Chymase-Dependent Angiotensin II Forming System in Humans

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Recent studies have provided evidence that human cardiovascular tissues contain components of the renin angiotensin system: angiotensinogen, renin, angiotensin I converting enzyme (ACE), chymase, and angiotensin (Ang) II receptors. It is likely that locally produced Ang II plays an important role in cardiovascular homeostasis in autocrine and paracrine fashions and may also be involved in remodeling of the heart and vasculature in pathological conditions. In addition to ACE, a cardiac Ang II-forming serine proteinase (human heart chymase) has been identified in the left ventricle of the human heart. The different cellular and regional distribution of ACE and heart chymase in the heart as well as in blood vessels implies distinct pathophysiological roles of these two Ang II-forming enzymes. Several reports indicate that both ACE dependent and ACE independent Ang II formation appears to take place in hypoxic or ischemic heart or blood vessel in vivo and seems to be involved in their pathological changes. However, chymase dependent Ang II formation, chymostatin sensitive but aprotinin insensitive, does not explain all of ACE independent Ang II formation. Therefore, it has become quite important to clarify the detailed mechanisms of the tissue Ang II formation in humans and their contribution to the pathophysiological changes in cardiovascular diseases. Am J Hypertens 1996;9:277-284

KEY WORDS: Chymase, angiotensin I converting enzyme, angiotensin II, heart, blood vessel.
Ang II forming system. This contention is substantiated by Okunishi et al., who described species differences between humans and rodents. These observations suggest that enzymes other than ACE may play a role in the conversion of Ang I to Ang II in various human tissues, particularly in the heart and blood vessels.

The first physiological description of an alternative Ang II formation pathway to ACE was reported by Cornish et al. in the hamster cheek pouch 12 and by Trachte et al. in the cat cardiac papillary muscle. 15 Cornish et al. also found an ACE independent Ang II formation in the hamster coronary artery. 14 ACE inhibitor-insensitive Ang II formation was further observed in the arteries of dog, monkey, and human, 15,16 in human plasma, 17 rat hindlimb, 18 ischemic dog heart, 19,20 hamster heart, 21 human detrusor smooth muscle, 22 marmoset, 23 and baboon, 24 as shown in Table 1. However, in none of these physiological studies the enzymes responsible for ACE independent Ang II formation were identified.

Urata et al. reported a dual pathway for Ang II formation in human hearts in vitro; approximately 80% of the total Ang II formation was due to a previously unknown serine proteinase, whereas ACE dependent Ang II formation activity only accounted for ≤11% of the total Ang II formation. 25 This unknown cardiac serine proteinase was purified to homogeneity and identified as a novel member of the chymase family and was subsequently referred to as human heart chymase. 26 Although several enzymes, such as trypsin, chymotrypsin, tonin, cathepsin G, kallikrein, and rat chymase I, can produce Ang II from Ang I in vitro, their physiological roles in the cardiovascular system in vivo have not been clarified. In addition, some of these enzymes, such as trypsin, chymotrypsin, and rat chymase I, also degrade Ang II, 27 which makes these enzyme’s physiological roles in the local Ang II formation doubtful. Chymase clearly distinguishes itself from these enzymes by its inhibitor sensitivity and substrate specificity for Ang I. 28 It is beyond the scope of this brief review to cover all of the Ang II forming enzymes. We will summarize the present knowledge about the chymase or chymase-like enzyme dependent Ang II formation in the cardiovascular system together with ACE dependent Ang II formation.

ACE AND CHYMASE IN THE HEART

All components of the RAS appear to be present in human hearts, indicating that the human heart is not only a target, but also an endocrine or paracrine organ for Ang II. Since several reviews are available for biochemical characteristics of two Ang II forming enzymes, ACE and chymase, 28,29 we would like to concentrate on the pathophysiological roles of these two Ang II forming enzymes. Characteristics of ACE and chymase are summarized in Table 2.

The regional distribution of these two Ang II forming enzymes in the human heart is considerably different. Levels of ACE activity were about three-fold

| TABLE 1. REPORTS ON ACE INDEPENDENT ANGIOTENSIN II FORMATION |
|-------------------------------|-----------------|----------------|
| Species                      | Tissues         | Experiments   |
| Hamster                      | cheek pouch     | contraction   |
| Hamster                      | coronary artery | contraction   |
| Cat                          | cardiac papillary muscle | contraction |
| Monkey                       | cheek pouch     | contraction   |
| Dog and monkey               | carotid artery  | biochemical   |
| Human                        | plasma          | RIA           |
| Dog                          | aorta           | biochemical   |
| Rat                          | hindlimb        | contraction   |
| Dog                          | coronary sinus  | RIA           |
| Rat                          | hindlimb        | RIA           |
| Human                        | heart           | biochemical   |
| Hamster                      | heart           | contraction   |
| Dog                          | renal artery    | RIA           |
| Human                        | plasma          | RIA           |
| Human                        | atrial papillary muscle | RIA  |
| Dog                          | coronary sinus  | RIA           |
| Human                        | gastroepiploic artery | contraction |
| Human                        | plasma in exercise | RIA  |
| Human                        | femoral circulation | RIA  |
| Human                        | plasma in exercise | RIA  |
| Human                        | detrusor smooth muscle | contraction |
| Marmoset                     | systemic circulation | blood pressure |
| Baboon                       | systemic circulation | blood pressure |

RIA, radioimmunoassay.
were two-fold higher in the right ventricle than in the atria; no significance difference existed between the ventricles. The findings indicate that chymase dependent Ang neurotensin and enzymatic activity were about two-fold higher in the right atrium than in the left ventricle and ventricle than in the other chambers.

These results indicate that ACE dependent Ang II formation appears to be more important in the left ventricle than in the other chambers.

In the human left ventricle ACE binding sites were approximately four times more numerous than in the rat heart. The lung:heart ratio of ACE binding site density was about 9:1 in humans and 100:1 in rats. These results indicate that ACE dependent Ang II formation in human hearts may differ considerably from that in rat hearts. In the rat heart, ACE mRNA expression and enzymatic activity were enhanced after cardiac hypertrophy due to aortic banding or congestive heart failure induced by coronary ligation. Local Ang II generation of the isolated perfused rat heart was almost completely blocked with ACE inhibitors. 

ACE inhibitors induced regression of cardiac hypertrophy even in a dose that did not affect blood pressure in rats with aortic banding, but this finding has not been confirmed in humans. ACE inhibitors were also beneficial to rats with low output heart failure, as well as high output heart failure. Altogether, ACE appears to be the major Ang II-forming enzyme both in the normal and hypertrophied rat heart.

In contrast, the increase in Ang II concentration in the dog coronary sinus after coronary ligation was not inhibited by ACE inhibitors, but was significantly decreased by the broad serine proteinase inhibitors chymostatin or aprotinin. This may imply that at least more than one serine proteinase besides ACE is involved in acute Ang II formation in the ischemic dog heart. Similar ACE independent Ang II formation has been reported in the cardiomyopathic hamster heart. Compared to the normal hamster heart, Ang I infusion in the presence of a high dose of ACE inhibitor induced an approximately three-fold higher positive inotropic response in isolated perfused cardiomyopathic hamster hearts. This result indicated that ACE independent Ang II-forming activity was significantly augmented in the cardiomyopathic hamster hearts. The pathophysiological significance of the elevated ACE independent Ang II-forming activity has to be clarified in future studies.

In human hearts, the cardiac ventricular tissue contains a dual enzymatic pathway for Ang II formation. The majority of the Ang II-forming activity in the human left ventricle was not inhibited by ACE inhibitors or aprotinin, but was inhibited by the serine proteinase inhibitors soybean trypsin inhibitor (SBTI) or phenylmethylsulfonyl fluoride (PMSF). Since aprotinin inhibits most of known Ang II-forming enzymes, including trypsin, chymotrypsin, cathepsin G, and kallikrein, but not chymase, it is most likely that heart chymase is the main enzyme involved in Ang II formation in the human heart in vitro. In normal human serum, however, Ang II formation was completely inhibited by captopril, but not by SBTI or PMSF, indicating that ACE is the main enzyme involved in the plasma Ang II formation.

Immunocytochemical and in situ hybridization studies for human heart chymase further suggested that chymase appears to be synthesized and stored in the secretory granules in mast cells, endothelial cells, and mesenchymal cells. Upon release from these cells, it is mainly localized in the interstitial region of the myocardium. This indicates that chymase dependent Ang II formation may take place in the interstitium of human myocardium since the majority of immunogold deposits were found in the interstitium and were bound to the extracellular matrix. Thus, chymase appears to be responsible for extracellular rather than intracellular Ang II formation at a basal condition.

These biochemical findings have recently been substantiated by several functional studies in the human heart. In the isolated human atrial trabeculae, Ang I produced positive inotropic and chronotropic responses, which were equipotent to Ang II. Captopril inhibited these responses by 70%, whereas an Ang II receptor antagonist, saralasin, abolished them completely. A selective substrate for chymase, [Pro11, D-Ala12]Ang I, was developed. Chymase, but not ACE, cleaves this substrate to yield Ang II from human ventricular membranes, the generation of Ang II from

**TABLE 2. CHARACTERISTICS OF SOMATIC ACE AND HUMAN HEART CHYMASE**

<table>
<thead>
<tr>
<th>Class</th>
<th>Serine</th>
<th>Chymase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular origin</td>
<td>Mast cell</td>
<td>Mast cell</td>
</tr>
<tr>
<td>Endothelial cell</td>
<td>Endothelial cell</td>
<td></td>
</tr>
<tr>
<td>Mesenchymal cell</td>
<td>T-lymphocytes</td>
<td></td>
</tr>
<tr>
<td>Histological distribution</td>
<td>Endocardium</td>
<td>Endothelium</td>
</tr>
<tr>
<td>Perivascular region</td>
<td>Intersitial region</td>
<td></td>
</tr>
<tr>
<td>Substrates</td>
<td>Ang I</td>
<td>Ang I</td>
</tr>
<tr>
<td>Neureotensin</td>
<td>Bradykinin</td>
<td></td>
</tr>
<tr>
<td>Substance P</td>
<td>LH-RH</td>
<td></td>
</tr>
<tr>
<td>Enkephalin</td>
<td>ACE inhibitors</td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibitors</td>
<td>Chymostatin</td>
<td>SBTI</td>
</tr>
<tr>
<td>PMSF</td>
<td>TPCK</td>
<td></td>
</tr>
<tr>
<td>Optimal pH</td>
<td>8-9</td>
<td>8</td>
</tr>
<tr>
<td>Molecular weight (kD)</td>
<td>29</td>
<td>150</td>
</tr>
<tr>
<td>Localization</td>
<td>RA = LA &lt; RV = LV</td>
<td>RALA &gt; RV &gt; LV</td>
</tr>
</tbody>
</table>

LH-RH, luteinizing hormone-releasing hormone; SBTI, soybean trypsin inhibitor; PMSF, phenylmethylsulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; TPCK, N-tosyl-L-phenylalaine chloromethyl ketone; RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle.
ACE AND CHYMASE IN THE BLOOD VESSEL

In the vasculature, ACE was located in the endothelium, whereas chymase or the chymase-like enzyme (CAGE) was found in the endothelium and in the adventitia of the dog aorta and the human coronary artery. The difference in the localization of chymase and ACE may imply that chymase-dependent Ang II formation is more prevalent in the adventitial regions of the vessels, whereas ACE dependent Ang II formation is more significant on the endothelial surface in normal physiological conditions. Several animal studies suggested that vascular ACE played a major role in maintaining high blood pressure in spontaneously hypertensive rats (SHR) and in rats with renal hypertension, since ACE inhibitors were highly effective in lowering blood pressure in renal hypertensive rats and in SHR. In SHR, the blood pressure lowering effect persisted even long after cessation of ACE inhibitors.

Persistent inhibition of vascular, but not plasma, ACE activity was reported after withdrawal of ACE inhibitors, which can explain the prolonged hypotensive effect. These experimental as well as clinical data suggested that ACE plays a major role in circulating Ang II formation.

It is generally accepted that after the denudation by balloon injury, smooth muscle cells (SMC) are activated, migrate to the lumen, and proliferate, leading to intimal hyperplasia. Ang II promotes migration and proliferation of SMC. Ang II also stimulates the synthesis of the extracellular matrix by SMC. In addition, it is reported that AT1-receptor levels increase in the region of the neointimal formation. Therefore, locally formed Ang II appears to play a role in neointimal formation mediated through specific Ang II receptors.

In rat vessels, ACE expression was induced 2 weeks after balloon injury. Cilazapril, administered 6 days before and continuously for 2 weeks after vascular injury, suppressed the neointimal hypertrophy in the rat carotid artery. Similar antiproliferative effects in rat models were also observed with other ACE inhibitors (captopril, quinapril, and ramipril), but not with verapamil or minoxidil despite comparable reduction of blood pressure. Cilazapril prevented neointimal proliferation in balloon-injured guinea pigs. These findings imply that ACE plays a pivotal role in the proliferative response to vascular injury in these animals.

The antiproliferative effects of ACE inhibitors in neointimal formation with vascular injury, however, have not been reproduced in other animal species, such as pigs, baboons, or dogs. In the balloon-injured carotid arteries of pigs, cilazapril failed to prevent intimal proliferation, despite a reasonable inhibition of plasma ACE activity. In baboons, whose vessels contain the chymase like enzyme, cilazapril administered for 3 months did not prevent intimal thickening after carotid artery endarterectomy, femoral artery balloon injury, or aortoiliac vascular grafting. The discrepancy of ACE inhibitor effects among different animal species may be partially explained by the following dog studies. Dog arteries have chymase-like enzyme, chymostatin-sensitive Ang II-generating enzyme (CAGE), as evidenced by biochemical and functional findings. After a balloon injury in the dog carotid artery, chymase-like enzymatic activities significantly increased, and, in this model, an AT1-receptor antagonist, but not an ACE inhibitor, was effective in reducing neointimal formation. This result suggested that ACE inhibitor insensitive Ang II formation played a significant role to develop neointimal formation in this model. The same group provided biochemical and functional evidence that there were remarkable differences in mechanisms of the vascular Ang II formation between humans and rodents.

In human vessels, Okunishi et al found that approximately 70% of the total Ang II formation was due to CAGE, whereas only 30% to 40% of it was due to ACE. Functional evidence of CAGE activity in the isolated human artery was demonstrated by the fact that captopril only partially inhibited Ang I-induced contraction of the human artery, while in rats it completely abolished the contraction induced by Ang I. It is likely that CAGE found by Okunishi et al is identical with chymase because of their similarities in biochemical and localization characteristics.

The dual pathway for Ang II formation in the vasculature was also reported in the vessels of the hamster cheek pouch. Conversion of Ang I to Ang II and vasconstriction produced by Ang I in the hamster cheek pouch were not inhibited by ACE inhibitors, but were completely inhibited by an Ang II receptor antagonist and an Ang II antisera. Recently, this enzyme in the hamster cheek pouch has been purified and identified as chymase. Hamster chymase is
mainly found in the heart, aorta, lungs, and alimentary tract. Although we need further studies to consolidate these results, hamsters could be a convenient model to investigate the pathophysiological roles of chymase in cardiovascular diseases.

**CLINICAL ASPECTS**

Although it has been established that ACE inhibitors are beneficial for the treatment of patients with cardiovascular diseases, the detailed mechanisms of their beneficial effects are still not fully understood. The discovery of a new Ang II-forming enzyme, chymase, in the heart and in blood vessels has made the investigation of this issue more complex. While ACE inhibitors are apparently beneficial to patients with left ventricular dysfunction, it should be noted that, despite their use, morbidity and mortality of the treated patients are still high. A recent study has shown that patients who deteriorated clinically during enalapril therapy had higher plasma Ang II levels than patients who remained stable. Since chymase is almost equally present in the failing and nonfailing human left ventricle, and since it is found in many organs, chymase dependent Ang II formation may be responsible for the inadequate suppression of plasma Ang II in unstable heart failure patients. These findings suggest a possible benefit of adding agents, such as AT₁-receptor antagonists, especially in patients who are unresponsive to ACE inhibitors.

Uncertainty still exists concerning the effects of ACE inhibitors after acute myocardial infarction. Several trials (Survival and Ventricular Enlargement [SAVE], Acute Infarction Ramipril Efficacy [AIRE], Survival of Myocardial Infarction Long-term Evaluation [SMILE]) have yielded positive outcomes, whereas no benefit was observed in the ACE inhibitor treated group in the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II) study. Although the reasons for this discrepancy are not clear, two recent studies in dogs suggest that non-ACE-dependent Ang II formation plays a role in the ischemic heart. After coronary ligation, ACE independent and chymostatin sensitive Ang II formation was shown in the dog heart, which may contribute to the remodeling of the ventricle.

A remarkably high incidence of restenosis (30% to 50%) remains a major problem after successful PTCA. Although both mechanical and pharmacological attempts to reduce the rate of restenosis have been made, none have been conclusively successful to date. The antiproliferative effects of ACE inhibition in balloon-injured animal models, such as rats and guinea pigs, have led to an investigation of the effects of ACE inhibitors in preventing restenosis after PTCA. In the Multicenter European Research Trial With Cilazapril After Angioplasty to Prevent Transluminal Coronary Obstruction and Restenosis (MERCATOR) study, patients were treated with 2.5 mg cilazapril immediately after PTCA and with 5 mg thereafter. A 6-month follow-up yielded no significant difference between cilazapril or placebo treated patients regarding the coronary diameter or the incidence of clinical events. This finding in humans contrasts sharply with the favorable results obtained from the animal studies.

Several hypotheses can be formulated to explain this discrepancy. First, the dosage of the ACE inhibitor may be insufficient since antiproliferative effects are dose-dependent, requiring a higher dosage than for the antihypertensive effect. Second, the timing of drug administration in relation to vascular intervention may be important. In most animal studies ACE inhibitors were given before the vascular injury, while in the MERCATOR study the drug was given after PTCA. However, in rats an ACE inhibitor prevented restenosis even when it was given 2 days after vascular injury. Further, the MERCATOR study, in which higher doses of cilazapril were given, showed no positive clinical outcome. In a more recent study, fosinopril administered in large doses at least 18 h before PTCA failed to prevent restenosis or decrease the rate of clinical events. Thus, dosage and timing of drug initiation may not be crucial. Third, an ACE independent pathway could be activated after balloon injury to produce Ang II, thus masking the effect of ACE inhibitors. This hypothesis appears to be particularly attractive since chymase-like activity has been detected in dog, monkey, and human arteries, but not in rodent arteries. High levels of chymase activity were detected in balloon injured dog arteries and an AT₁-receptor antagonist prevented neointimal formation, while an ACE inhibitor did not. Thus, it is likely that an ACE independent Ang II forming pathway is involved in the neointimal formation after PTCA in humans. AT₁-receptor antagonists developed recently, therefore, could be more effective in neointimal prevention since they inhibit effects of ACE-independent Ang II formation, and, simultaneously, augment Ang II effects on AT₂-receptors that appear to be involved in antiproliferative actions in cultured coronary endothelial cells.

The ACE independent Ang II formation increased during exercise in the plasma of normal individuals and this Ang II formation was reduced partially, but not completely, by the treatment with nafamostat, a broad serine proteinase inhibitor, indicating the existence of two additional independent Ang II forming pathways other than the ACE dependent pathway. This finding was further supported by the fact that nafamostat also improved peripheral circulation in patients with arteriosclerosis obliterans, probably by inhibiting ACE and chymase independent Ang II formation because nafamostat does not inhibit ACE and chymase (personal communication with Drs. Ki-
inhibitor and an AT,-receptor antagonist or AT,-
the conventional ACE dependent route, the second to the
chymase dependent pathway, and the third to the
aprotinin or nafamostat sensitive pathways. Since
zymes, including trypsin, tonin, kallikrein and cathep-
sin G, these enzymes could be involved in the third
pathway. This data, combined with that from the dog
studies,2,9,20,51 indicates that some triggering stimuli,
such as exercise, ischemia, or mechanical damage,
would be required to further activate three indepen-
dent Ang II forming cascades in the heart and vascu-
lature. Clinical trials with a combination of an ACE
inhibitor and an AT,-receptor antagonist or AT,-
receptor antagonist alone should be attempted in car-
diovascular diseases, such as congestive heart failure,
myocardial infarction, and restenosis after PTCA.

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