

ANGIOTENSIN-CONVERTING ENZYMES - BIOCHEMICAL ROLE AND FUNCTIONAL IMPORTANCE

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SUMMARY

This paper reviews the new data regarding angiotensin-converting enzyme (ACE) and its relatives. Somatic ACE, well known component of renin-angiotensin system (RAS), is membrane-bound metalloenzyme localized on endothelial cells. ACE converts angiotensin I (ANG I) to angiotensin II (ANG II)- potent vasoconstrictor. This enzyme is also involved in the catabolism of the vasodilator bradykinin and thus plays a dual role in the regulation of blood pressure, pathogenesis of arterial hypertension, and related cardiovascular disorders. ACE is enzyme of tissue RAS in brain, kidney, heart, blood vessels, adipose tissue, where it is responsible for local ANG II production. Testicular ACE is isozyme of ACE important for male fertility, insensitive to conventional ACE inhibitors. The newly discovered homolog of ACE, ACE2, has been expressed in the heart, kidney, testis, brain. This enzyme metabolizes ANG I to ANG-(1-9), and ANG II to vasodilator ANG-(1-7). ACE2 is not inhibited by "classical" ACE-inhibitors, and plays a critical role in regulating the balance between vasoconstrictor and vasodilator effects within the RAS cascade.

Keywords: angiotensin- converting enzyme, ACE2, angiotensin -(1-7)

I. Angiotensin- converting enzyme (ACE; EC 3.4.15.1)

The classical function of the ACE, well known component of renin-angiotensin system (RAS), is to convert angiotensin I (ANG I) to vasoconstrictor angiotensin II (ANG II). ACE is dipeptidase capable of hydrolyzing a variety of other peptide substrates as vasodilators bradykinin (ACE more readily hydrolyzes bradykinin than it does ANG I), and angiotensin-(1-7) (ANG-(1-7)), as well angiotensin-(1-9) (ANG-(1-9)). ACE degrades hematopoietic factor Ac-SDKP- potent natural inhibitor of hematopoietic stem cell proliferation. ACE is ectoenzyme, membrane-bound metalloproteinase, which is predominantly expressed in high concentrations on the surface of endothelial cells in the pulmonary circulation. This enzyme, so-called somatic ACE (sACE), is a single polypeptide chain. It consists of an intracellular domain, transmembrane domain, and 2 extracellular catalytic domains, often termed the N-terminal and C-terminal domains. Each of these domains binds zinc and is able to independently convert ANGI. The extracellular localization of ACE on endothelial cells positions it optimally for interaction with its substrate ANGI. A single passage of blood through the pulmonary vasculature is sufficient to convert all circulating ANGI to ANGII.

A soluble form of ACE (soluble or plasma ACE) is also present in the serum and other body fluids (cerebrospinal and bronchoalveolar fluids in disease states). It is derived from the membrane-bound form through the action of the ACE-secretase.

The presence of tissue RAS that could produce locally acting ANGII has been demonstrated. ACE, as all the components of RAS, have been found in cells and tissues non implicated in classical RAS.

In cardiovascular system ACE is detectable not only in the endothelium, but in other layers of vascular wall also. Vascular smooth muscle cells, which do not appear to express ACE, can do so in certain pathophysiological situations. Local ACE production in the heart can be easily detected by number of methods. Predominant sources of ACE are cardiac blood vessels and the endocardium, ACE is present in cultured cardiomyocytes and viable human cardiomyocytes after myocardial infarction. For > 80% of locally produced ANG II in the heart is responsible chymase- another ANG II- forming enzyme (8).

ACE in brain is colocalized with renin in synaptosomal fractions of circumventricular organs (vascular organ of the lamina terminalis, subfornical organ, area postrema)- brain structures involved in the control of cardiovascular function and hydroelectrolyte balance; as in choroid plexus, pineal gland, pituitary gland.

The kidney contains considerable ACE not only in renal vascular endothelium, but in the brush border of proximal tubule also.

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Expression of ACE has been demonstrated in isolated human adipocytes and cultured adipose cells. RAS in adipose tissue regulates cell growth and differentiation.

Regulation of plasma and tissue ACE

Plasma and tissue ACE levels are under control with genetic origins. The function of ACE gene was tested in mice having 1, 2, or 3 functional copies of the gene at its normal chromosomal location. Serum ACE activity increased progressively from 1-copy animals to the 3-copy animals. In humans polymorphism in the 16th intron of ACE gene involving the presence(insertion, I) or absence(deletion, D) of a 287-bp sequence of DNA explains the variance of blood ACE levels between individuals. Homozygosity for the D allele is associated with ACE activity levels twice that found in II carriers. DD genotype determinates also higher levels of ACE expression in some cells - adipocytes, cardiomyocytes, T-lymphocytes(9).

ACE activity in endothelial cells is positively regulated by beta-adrenergic stimulation signaling through c-AMP. Hormonal influences are involved in control of ACE in plasma and tissues. The effects of estrogens has been demonstrated in ovariectomized rats - estrogens replacement reduces ACE in the heart, angiotensinergic brain nuclei, kidney, abdominal aorta. Reduction in serum ACE has been reported after hormone replacement therapy in postmenopausal women (2).

ACE levels depend on body mass. Obese individuals have higher ACE than non-obese, in turn, it declines with dietary weight loss (6). ACE is influenced by dietary zinc.

Drug treatment with ACE- inhibitors and AT1 receptor antagonists modify the levels of ACE in plasma and tissues.

Mechanisms of action

Two mechanisms are responsible for ACE effects in disease states: 1) the synthesis of ANG II; 2) the newly recognized signaling by ACE ("outside-in signaling").

The majority of the actions of Ang II have been demonstrated to be mediated by the AT1 receptor, including growth promotion, vasoconstriction, antinatriuresis, aldosterone secretion, salt appetite, thirst, sympathetic outflow and inhibition of renin biosynthesis, and secretion.

ANG II, produced by activated endothelial ACE, impairs nitric oxide bioactivity, mainly because of oxidative stress through the ANG II- induced production of superoxide radicals that can scavenge nitric oxide. There is evidence that ACE expression is increased in atherosclerosis and that ANG II may contribute to disease progression by increasing oxidative stress, chemoattractant and adhesion molecule expression, leading to inflammation. ANG II can also favor proliferative, inflammatory and prothrombotic actions.

AT2 receptor stimulation engenders an autacoid vasodilator cascade composed of bradykinin (BK), nitric oxide (NO), and guanosine cyclic 3', 5'-monophosphate (cGMP). The AT2 receptor is counter-regulatory to the vasoconstrictor actions of Ang II at the AT1 receptor and

may provide at least some of the hypotensive benefit of AT1 receptor blockers in hypertension.

ACE-inhibitor-induced signaling process was described. The binding of an ACE inhibitor to ACE results in the activation and accumulation of c-Jun in the nucleus, which has been reported to activate transcription of ACE itself and cyclooxygenase-2, resp. production of prostacyclin (5)

Mice completely lacking all ACE(ACE - null) demonstrate the functional role of the enzyme - they have low systolic blood pressure, they are unable to effectively concentrate urine because of renal structural defect, they are mildly anemic, they also have an increase of serum and urinary potassium levels, male ACE-null mice have a marked defect in fertility (1).

II. Testicular ACE (tACE)

Testicular ACE is isozyme expressed at high levels by male germ cells during spermiogenesis. The tACE proteins are unique in that they are produced from exons identical to those used to assemble the carboxyl terminal half of somatic ACE. tACE has only a single catalytic domain, tACE substrate is unknown. Zinc deficiency leads to a reduction of ACE mRNA. Sperm lacking tACE are deficient in transport and attachment to zonae pellucidae of oocytes, and male mice lacking ACE are infertile even though they have normal testis structure, sperm count, sperm morphology and sperm motility. The selective tACE expression in mice lacking ACE results in normalized fertility. There is no indication that conventional ACE inhibitors have any effect on male fertility.

III. Angiotensin-converting enzyme 2 (ACE2)

A new enzyme, ACE2, homolog of ACE has recently been cloned (3, 11). ACE2 was first shown to cleave ANG I to ANG-(1-9). ACE2 had substrate preference for ANG II, which it hydrolysed to ANG-(1-7) at a high rate. ACE2 can cleave the C-terminal residues from several other peptides such as apelin, ghrelin, opioids. ACE2 contains a single zinc-binding domain which is homologous to one of the active sites of ACE and had 40% overall identity to ACE. ACE2 cannot be inhibited by ACE-inhibitors.

In contrast to the near ubiquitous expression of somatic ACE, ACE2 gene expression was localized predominantly to the heart, kidney, testis, brain.

ANG II may be a stimulus determining ACE2 gene expression, because either reduction in its levels or prevention of ANG II binding to the AT1 receptor increased ACE2 mRNA. ACE2 gene expression can be activated by increased wall stress or other factors associated with cardiac and vascular remodeling.

The importance of ACE2 in regulating cardiovascular function is highlighted by the phenotype of the ACE2 knockout mouse, which showed left ventricular dilation and impaired contractility. The phenotype was rescued by crossing with the ACE knockout mouse. This finding suggests that increased local cardiac ANG II was the cause of the cardiac abnormalities in the ACE2 knockout. The ab-

sence of functional ACE2 causes enhanced susceptibility to ANG II-induced hypertension.

III. Angiotensin - (1-7), (ANG- (1- 7)

ANG-(1-7) can be generated from ANG II by ACE2. ANG-(1-9), whose activity on the vascular system is not known, is formed from ANGI by ACE2., hydrolyzed further and loses 2 amino acids by the action of ACE to generate ANG-(1-7). Endopeptidases other than ACE2 and ACE, including neprilysin, prolyl endopeptidase, and thimet oligopeptidase can also generate ANG-(1-7) directly from ANG I. Levels of ANG-(1-7) increase significantly during ACE inhibition because of an increase in the ANG-(1-7) precursor (ANG I) and also because ACE is one of the main metabolizing enzymes of ANG-(1-7). Ang-(1-7) mediates specific effects through its recently identified receptor, the mas oncogene product (MAS). Through this receptor, ANG-(1-7) may stimulate nitric oxide synthase (NOS) and counteract the effects of ANG II. The effects of ANG-(1-7) may also involve cross-talk with the angiotensin type 2 receptor (AT2R), and the bradykinin type two receptor (BK2R). For example, it has been demonstrated that the vasodilator action of ANG-(1-7) can be blocked by the B2 receptor

ANG-(1-7) reduced the blood pressure of hypertensive rats, inhibited renal fluid absorption, caused vasodilation and participated in the antihypertensive responses to ACE inhibition or AT1receptor blockade. ANG-(1-7) inhibits the growth of vascular smooth muscle cells, cardiomyocytes, cardiac fibroblasts. ANG-(1-7) acts as a physiological modulator of ANG II, with opposing actions on body fluid volume, blood pressure, and cell growth(4).

IV. ACE и ACE2 under pathological states

As a component of RAS, ACE participates in the pathogenesis of hypertension.

The elevation in vascular ACE activity may play an important role in the maintenance of high blood pressure in case of hypertension without activation of "classical" RAS in the circulation, like in late stage of renal hypertension (7). Association has been suggested between ACE in adipose tissue and obesity hypertension (10). ACE2, through the generation of vasodilator ANG-(1-7) and by hydrolyzing part of ANG II, counterbalances the vasopressor effect of ACE that is mediated by ANG II. In different model systems of experimental genetic hypertension ACE2 levels are diminished, whereas, in normotensive strains higher ACE2 expression could account for the "resistance" to hypertension. ACE of local RAS is a pivotal enzyme in cardiac remodeling after myocardial infarction or in failing heart. The cellular events in the myocardial tissue triggered by ANGII, while initially compensatory, may lead to eventual loss of cellular function, contractility, and viability. ACE2 plays an important role in counter-regulatory response to ANGII-mediated effects.

ACE2 activity may counterbalance the ANGII-promoting effect of ACE in diabetic and non-diabetic kidney disease(12).

V. Perspectives

The discovery of ACE2 and its product ANG-(1- 7) shed new light on our understanding of pathophysiology of cardiovascular system, and mechanisms of pharmacological blockade of RAS. A further clarification of the role of ACE2 and ANG-(1-7) may hopefully lead to the development of new therapeutics.

REFERENCES

1. Bernstein, K.E. - Views of the Renin-Angiotensin System: Brillig, Mimsy, and Slithy Tove - Hypertension, 47: 509- 514, 2006
2. Dean, S.A., J. Tan, E.R.O'Brien et al - 17beta-Estradiol downregulates tissue angiotensin-converting enzyme and ANG II type 1 receptor in female rats - Am.J. Physiol. Regul. Integr. Comp. Physiol., 288: R759-R766, 2005.
3. Donoghue M., F.Hsieh, E. Baronas et al - A Novel Angiotensin-Converting Enzyme-Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1-9- Circ. Res.;87: E1-E9, 2000
4. Ferrario, C.M. - Angiotensin - Converting Enzyme 2 and Angiotensin-(1-7) An Evolving Story in Cardiovascular Regulation- Hypertension, 47: 515 - 521, 2006
5. Fleming, I. - Signaling by the Angiotensin-Converting Enzyme - Circ. Res., 98:887 -896, 2006
6. Harp, J.B., S.A. Henry, M. DiGirolamo - Dietary weight loss decreases serum angiotensin-converting enzyme activity in obese adults - Obes. Res., 10: 985-990, 2002
7. Miyazaki, M., T. Okamura, N. Toda - Role of vascular angiotensin-converting enzyme in hypertension - J. Hypertension, 6(suppl 3): S13-S15, 1988
8. Paul, M., A. P. Mehr, R. Kreutz - Physiology of Local Renin-Angiotensin Systems - Physiol. Rev., 86: 747-803, 2006
9. Sayed-Tabatabaei, F.A., B.A. Oostra, A. Isaacs et al. - ACE Polymorphisms - Circ. Res., 98:1123 - 1133, 2006
10. Strazzullo, P., R. Iacone, L. Iacoviello et al - Genetic variation in the renin-angiotensin system and abdominal adiposity in men: the Olivetti prospective heart study - Ann. Intern. Med., 138: 17-23, 2003
11. Turner, A.J., S.R. Tipnis, J.L. Guy et al - ACEH/ACE2 is a novel mammalian metallo-carboxypeptidase and a homologue of angiotensin-converting enzyme insensitive to ACE inhibitors - Can. J. Physiol. Pharmacol., 80: 346-353, 2002
12. Wysocki, J., M. Ye, M. J. Soler et al - ACE and ACE2 Activity in Diabetic Mice -Diabetes, 55: 2132-2139, 2006