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Angiotensin I-converting enzyme (kininase II) in cardiovascular and renal regulations and diseases

O COSTEROUSSE, J ALLEGRINI, W HUANG and F ALHENC-GELAS*

Institut National de la Santé et de la Recherche Médicale,
Unité 367, Paris, France

The angiotensin I-converting enzyme (kininase II, ACE) is the major angiotensin II forming and kinin degrading enzyme in the circulation, and has other physiological peptide substrates. Recent studies have established that the interindividual variability in the levels of ACE in plasma and tissues is under the influence of a genetic polymorphism. The genetic polymorphism of ACE levels has been linked in case-control studies to the susceptibility of developing cardiovascular diseases, especially myocardial infarction and diabetic nephropathy, and to the risk of progression of renal diseases. The new concept that the level of ACE in peripheral circulations and tissue interstitium is an important factor in the determinism of the local concentration of peptides and their putative protective / deleterious effects, especially in the kidney and the heart, will be further appraised.

Key terms: *angiotensin I converting enzyme, angiotensins, diabetic nephropathy, genetic polymorphism, kidney, kininase II, kinins, myocardial infarction*

INTRODUCTION

The angiotensin I-converting enzyme (dipeptidylcarboxypeptidase I, kininase II, EC 3.4.15.1, ACE) is a transmembrane ectopeptidase of vascular cells, also secreted as a so-called soluble form in plasma. This enzyme plays an important role in cardiovascular homeostasis through its action on angiotensin I and bradykinin (Skeggs *et al*, 1956; Erdös, 1991). Angiotensin I is converted into the vasopressor and aldosterone stimulating peptide angiotensin II by removal of a single carboxy-terminal dipeptide. ACE also cleaves sequentially two carboxy-terminal dipeptides from bradykinin suppressing the vasodepressor action of this peptide. Because ACE is a relatively unspecific peptidase *in vitro*, and possesses two active sites, it may have other physiological functions not all yet identified (Jaspard *et al*, 1993; Azizi *et al*, 1996). Numerous studies using competitive ACE inhibitors indicate that ACE participates in the control of vascular tone, and maintains vasoconstriction, especially in physiological situations where renin secretion, and therefore angiotensin I production, are stimulated. These inhibitors became widely used as therapeutic agents to reduce blood pressure in hypertension, or decrease vascular resistance in cardiac insufficiency (Cushman & Ondetti, 1980). Recently it has been demonstrated that they protect against degradation of renal function in type I diabetes with incipient diabetic nephropathy, and also in other types of renal diseases (Marre *et al*, 1988; Lewis *et al*, 1993; Maschio *et al*, 1996; Gisen group, 1997).

* **Correspondence to:** Dr François Alhenc-Gelas, INSERM U 367, 17 rue du Fer à Moulin, 75005 Paris, France. E-mail: fagu367@idf.inserm.fr

In the cardiovascular system, the ACE gene is constitutively expressed in endothelial cells, smooth muscle cells (at least in the rat) and fibroblasts, as well as in some subtypes of mononuclear cells (Costerousse *et al*, 1993a). In the kidney it is also expressed in the epithelial cells of the proximal tubule which, like the other absorptive epithelia, contain high levels of ACE in the brush border (Erdös, 1991; Sibony *et al*, 1993). A soluble form of ACE circulates in plasma at the relatively high concentration of 10^{-9} M, although the plasma enzyme was considered physiologically less important for the processing of peptide in the circulation than the membrane bound endothelial enzyme. The circulating ACE is most likely released from the cells, mostly endothelial cells, but also perhaps mononuclear cells, by a proteolytic cleavage of the membrane anchor, involving another membrane metalloprotease, not yet identified (Wei *et al*, 1991a).

Plasma ACE levels can be studied by a number of enzymatic or immunological assays in healthy subjects and in disease. Past and recent work have established that the interindividual variability in plasma ACE levels in human is under the influence of a major gene. Genomic markers for this effect have been identified in the ACE gene, and are associated to ACE levels. The genetic polymorphism of ACE levels has been linked to the susceptibility of developing degenerative vascular diseases, especially myocardial infarction and diabetic nephropathy. It may also be involved in the progression of several types of glomerular diseases. While this should be confirmed in prospective studies of cardiovascular and renal risk factors, the role of plasma and tissue ACE levels in cardiovascular and renal homeostasis, and the pathophysiological bases of a putative deleterious effect of the enzyme in the heart and kidney should be established (Costerousse *et al*, 1997).

GENETIC POLYMORPHISM OF ACE LEVELS

The interindividual variability of plasma ACE levels is very high in healthy subjects, as those levels can differ up to 6-fold among subjects (Alhenc-Gelas *et al*, 1991). When measured repeatedly in a given subject, plasma ACE level remains remarkably constant. No association with hormonal candidate or environmental parameters was found to explain this large interindividual variability (Alhenc-Gelas *et al*, 1991). To test the hypothesis that the interindividual variability in ACE levels was genetically determined, a study of plasma ACE levels has been conducted in nuclear families. Intrafamilial correlation between genetically related members was indeed observed, and the analysis of several transmission models suggested that a major gene effect was responsible for a large part of the interindividual variability in ACE levels (Cambien *et al*, 1988).

As the ACE gene was a logical candidate for this effect, polymorphism of this gene was studied after cloning of the ACE cDNA (Soubrier *et al*, 1988; Lattion *et al*, 1989). A major polymorphism was recognized, corresponding to the presence or absence of a 287 base pairs Alu-type sequence located in intron 16, and this polymorphism was shown to be associated with the concentration of ACE in plasma. Subjects homozygotes for the insertion have lower levels than those homozygotes without the insertion, heterozygotes displaying intermediate levels (Rigat *et al*, 1990). These findings have been extended to the membrane bound form of ACE by studying circulating mononuclear cells, where ACE was found to be synthetized mostly in T lymphocytes. The membrane bound ACE levels in T lymphocytes, like the levels of soluble ACE in plasma, are highly variable from one subject to the other and associated with the ACE gene polymorphism (Costerousse *et al*, 1993b). These observations have been extended to the human myocardium (Danser *et al*, 1995). They suggest that in most, if not all, tissues synthetizing the enzyme the interindividual variability in ACE level is large and, for a part, genetically determined.

The mechanisms linking the insertion deletion (I/D) polymorphism to ACE levels remain unknown. The insertion is an Alu repeat type sequence, located in the 16th

intron of the ACE gene, and not found in the ACE gene of other primates (Batzer *et al*, 1994). It is unlikely to directly affect the level of transcription of the ACE gene promoter, but it may possibly affect the transcription and maturation process of the ACE pre-mRNA. This remains, however, hypothetical. The I/D polymorphism may also be only a neutral marker in linkage disequilibrium with a causal variant. Although other polymorphisms have been found in the ACE gene, all in non-coding sequences, the putative variant affecting the transcription of the promoter has not yet been identified (Doria *et al*, 1994; Villard *et al*, 1996).

The ACE gene I/D polymorphism, initially identified by RFLP (restriction fragment length polymorphism) analysis, can be more conveniently studied in large series of subjects by PCR (polymerase chain reaction) amplification of the 16th intron, preferably using flanking primers for primary amplification and an insertion, or allele specific oligonucleotide for secondary amplification, or for dot blot hybridization (Ludwig *et al*, 1995; Marre *et al*, 1997) The ACE levels can be followed in parallel by enzymatic activity measurement on synthetic tripeptide derived substrates, or by immunological assays (Cushman & Cheung, 1971; Alhenc-Gelas *et al*, 1983).

ASSOCIATION OF THE GENETIC POLYMORPHISM OF ACE LEVELS WITH DISEASES

Because of its involvement in vasoactive peptides metabolism, the ACE gene is a candidate gene for cardiovascular and renovascular diseases, and for hypertension. The ACE gene polymorphism and plasma ACE levels have, therefore, been studied in case control studies of cardiovascular diseases.

In a large multicenter study of patients having suffered from a myocardial infarction compared to appropriate controls (the ECTIM study), an abnormal proportion of subjects of the DD genotype, and of young subjects of all genotypes with high plasma ACE levels, was observed among cases, independently of blood pressure level, or of other classical risk factors (Cambien *et al*, 1992, 1994). An abnormal proportion of subjects carrying the D allele was also observed among subjects with a family history of myocardial infarction (Tiret *et al*, 1993). This study suggests that ACE is a risk factor for myocardial infarction. Numerous other studies have been carried out since then, sometimes with conflicting results (Lindpaintner *et al*, 1995), but there seems to be growing evidence in favour of an association of the ACE gene polymorphism with myocardial infarction (Samani *et al*, 1996; Staessen *et al*, 1997). These studies are all, however, case control studies, or in one case of post-hoc analysis of therapeutic intervention trial in highly selected populations (Lindpaintner *et al*, 1995), can be subjects to bias, and the role of ACE as a cardiovascular risk factor should, therefore, be definitely established in prospective studies designed for the detection of such factors.

The genetic polymorphism of ACE levels was also associated with the left ventricular size in middle aged normotensive and hypertensive populations (Schunkert *et al*, 1994), and to the development of exercise induced left ventricular hypertrophy in young males (Montgomery *et al*, 1997).

Constitutive ACE levels, and their genetic polymorphism were also associated with a peculiar renovascular disease, diabetic nephropathy in type I diabetes, and subsequently to the progression of other types of glomerular diseases, suggesting that ACE can have a deleterious effect not only on the coronary, but also on the renal circulation. In type I diabetic patients, the risk of diabetic nephropathy, a major complication of poorly controlled diabetes which is linked to cardiovascular (especially coronary) mortality, is probably for a part genetically transmitted. Vasoactive peptide systems affecting glomerular hemodynamics may be involved in the development of diabetic nephropathy.

In a case control study of type I diabetic patients with microvascular complications, an abnormal proportion of subjects carrying

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the D allele, or of subjects with high plasma ACE levels was observed in nephropathic subjects, but not in subjects with retinopathy, compared to paired non-albuminuric diabetic patients (Marre *et al*, 1994). A similar independent study also found an association between an ACE gene polymorphism and diabetic nephropathy in North American type I diabetic patients (Doria *et al*, 1994). Since then several studies have addressed the question in both insulin and non insulin dependant diabetic patients, again with conflicting results (Fogarty *et al*, 1994; Powrie *et al*, 1994; Tarnow *et al*, 1995; Schmidt *et al*, 1995; Mizuiri *et al*, 1995; Doi *et al*, 1996; Dudley *et al*, 1995). Here again there seem to be growing evidence in favor of an association between genetic polymorphism of ACE levels and diabetic nephropathy (Fusijawa *et al*, 1998). Recently, a large multicenter case control study of 494 type I diabetic patients selected for high risk of nephropathy because of poor glycemic control, all with proliferative retinopathy, did confirm the association, and suggested also a dominant effect of the D allele on the progression of the disease (Marre *et al*, 1997). These findings in diabetic nephropathy were subsequently extended to other types of glomerular diseases. Association between the ACE D allele and the rate of decline in renal function was indeed observed in studies of other glomerular diseases, especially IgA nephropathy (Harden *et al*, 1995; Yoshida *et al*, 1995; Van Essen *et al*, 1996).

As for myocardial infarction, however, these studies concerning diabetic nephropathy, and non-diabetic glomerular diseases, are case control studies that need to be confirmed in follow up studies of renal function in exposed patients.

Although there is a theoretical possibility that the polymorphisms of the ACE gene are in linkage disequilibrium with polymorphisms of another unknown but closely located gene, that would be responsible, at least in part, for the susceptibility to diseases, the association of the ACE gene I/D polymorphism with ACE levels in plasma and tissue, and the strong association between these levels and diseases suggests a pathogenetic role for ACE (Cambien *et al*, 1994; Marre *et al*, 1994). This role may be mediated by angiotensin II production and kinin inactivation.

PHYSIOLOGICAL AND PUTATIVE PATHOGENETIC CONSEQUENCES OF THE INTERINDIVIDUAL VARIABILITY IN CONSTITUTIVE AND REGULATED ACE LEVELS

The conversion of angiotensin I is generally not considered as a rate limiting step in angiotensin II formation because of the high abundance of ACE in the lung and the large capacity of the pulmonary circulation for angiotensin I conversion. Two studies have assessed the conversion of intravenously injected angiotensin I as it occurs through the lung, large arteries and forearm circulations, according to the ACE gene polymorphism. They provided different results, perhaps because of different design and basal state of activation of the renin-angiotensin system (Lachurie *et al*, 1995; Ueda *et al*, 1995). The fact that the D allele does not seem to be associated with higher blood pressure and the ACE gene locus is not linked to high blood pressure in most studies, except perhaps in subjects of African descent (Cambien *et al*, 1992; Jeunemaître *et al*, 1992; Duru *et al*, 1994; Rotimi *et al*, 1996), can suggest that the interindividual variability in ACE levels has only minimal consequences on the overall rate of angiotensin I conversion through the lung.

In spite of the above, angiotensin I is produced in arteries and in peripheral vascular beds, as well as in the interstitium of tissues, and a large part of it is produced in the heart and kidney (Danser *et al*, 1992). The conversion of angiotensin I may be a critical, rate limiting step in angiotensin II production in these locations. This can be especially the case in the kidney (Vane, 1972; Alhenc-Gelas *et al*, 1989) where angiotensin I levels can be high, and ACE levels in renal vessels are very low, at least in the human species. The conversion of angiotensin I in the kidney, especially at the glomerular level, may

depend mostly on circulating ACE, and remain low (Alhenc-Gelas *et al*, 1989). This hypothesis was further strengthened by the observation that the distribution of endothelial ACE in the human vasculature is highly heterogeneous, with undetectable levels of immunoreactive ACE in the renal vessels, in both the glomerular and peritubular circulations, whereas the enzyme is readily synthesized in muscular arterioles and part of the capillary network in other vascular territories (Franke, Metzer, Bohle, Reuter, Alhenc-Gelas & Danilov, Ms submitted for publication).

Bradykinin is another ACE substrate, and possesses vasodilator and antiaggregant properties. Intrarenal kinins may participate in the control of renal circulation (Roman *et al*, 1988). Kinins are also perhaps produced in the heart and have a protective effect in experimental cardiac ischemia (Linz *et al*, 1996). As ACE is the major kinin inactivating enzyme in the circulation it may limit the beneficial effects of kinins in the heart and kidney. It is of interest to note that induction of ACE gene expression occurs together with interstitial activation in the ischemic or hypertrophied myocardium, especially in macrophages and perhaps also in fibroblasts and vascular endothelial cells (Schunkert *et al*, 1990; Johnston, 1994; Challah *et al*, 1995). It is also observed in the hypertrophied aortic smooth muscle (Arnal *et al*, 1994). ACE induction is also observed in interstitium and arteries of the hypertensive rat kidney (Vio *et al*, 1997). In all these situations, the enzyme may play a pathogenetic role through kinin inactivation, or hydrolysis of other physiological substrates of the two ACE active sites, some of them being perhaps still unknown (Wei *et al*, 1992b; Jaspard *et al*, 1993; Azizi *et al*, 1996).

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