Vasoconstrictor Action of Angiotensin I–Convertase and the Synthetic Substrate (Pro\textsuperscript{11},D-Ala\textsuperscript{12})-Angiotensin I


Abstract A chymase (also referred to as angiotensin I–convertase) specific for the conversion of angiotensin (Ang) I to Ang II has been identified in human heart. This serine protease is also present in dog and marmoset vasculature. We examined the vasoconstrictor effects of Ang II putatively generated from an angiotensin-converting enzyme (ACE)–resistant convertase synthetic substrate (SUB) in vivo and in vitro. In marmosets, SUB (7 to 700 [\mu g/kg IV) or Ang I (0.1 to 30 [\mu g/kg) caused similar dose-dependent increases in mean arterial pressure (10 to 100 mm Hg) and decreases in heart rate. Pressor effects of SUB were slightly attenuated at low (but not high) doses by captopril (1 mg/kg IV) and blocked by losartan (5 mg/kg IV); in contrast Ang I pressor effects were substantially blocked by both. In isolated canine superior mesenteric artery, Ang I–induced contraction was eliminated by losartan and reduced but not eliminated by 10 [\mu mol/L CAP. When combined with the serine protease inhibitor chymostatin, CAP eliminated Ang I–induced contraction, but chymostatin alone had no effect. SUB-induced contraction was not blocked by CAP but was equally blocked by chymostatin (25 [\mu mol/L) alone or by the combination of CAP (10 [\mu mol/L) and chymostatin (25 [\mu mol/L); losartan (10 [\mu mol/L) eliminated SUB–induced responses. Previous studies have suggested that Ang I–convertase is important for production of Ang II in the heart. Our results are consistent with a potential role for Ang I–convertase in the production of Ang II in the vasculature, resulting in Ang II–mediated vasoconstriction. (Hypertension. 1994;23(part 2):857–860.)

Key Words • angiotensins • vasoconstriction • heart • mast cells • hypertension, essential

In the present view of the renin-angiotensin biochemical cascade, angiotensin (Ang) I is primarily, if not exclusively, converted to Ang II by angiotensin-converting enzyme (ACE) (EC.3.4.15.1). Although other enzymes (eg, cathepsin G, tonin, trypsin, kalikrein) can cause the synthesis of Ang II in vitro, their physiological function in this pathway is unclear because many of these enzymes also degrade Ang II.

An exception to this rule is the chymotryptic proteinase (chymase), which is also called human heart chymase or Ang I–convertase.\textsuperscript{1} Ang I–convertase has been localized immunocytochemically to cardiac endothelial cells, fibroblasts, and the granules of mast cells; in addition its synthesis in these cell types has been suggested in situ hybridization studies of Ang I–convertase mRNA.\textsuperscript{2} Primate Ang I–convertase is highly specific for the conversion of Ang I to Ang II, with a \(K_m\)/\(K_{m,\text{cat}}\) value higher than that of ACE (198 min\textsuperscript{-1} for convertase versus 125 min\textsuperscript{-1} for ACE). Furthermore the convertase does not utilize angiotensinogen as a substrate, it does not degrade Ang II, and unlike ACE it does not degrade bradykinin or enkephalins.\textsuperscript{1}

Although the presence and specificity of Ang I–convertase were first demonstrated definitively in human heart, which is why it is called human heart chymase, Okunishi et al\textsuperscript{2} recently suggested that Ang II formation in human arteries depends more on convertase than on ACE. In addition, the convertase recently has been observed in a number of other tissues, with high concentrations occurring in the marmoset\textsuperscript{4} and dog aorta (K.W. Hoover and A.L. Rauch, unpublished observations). The presence of the convertase in the vasculature led us to consider whether it might produce vascular Ang II and therefore vasoconstriction. We used the ACE-resistant convertase synthetic substrate [(Pro\textsuperscript{11},D-Ala\textsuperscript{12})-Ang I] (SUB) to assess the in vivo pressor and in vitro vasoconstrictor potential of the Ang I–convertase.

Methods

Animals

Male and female marmosets (weight, 350 to 475 g) were obtained from Charles River Laboratories and housed two to a cage in the Pfizer Central Research colony for at least 30 days before surgery. Because of weak responses and tachyphylaxis to Ang II, marmoset aortic rings could not be used for isolated smooth muscle studies. Instead we used superior mesenteric arteries from beagles weighing 10 to 12 kg. They were housed in the Pfizer animal colony before use. All protocols were approved by the Pfizer Central Research Animal Care and Use Committee and are in accordance with federal regulations governing the use of laboratory animals.

Biochemistry

We attempted to determine the \(K_m\) value of SUB for both marmoset cardiac left ventricular Ang I–convertase and rabbit lung ACE (Sigma Chemical Co); the latter is a standard preparation used in studies of the effects of converting enzyme. Left ventricular Ang I–convertase was prepared by extraction of membranes with 2 mol/L KCl and 1% Triton
X-100. Ang I–convertase activity was assayed by incubating the enzyme at 37°C with a range of concentrations of SUB in 20 mmol/L Tris-HCl (pH 8.0) with 0.25% Triton X-100 and 0.5 mmol/L KCl in a final volume of 100 μL. The reaction was terminated by the addition of 100 μL of 2 mmol/L phenylmethylsulfonyl fluoride (PMSF) in 50 mmol/L Tris-HCl (pH 7.2) with 5 mmol/L MgCl₂ and 0.25% bovine serum albumin (BSA) and being chilled on ice. The ability of ACE to metabolize SUB (100 μmol/L) was determined by incubation with ACE (2 μg/mL) in 50 mmol/L HEPES and 300 mmol/L NaCl for 20 minutes at 37°C (pH 7.5). The reaction was started by the addition of ACE and terminated by the addition of captopril (10 μmol/L) and being chilled on ice. Ang II was measured with an Ang II radioreceptor assay using Ang II receptors from rat liver microsomes.

Tissue Studies

Dogs of either sex were anesthetized with sodium pentobarbital (35 mg/kg) and killed by exsanguination. Distal sections of mesenteric artery were rapidly excised and immediately placed in ice-cold, modified Krebs’ physiological salt solution (MKPSS) of the following composition (in mmol/L): NaCl 118.3, KCl 4.69, MgSO₄ 1.4, CaCl₂ 2.5, KH₂PO₄ 1.18, NaHCO₃ 25.0, and dextrose 11.66. Each vessel was cleaned, cut into a 1-mm-long piece, and denuded of endothelium. The vessel was then maintained with a solution of 7.4), maintained at 37°C, and oxygenated continuously with 95% O₂-5% CO₂. Responses were recorded with isometric techniques using either Grass FT03C force displacement transducer signal was amplified by Gould transducer preamplifiers and passed to a Po-Ne-Mah data acquisition system. Signals were displayed on a CRT monitor and stored on magnetic media. Signals were recorded using three-way analysis of variance with repeated measures on one factor and Student’s t-test. A value of P<.05 was considered significant. All data are expressed as mean±SEM.

Results

Biochemistry

The Kₘ value of Ang I–convertase for SUB was 41 μmol/L. We were unable to determine a Kₘ value for ACE using SUB because little or no Ang II was produced even at the highest concentration of SUB (100 μmol/L).

In Vitro Responses

In endothelium-denuded distal sections of superior mesenteric arteries, Ang I (1 μmol/L) produced vasostenosis equal to 42±4% of the response to 50 mmol/L KCl. Captopril significantly reduced this to 7±2% of the response to KCl, whereas losartan or captopril plus chymostatin caused virtually 100% inhibition of the response (Fig 1, top). Chymostatin alone (25 μmol/L) did not affect the response to Ang I. The vasoconstrictor response to SUB (2.4 μmol/L) was similar to that of Ang I (38±4% of the KCl response); however, the response to SUB was not significantly affected by captopril (Fig 1, bottom). In addition, contractile responses to SUB were significantly reduced by the combination of captopril plus chymostatin (a serine protease/chymase inhibitor) to 9±2% of the KCl response (Fig 1, bottom); chymostatin alone (25 μmol/L) had a similar effect. The responses to SUB were totally eliminated by 10 μmol/L losartan.

In Vivo Responses

Intravenous Ang I caused dose-dependent pressor responses in marmosets with a maximal increase of ~100 mm Hg at 30 μg/kg. These responses were completely eliminated by captopril (Fig 2, top left). In a separate group of marmosets, SUB also caused pressor responses of a similar magnitude; however, captopril had only a small, albeit significant, inhibitory effect at the three lowest doses (Fig 2, bottom left). In a third and fourth group of marmosets, the pressor responses to Ang I (Fig 2, top right) and SUB (Fig 2, bottom right) were significantly inhibited by losartan.

Discussion

In 1990, Urata et al provided persuasive evidence that Ang I–convertase (chymase) was a significant and functional Ang II–producing enzyme in vitro; however, in the absence of a suitable in vivo Ang I–convertase inhibitor or an ACE-resistant substrate, the study of the enzyme’s physiological function has lagged. The advent of the ACE-resistant Ang I–convertase substrate (Pro¹¹-D-Ala¹²)-Ang I (SUB) was reported by Hoit et al. Therefore
our first objective was to confirm that SUB was ACE resistant. We found that SUB was not an effective substrate for ACE in vitro; however, it was an excellent substrate for marmoset Ang I–convertase, with a K<sub>m</sub> value of 41 μmol/L. Thus this compound apparently is converted to Ang II by Ang I–convertase but not ACE in vitro. It therefore is a useful tool to study the actions of Ang I–convertase without serious interference by ACE.

In view of recent findings that Ang I–convertase is present in the marmoset and dog (K.W. Hoover and A.L. Rauch, unpublished observations) vasculature in high concentrations, we hypothesized that it might have a role in the synthesis of vascular Ang II and therefore in vasoconstriction. Therefore our second objective was to determine whether SUB in vitro had vasoconstrictor actions through Ang I–convertase and AT<sub>1</sub> receptors. Because the biochemical and in vivo data were obtained in marmosets, it would have been preferable to use marmoset aorta for the in vitro vascular contraction studies; however, we were unable to obtain repeatable vasoconstrictor responses to Ang I or Ang II in isolated marmoset vessels. Therefore isolated vascular contraction studies were conducted with canine mesenteric vessels, as occurred in marmosets.

The actions of SUB in studies of dog superior mesenteric artery indicate that it apparently is converted by Ang I–convertase to vasoconstricting quantities of Ang II. Similar to Ang I, SUB caused vasoconstriction in distal sections of superior mesenteric artery to levels approximately 38% of that attainable with a 50-mmol/L KCl vasoconstriction. This action of SUB was not blocked by captopril but was greatly attenuated by the serine protease/chymase inhibitor chymostatin or by the combination of chymostatin plus captopril. The vasoconstrictor action of SUB was eliminated by the AT<sub>1</sub> receptor antagonist losartan. Thus the vasoconstrictor action of SUB in vitro appears to stem largely from its conversion to Ang II by Ang I–convertase. Like SUB, the vasoconstrictor action of Ang I in distal sections of superior mesenteric artery was completely blocked by losartan; unlike SUB, vasoconstrictor to Ang I was 85% blocked by captopril. Addition of chymostatin to captopril completely eliminated Ang I–mediated vasoconstriction. Thus it appears that in the presence of captopril, a portion of the vasoconstrictor action of Ang I results from Ang II production by Ang I–convertase. In the absence of captopril, however, chymostatin did not by itself reduce the vasoconstrictor response to Ang I. Together with the finding that captopril alone blocked 85% of the Ang I response, these data suggest that ACE is normally responsible for most of the conversion of exogenously administered Ang I to Ang II in the vasculature; however, in the presence of captopril, Ang I–convertase could take on a significant Ang II–producing role.

It is worth noting that our in vitro isolated vasculature data were obtained in endothelium-denuded vessels; this was done to ensure that the inhibition by captopril of the contractile response to Ang I or SUB occurred exclusively through decreased formation of Ang II with-
out contributions from kinin-mediated formation of nitric oxide.

Consistent with our findings in vitro, we found in marmosets that intravenous SUB generated a dose-dependent pressor response. A third objective was to consider for Ang I and SUB the amount of the vasopressor response that is due to convertase-produced Ang II versus ACE-produced Ang II. Because captopril eliminated the response to intravenous Ang I, it is apparent that the role of Ang I-convertase in the conversion of circulating Ang I to Ang II in the marmoset may be small at best. Nonetheless, it is possible that even in the absence of a pressor response to intravenous Ang I, Ang I-convertase-produced Ang II could cause mesenteric vasoconstriction that is undetectable by the gross measurement of blood pressure. This would be consistent with our results in vitro and allow the possibility of a small role of Ang I-convertase in the production of circulating Ang II from intravenously administered Ang I. The role of Ang I-convertase in creating tissue Ang II may be somewhat larger. The in vitro results are consistent with this hypothesis; in distal sections of superior mesenteric artery, the vasoconstrictor action of Ang I was only about 85% blocked by captopril, with the remainder eliminated by chymostatin.

In vivo the pressor action of SUB was far more resistant to blockade by captopril than Ang I but was slightly attenuated by captopril; although this may indicate some in vivo susceptibility to ACE, it also may reflect other antipressor actions of captopril (eg, bradykinin/nitric oxide potentiation). The resistance of SUB to captopril blockade suggests that the pressor response was due to production of Ang II by Ang I-convertase, yet it is paradoxical that the Ang I pressor response was eliminated by captopril and thus largely independent of Ang I-convertase. In other words, if the failure of captopril to block the pressor response to SUB indicates that Ang I-convertase can produce pressor amounts of Ang II from SUB, we would expect the convertase to produce some Ang II from Ang I in the presence of captopril. It may be that exogenously administered Ang I has easier access to ACE than to convertase, an hypothesis compatible with the findings of Okunishi et al\(^6\) suggesting a primarily adventitial location for convertase versus an endothelial location for ACE; on the other hand, the present study suggests that captopril remains highly effective in endothelium-denuded vessels, indicating that ACE is not confined to the endothelium. This question may be resolved by measurements of both plasma and tissue Ang II after administration of intravenous SUB as well as by the advent of an effective in vivo chymase inhibitor. It is worth noting, however, that although there may be uncertainties about the mechanism of action of intravenous SUB, a large proportion of its vasoconstrictor effect in vitro appears to be the result of Ang I-convertase action.

Although our results suggest that the Ang I-convertase may have a role in the regulation of blood pressure, we did not determine the hemodynamic basis of the pressor response; however, our in vitro findings suggest that SUB caused an increase in blood pressure through vasoconstriction. The present results also are consistent with the findings of Hoit et al\(^7\) that SUB increases left ventricular end-systolic pressure in baboons, a finding that suggests a parallel increase in systemic arterial pressure. In addition our findings are consistent with the demonstrations by Urata et al\(^2\) of the inability of captopril to block generation of Ang II in primate heart membranes and of the specificity and efficiency of chymase for the generation of Ang II. Furthermore, our results are in agreement with the findings of Okunishi et al\(^8\) showing that captopril only partially blocks the vasoconstrictor response to Ang I and that only a serine protease such as Ang I-convertase could be responsible for the remainder of the response. Our results combined with the results of these studies suggest that production of Ang II by the Ang I-convertase may be responsible for the reported inability of ACE inhibition to suppress plasma Ang II during long-term therapy\(^9,10\) and prevent restenosis in human clinical trials.\(^11\) The latter hypothesis is supported by the findings that the levels of both ACE and Ang I-convertase (and their respective mRNAs) in balloon-injured canine arteries were increased\(^1\) and only 30% to 40% of the vasoconstrictor response to Ang I in isolated human gastroepiploic, superior rectal, and ileocolic arteries was inhibited by captopril, whereas 60% to 70% was inhibited by chymostat.\(^3\) These results suggest that for maximal suppression of Ang II generation, it may be desirable to combine ACE inhibition with Ang I-convertase inhibition.

References

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(Pro11,D-Ala12)-angiotensin I.
M L Mangiapano, A L Rauch, J T MacAndrew, S S Ellery, K W Hoover, D R Knight, H A
Johnson, W P Magee, D J Cushing and R A Buchholz

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