UA myocardial specimens without any significant changes in AT2 mRNA levels. The small size of biopsy specimens did not allow binding studies; thus, the function or density of AT1 receptors could not be investigated. A selective increase in the number and gene expression of AT1 receptors was found in human heart after acute myocardial infarction (23), and an enhanced myocardial expression of AT1 was reported in rats after experimental ischemia/reperfusion (24). The selective AT1 gene overexpression may depends on a different regulation of the mechanism for the Ang II subtype receptors since a different molecular mechanism was found to regulate Ang II subtype receptor expression in the rat heart in a cell and subtype specific fashion (25). Angiotensin II has been reported to be a potent negative regulator of AT1 receptor gene in neonatal rat myocytes (26). However, an enhanced density of Ang II receptors and an upregulation of the AT1 gene in the presence of increased Ang II formation were found in isolated myocytes in various experimental conditions, including myocardial infarction and heart failure (27,28). Therefore, the downregulation of an AT1 receptor by Ang II might be confined to neonatal rat myocytes. In agreement with this hypothesis, in adult rat heart subjected to an acute pressure overload, Ang II levels were found to be directly related with AT1 promoter activity (29). Moreover, this finding suggests that Ang II provides a positive feedback loop for potentiation of its effects through the upregulation of the AT1 gene. However, only specific studies will be able to clarify this particular issue.

**The sites of Ang II formation.** In the heart, Ang II may be formed either in the intravascular or the interstitial compartment, and both angiotensins, when injected intracoronary, do not readily pass from one to the other compartment (30). There is experimental evidence that Ang II formed by renin uptaken from the plasma and bound to the endothelial cells may pass into the interstitial compartment (31). Angiotensinogen and ACE mRNAs in biopsy specimens were overexpressed in the interstitial cells and in the vascular cells of coronary microvessels (Fig. 3D–F) near the sites for binding renin uptaken from plasma (20,21), thus suggesting that both angiotensins are probably produced in the boundary between vascular and interstitial compartments.

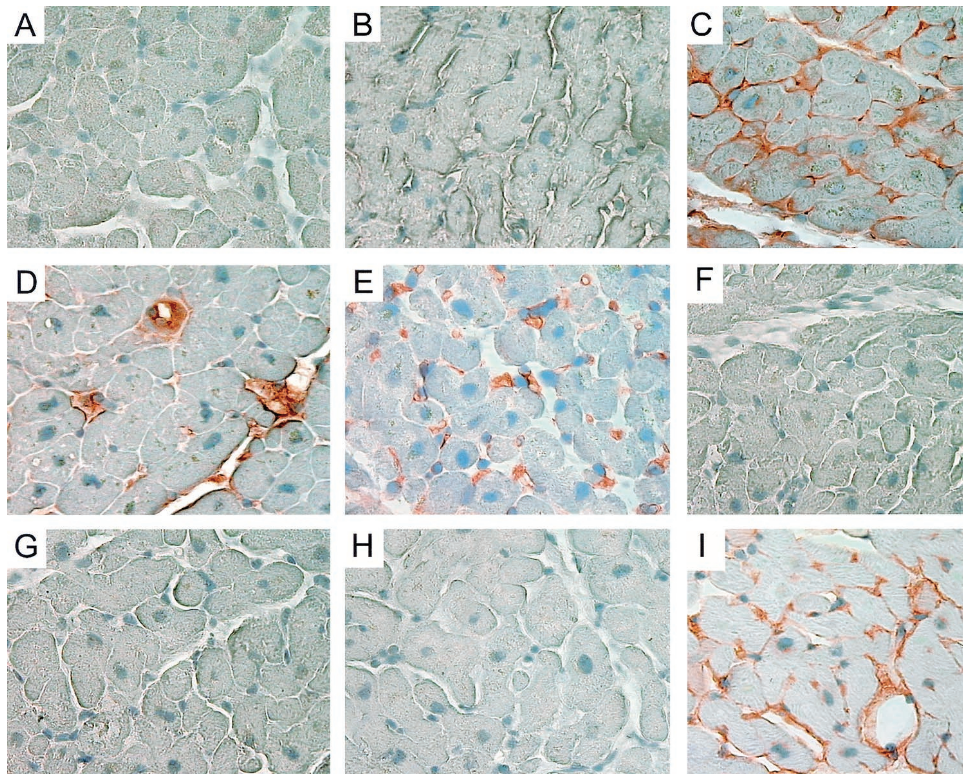


**Figure 3.** In situ hybridization for angiotensinogen (AGTN) **(A,D,G)**, angiotensin-converting enzyme (ACE) **(B,E,H)**, Glyceraldeyde-3-phosphate dehydrogenase **(C,I)** and plasmid vector pB322 **(F)** in controls **(A,B,C)** and in patients with unstable **(D,E,F)** and stable **(G,H,I)** angina. Magnification × 400. Positive messenger RNA (mRNA) signal for AGTN **(D)** and ACE mRNA **(E)** was detectable in the interstitial and endothelial cells of patients with unstable angina. On the contrary, mRNA for AGTN **(G)** and for ACE **(H)** was not expressed in myocardial biopsy specimens from patients with stable effort angina.

In spite of the increase in Ang II formation, CVR in patients with UA did not differ from that in patients with SA or in controls. This paradoxical finding may be explained by Ang II production in coronary microvessels downstream from the resistance vessels, even if the formation of vasodilating agents, such as nitric oxide, which has been demonstrated to increase during hypoxia (32), cannot be ruled out. However, this hypothesis remains to be proved, and it is worth stressing that Ang II depresses constitutive nitric oxide synthase (33) and nitric oxide activity through the increased production of anione-superoxide via membrane-bound NADH-NADPH driven oxidases (34).

**Significance and mechanism(s) of the increased Ang II formation.** The increased Ang II formation does not appear to be either an epiphenomenon of the ischemic episodes, because it was also detectable in patients who had been free from ischemia on the third and fourth days of Holter monitoring preceding the study, or a consequence of myocardial necrosis, because patients investigated had myocardial enzymes within the normal range. Although we cannot totally rule out the possibility that heparin modulates RAS activity, only a proportion of patients with UA received heparin, whereas all the patients showed RAS activation. Therefore, it is highly unlikely that heparin is responsible for the increased Ang II formation. Unstable angina is associated with an acute systemic inflammatory reaction involving acute phase proteins (11), cytokine formation (12) and circulating lymphocytes (10). Cardiac RAS activation might be, therefore, another expression of this reaction. However, the demonstration that Ang II formation by forearm vascular tissues in patients with UA did not differ from that in patients with SA or in controls goes against this hypothesis. The selective activation of cardiac RAS indicates that local factors play a major role in cardiac RAS activation. Cardiopulmonary bypass induces an inflammatory reaction (35), which might contribute to the increased ACE and AGTN mRNA expression found in UA biopsy specimens. However, enhanced Ang II formation was detected in all the patients with UA investigated at least five days before surgery. Therefore, even if cardiopulmonary bypass may have magnified the expression of ACE and AGTN genes, cardiac RAS activation preceded surgery.

The positive relationship between the amount of Ang II formed and the burden of ischemia suggests an important role of myocardial ischemia in RAS activation. This role needs, however, to be further investigated since no relationship was found between the amount of Ang II formed or the expression levels of mRNA for AGTN and ACE and the interval free from anginal attack before cardiac RAS study. Thus, myocardial ischemia appears to act as a trigger activating intermediate mechanisms and mediators that

seem more directly responsible for the RAS activation. Since UA may be considered as a series of episodes of ischemia/reperfusion, we may speculate that cardiac RAS activation occurs through the production of reactive species of oxygen that are able to activate the redox sensitive AP-1 promoter of AGTN, ACE and AT1 genes (36). This explanation, however, remains unsubstantiated and requires direct evidence.

**Conclusions.** These results extend current knowledge on the pathophysiology of UA. Strong evidence has been found that cardiac Ang II formation is enhanced, AT1 receptor mRNA levels are selectively increased and coronary microvessels and myocardial interstitial cells are involved in the bursts of UA overexpressing mRNA for ACE and AGTN. Because of the capacity of Ang II to operate both in an autocrine-paracrine and intracrine fashion, locally increased Ang II formation may have important consequences for cardiac function, microvessel adaptation responses to the increased oxygen demand and fibroblast activation.

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