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Angiotensin-Converting Enzyme: New Concepts Concerning Its Biological Role†

Mario R. W. Ehlers‡ and James F. Riordan*

Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, Boston, Massachusetts 02115

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Angiotensin-converting enzyme (ACE,[1] EC 3.4.15.1) is a zinc metalloprotease catalyzing the hydrolysis of carboxy-terminal dipeptides from oligopeptide substrates, most notably the decapeptide angiotensin I (AI) and the nonapeptide bradykinin (BK) (Soffer, 1981). ACE is a component of the renin–angiotensin system (RAS), and in view of recent advances in our understanding of the physiology and pathophysiology of the RAS (Campbell, 1987; Dzau, 1988), it is important that the implications of these new concepts are explored in terms of what is known about the biochemistry of ACE. Further, there has been a dramatic increase in the use of potent orally effective, active-site-directed ACE inhibitors (Johnston, 1988) in the absence of a complete understanding of the molecular basis for their activity or of the full physiological significance of their use. The biochemical and enzymatic properties of ACE have recently been reviewed in extensive detail (Soffer, 1976, 1981; Ondetti & Cushman, 1982; Erdös, 1987; Ehlers & Riordan, 1989) and need not be reiterated here. However, a perspective in terms of the role of ACE, both within and independent of the framework of the RAS, would appear to be both timely and useful.

Although ACE was discovered in 1954 (Skeggs et al., 1954), more than a decade elapsed before it became clear that ACE plays a critical role in the RAS, after the finding that the most important site for the generation of circulating AII was the lungs (Ng & Vane, 1967, 1968). In this organ ACE is both abundant (Cushman & Cheung, 1971; Lieberman & Sastre, 1983) and uniquely positioned to act on circulating substrates by its localization on the luminal surface of endothelial cell plasma membranes in the lung's rich vasculature (Ryan et al., 1975; Caldwell et al., 1976; Wigger & Stalcup, 1978). ACE was therefore incorporated into the central dogma of the circulating RAS: the acid protease renin is elaborated into plasma from the juxtaglomerular apparatus in the kidney in response to stimuli such as hypotension and diminished delivery of sodium to the distal tubular macula densa sites. Renin acts on liver-derived angiotensinogen (a 50-kDa α_2-globulin) to release the largely inactive AI, which is then converted to the active peptide hormone AII by ACE in the pulmonary vascular bed (Oparil & Haber, 1974a,b). AII, the most important effector of the RAS, is a potent vasoconstrictor and also stimulates the release of the sodium-retaining steroid hormone aldosterone from the adrenal cortex (Sancho et al., 1976). The circulating RAS was thus considered to play a central role in the maintenance of blood pressure and in fluid and electrolyte homeostasis, as well as in the etiology of at least some forms of hypertension (Skeggs et al., 1981).

This classical endocrine view of the RAS has recently been challenged by newer concepts that have emerged as a result of the cumulative weight of data acquired over the past 10–15 years from such sources as the purification and characterization of all the components of the RAS, the production of antibodies which allow the use of sensitive radioimmunoassay and immunohistochemical methods for the detection and quantitation of every component of the system, the development of multiple inhibitors such as the orally active ACE inhibitors and the AII antagonist saralasin, and the introduction of molecular biological techniques that provided clearer insights at the molecular level into some of the components of the system. Together, these studies have expanded the traditional systemic (endocrine) mode of action of the RAS to include effects on local tissues (paracrine), on their cells of synthesis (autocrine), and perhaps even on intracellular

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* Address correspondence to this author at the Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, 250 Longwood Ave., Boston, MA 02115.

‡ Associated with the MRC Liver Research Centre, Department of Medicine, University of Cape Town Medical School, Cape Town, South Africa, and supported in part by the South African Medical Research Council.

[1] Abbreviations: ACE, angiotensin-converting enzyme; AI, angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu); AII, angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe); AIII, angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe); BK, bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg); LH-RH, luteinizing hormone-releasing hormone (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2); RAS, renin–angiotensin system; SP, substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2).

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events (intracrine) (Re, 1984; Re & Rovigatti, 1988). Further, while the importance of the RAS for blood pressure and fluid and electrolyte homeostasis remains undisputed, the RAS and, consequently, ACE have been implicated in at least two other areas, unrelated to the former or each other, both of major physiological and pathophysiological interest: reproduction and immunity (Weinstock, 1986). In addition to its expanding roles as a member of the RAS, ACE has also been localized to at least one tissue containing no other components of the RAS, notably the basal ganglia in the brain, where ACE may hydrolyze non-angiotensin peptides such as substance P (SP) (Strittmatter et al., 1985b) or opioid peptides (Stewart et al., 1981; Kase et al., 1986).

Biochemistry of ACE

ACE is an acidic glycoprotein composed of a single, large polypeptide chain and 1 mol/mol zinc [for reviews, see Soffer (1976, 1981), Ondetti and Cushman (1982), Erdös (1987), Ehlers and Riordan (1989)]. In most mammalian species the predominant form appears to have a molecular weight of 140000–160000. It occurs in most tissues, although in humans primarily in kidney and lung. A lower molecular weight form has been shown to be present in testis (El-Dorry et al., 1982a,b; Lanzillo et al., 1985; Soffer et al., 1987). An mRNA encoding ACE was first isolated from bovine lung tissue (Deluca-Flaherty et al., 1987), and the corresponding cDNA has been partially sequenced. An ACE cDNA has also been obtained by using mRNA isolates from mouse kidney (Bernstein et al., 1988) and from rabbit testes (Roy et al., 1988). The complete amino acid sequence of human ACE has recently been determined on the basis of the DNA complementary to mRNA obtained from human umbilical vein endothelial cells (Soubrier et al., 1988). The initial translation product contains 1306 amino acids including an N-terminal 29-residue signal peptide. There is a hydrophobic region near the C-terminus, which is thought to be involved in binding to the plasma membrane. There is a high degree of internal homology indicative of gene duplication, and within each half of the molecule there is a region corresponding to the putative metal binding sites of several metalloproteins. This raises the intriguing question of why ACE appears to contain only a single catalytically active zinc atom. Genomic DNA analysis by Southern blotting indicates a single ACE gene is present in both the human and mouse genome (Soubrier et al., 1988; Bernstein et al., 1988). Differential splicing of the gene transcript has been proposed to account for the generation of the lung/kidney and testicular forms of the enzyme (Soubrier et al., 1988).

ACE and the RAS

By enzymatic activity, immunoreactivity, and/or the presence of mRNA, all the components of the RAS (renin, angiotensinogen, AI and AII, AII receptors, and ACE) have been found to coexist locally in various tissues, including blood vessels, kidney, adrenal, heart, and brain [reviewed by Dzau (1988)]. These intrinsic tissue RASs form autocrine or paracrine systems that provide tonic control of vascular resistance and influence local tissue function, while the circulating or endocrine RAS serves to provide short-term cardiorenal homeostasis (Dzau, 1988).

It is becoming increasingly accepted that many of the most important effects of the RAS are exerted by local tissue systems, rather than by the circulating RAS (Dzau, 1986; Campbell, 1987; Dzau, 1988). However, the implications of these revised concepts for our understanding of ACE have not been fully explored. Most radical is the notion that in some tissues the entire RAS, including ACE, may be localized and perform their functions intracellularly (Inagami et al., 1983); the evidence for and implications of this view are discussed below.

ACE and an Intracellular RAS

Consequent to the development of the concept of multiple local tissue RASs is the hypothesis of the complete intracellular generation of AI and AII, implying the intracellular localization of some or all of the components of the RAS, including ACE (Inagami et al., 1983; Re, 1984). By immunoreactivity and/or enzymatic activity, the various components of the RAS have been colocalized in cell cultures of neuroblastoma × glioma cells (Fishman et al., 1981), neuroblastoma cells (Okamura et al., 1981), and murine Leydig tumor cells (Pandey & Inagami, 1986) and in tissue sections of rat kidney juxtaglomerular cells (Celio & Inagami, 1981; Naruse et al., 1982). While the intracellular generation of angiotensins is one explanation for these data, no conclusive evidence has been provided that the entire renin–angiotensin cascade occurs intracellularly, since the uptake of extracellularly produced angiotensin is equally likely (Campbell, 1987). Indeed, one recent study (Urata et al., 1988) provided strong evidence that in the rat adrenal zona glomerulosa AII generation occurred extracellularly rather than intracellularly and that intracellular AII probably represents receptor endocytosed hormone. However, the possibility of intracellular ACE is an intriguing one which deserves to be explored.

ACE is generally thought, in keeping with its extensive glycosylation, to be a transmembrane peptidase, bound to the external surface of plasma membrane of varius cell types by a hydrophobic anchor (Erdös, 1987). This is consistent with a stretch of hydrophobic amino acids at the C-terminal region of ACE (Soubrier et al., 1988). Numerous immunohistochemical studies have confirmed this extracellular membrane-bound position on endothelial, epithelial, neuroepithelial, and miscellaneous other cell types (Ryan et al., 1975; Caldwell et al., 1976; Wigger & Stalcup, 1978; Defendini et al., 1983; Danilov et al., 1987). However, two studies have demonstrated the existence of large amounts of ACE in the cytoplasm of swine and rabbit spermatids and spermatozoa and in their discarded cytoplasmic droplets (Yotsumoto et al., 1984; Brentjens et al., 1986). Further, ultrastructural immunohistochemical studies have localized the enzyme to intracellular positions in the endocytoplasmic reticulum, nuclear envelope, and endocytotic vesicles of human renal vascular and proximal tubular epithelial cells (Bruneval et al., 1986). While this may merely reflect synthesis and cellular processing of ACE, it is equally conceivable that some of the ACE, following processing in the Golgi apparatus, remains sequestered in intracellular vesicles and is copackaged with other components of the RAS, namely, renin and angiotensinogen, with subsequent generation of AII (Re, 1984). Alternatively, endocytosis of extracellular membrane-bound ACE, with or without the other components of the RAS, can be envisaged. Intracellularly generated AII may be exported to serve local paracrine or autocrine functions, or it may even remain and exert its effects intracellularly. The latter possibility is supported by evidence that internalized AII localizes to mitochondria and nuclei (Robertson & Khairallah, 1971), by preliminary evidence suggesting that nuclear chromatin contains AII receptors (Re et al., 1984), and by the finding of a cytosolic AII-binding protein in rabbit liver (Rosenberg et al., 1988).

The evidence presented above suggests that ACE may exist intracellularly in microsomes or endocytotic vesicles, that is, in an environment which is essentially extracellular in its properties, since it lies outside the cytosol. It certainly would

be highly unlikely that a heavily glycosylated protein such as ACE is active in both an extracellular, oxidizing environment, where it is known to act on various peptide substrates in flux, and in an intracytoplasmic, reducing environment, which would likely profoundly affect the enzyme's folded conformation required for activity (Creighton, 1984).

Lastly, any hypothesis postulating an intracellular location for ACE must take into account the anion requirement of the enzyme. This is a complex phenomenon that involves both essential and nonessential mechanisms of activation, dependent on substrate, pH, and probably enzyme source (species) (Cheung et al., 1980; Bünning & Riordan, 1983; Shapiro et al., 1983; Ehlers & Kirsch, 1988). Although a great deal of kinetic data has been collected, the molecular basis for this activation remains obscure and its physiological relevance, if any, unclear. Studies with rabbit ACE led to the suggestion that chloride activation represents a potential regulatory mechanism (Bünning & Riordan, 1983; Shapiro et al., 1983), but recent data obtained for the conversion of AI to AII by human ACE indicate that maximal activation for this substrate is achieved already at 30 mM Cl^- at physiological pH (Ehlers & Kirsch, 1988). Thus, anion activation is unlikely to play a regulatory role in the extracellular sites where ACE is known to occur, with the possible exception of the intestinal microvilli. On the other hand, it is conceivable that chloride and hydrogen ion concentration fluxes may regulate ACE intracellularly, where the ionic composition differs significantly from that of the extracellular milieu: $[Cl^-]$ is approximately 4 mM, and there are marked intercompartmental pH differences (Darnell et al., 1986). Endosomes are acidic with the pH as low as 5.0 (Helenius et al., 1983), while a pH of 5.5 has been noted for some secretory vesicles (Russell, 1984). Such environments would lead to near-total inhibition of ACE activity (Bünning et al., 1983; Ehlers & Kirsch, 1988). However, other vesicles, such as the small vesicles and tubules associated with the Golgi complex, have a pH of 6.4 (Yamashiro & Maxfield, 1984), and it is tempting to speculate that if ACE is indeed active intracellularly, it is regulated by transient intravesicular hydrogen and chloride ion concentration changes.

ACE and the RAS: Functions Unrelated to Blood Pressure and Fluid and Electrolyte Homeostasis

The circulating RAS and the local tissue RASs described above, i.e., in the kidney, brain, adrenal, heart, and vessel walls, are concerned with the maintenance of blood pressure and fluid and electrolyte homeostasis by such mechanisms as vasoconstriction, aldosterone release resulting in sodium and water retention, regulation of intrarenal hemodynamics, stimulation of thirst, and release of vasopressin and catecholamines (Dzau, 1988; Unger et al., 1988). Further, the ACE found in high concentrations on epithelial surfaces such as the intestinal, choroid plexus, and placental brush borders, probably coexisting with other components of the RAS, is, at least in part, also involved in fluid and electrolyte balance at these fluid/membrane interfaces (Defendini et al., 1983; Erdös, 1987). On the other hand, ACE and the RAS have recently been linked to two functions unrelated to fluid and pressure balance: reproduction and immunity.

Reproduction

Testicular ACE is of great interest: it is 30% smaller than its pulmonary counterpart, has different N- and C-terminal sequences and mRNA, yet arises from a common gene (Soubrier et al., 1988). It probably represents an internal portion of pulmonary ACE and has similar enzymatic properties (El-Dorry et al., 1982a,b; Iwata et al., 1982). Further, testicular ACE is under hormonal control: it is absent in immature rats and develops with puberty (Cushman & Cheung, 1971; Strittmatter et al., 1985a; Velletri et al., 1985). Testicular ACE has been localized to the cytoplasmic droplets of sperm (Yotsumoto et al., 1984; Brentjens et al., 1986) and to Leydig cells (Brentjens et al., 1986; Pandey et al., 1984). A complicating factor in the male genital tract is that ACE is also very abundant in the prostate and epididymis, from which it is secreted or sloughed off into the seminal plasma (Yokoyama et al., 1982; Strittmatter et al., 1985a), and this ACE is almost certainly of the lung, rather than testicular, type (El-Dorry et al., 1983; Strittmatter & Snyder, 1984). Recently other components of the RAS have been localized to the testis: renin mRNA has been detected by Northern blot analysis in testis (Pandey et al., 1984) and by in situ hybridization in Leydig cells (Deschepper et al., 1986); AI, AII, and AIII immunoreactivity was found in Leydig cell homogenates (Pandey et al., 1984); and AII receptors were identified in rat and primate Leydig cells (Khanum & Dufau, 1988; Millan & Aguilera, 1988). While prostatic and epididymal ACE may primarily be involved in the regulation of fluid and electrolyte balance of the seminal plasma, testicular ACE, in the germinal and Leydig cells, may play a more direct role in testicular function. It has been thought that ACE may contribute to male fertility by affecting sperm motility and capacitation through BK inactivation (Hohlbrugger & Dahlheim, 1983). It can also play a role in the modulation of steroidogenesis and/or regulation of cell growth and differentiation (Millan & Aguilera, 1988). In this latter regard its actions may be analogous to those of ovarian ACE, discussed below.

Ovaries appear to contain all the components of the RAS, as evidenced by the detection, in rat and human ovaries, of angiotensinogen mRNA (Ohkubo et al., 1986); prorenin and renin activity, immunoreactivity, and mRNA (Glorioso et al., 1986; Do et al., 1988; Kim et al., 1987); AII and AII receptors (Husain et al., 1987; Pucell et al., 1987; Speth & Husain, 1988); and ACE by immunohistochemistry (Brentjens et al., 1986) and in vitro autoradiographic localization by ^{125}I-labeled inhibitor binding (Speth & Husain, 1988). The RAS may have important paracrine functions in the ovary, since it has been shown that AII increases steroidogenesis (Pucell et al., 1988), and thus AII may be involved in follicular development (Speth & Husain, 1988). Indeed, the AII antagonist saralasin blocks ovulation in the rat (Pellicer et al., 1988). The wider implications of these findings for the use of ACE inhibitors and their effects on both the male and female reproductive systems will be discussed below. Lastly, it is worth noting that estrogens stimulate an increased rate of angiotensinogen synthesis in the liver and elevate plasma angiotensinogen levels (Tewksbury, 1981), and thus there appears to be a general link between the RAS and steroidogenesis.

Immunity

Since Lieberman (1975) reported the association between an elevated level of serum ACE and the granulomatous disease sarcoidosis, numerous studies have localized ACE to cells of the monocyte lineage, including monocytes, macrophages, and their derivatives, and ACE is thought to be important in mediating and/or modulating inflammation [reviewed by Weinstock (1986)]. One mechanism is through the conversion of AI to AII: angiotensinogen, renin-like activity, ACE, and AI, AII, and AIII are all present within granulomas of murine schistosomiasis (Weinstock & Blum, 1983); AII is synthesized by granuloma macrophages (Weinstock & Blum, 1987) and enhances phagocytotic activity of such macrophages (Foris et al., 1983); and AII is chemotactic for a T-lymphocyte subset

(Weinstock et al., 1987). Alternatively, the effect of ACE on inflammation may be due either to the inactivation of BK, a proinflammatory vasoactive peptide, or to the metabolism of neuropeptides such as SP and neurotensin (Weinstock, 1986).

Non-RAS ACE Functions

Although ACE is increasingly being described as a ubiquitous mammalian dipeptidyl carboxypeptidase, present in most tissues and body fluids (Erdös, 1987), it is also becoming increasingly evident that in the great majority of instances it is colocalized with some or all of the components of the RAS, and it is likely that its most important function remains its critical role in that system. Nevertheless, a search for roles outside the RAS was stimulated not only by its widespread tissue distribution but also by the apparent lack of substrate specificity. Thus, in addition to AI and BK, ACE also hydrolyzes, in vitro, other biologically active peptides such as the enkephalins (Stewart et al., 1981) and neurotensin (Skidgel et al., 1984), as well as the B chain of insulin (Igic et al., 1972). Further, although classically regarded as a dipeptidyl carboxypeptidase with a requirement for a free C-terminal carboxyl, ACE can cleave amidated peptides such as SP (Yokosawa et al., 1983; Cascieri et al., 1984; Skidgel et al., 1984; Thiele et al., 1985) and substance K (Thiele et al., 1985). Also, tripeptidyl carboxypeptidase activity is observed with SP (Cascieri et al., 1984; Thiele et al., 1985), des-Arg^9-BK (Inokuchi & Nagamatsu, 1981), and LH-RH (Skidgel & Erdös, 1985); ACE even exhibits tripeptidyl aminopeptidase activity, as with LH-RH which has a blocked N-terminus (Skidgel & Erdös, 1985). However, while these activities have been observed in vitro, there is no convincing evidence that most of these are physiologically relevant, and only BK and SP can seriously be considered, in addition to the well-established AI, as biological substrates of mammalian ACE.

BK is a nonapeptide that is inactivated by ACE by sequential cleavage of two C-terminal dipeptides (Cushman & Ondetti, 1980). It is an interesting substrate because, in common with other "class II" substrates (Shapiro et al., 1983), it is tight binding ($K_m \sim 10^{-6}$ M) with a favorable k_{cat}/K_m ($\sim 10^8$ M^{-1} min^{-1}) (Bünning et al., 1983) and its hydrolysis is not absolutely dependent on chloride, occurring maximally at a low concentration of ~10 mM (Dorer et al., 1974; Bünning et al., 1983). Twenty years ago it was suggested that the pulmonary inactivation of BK and the conversion of AI to AII were due to the same enzyme (Ng & Vane, 1967, 1968) and that this enzyme was ACE (Yang et al., 1972). However, while it is an attractive idea that ACE performs its blood pressure regulating role by both generating the vasoconstrictor AII and inactivating the vasodilator BK, the physiological importance of ACE in the inactivation of BK remains debatable. Thus, inhibition of ACE in isolated rat lungs has little effect on the rate of BK inactivation (Ryan et al., 1970), studies on plasma BK levels during administration of ACE inhibitors have yielded conflicting results [reviewed by Filep et al. (1987)], and urinary kinins are not increased following ACE inhibition in the spontaneously hypertensive rat (Filep et al., 1987). It should be noted, of course, that BK is susceptible to the action of proteases and peptidases other than ACE.

SP, an amidated undecapeptide, is a potential neuropeptide transmitter in the central and peripheral nervous system (Nicoll et al., 1980). The k_{cat}/K_m for the ACE-catalyzed hydrolysis of SP is 2×10^5 M^{-1} min^{-1} (Cascieri et al., 1984), which is 50 times less than the $\sim 10^7$ M^{-1} min^{-1} determined for AI (Bünning et al., 1983; Ehlers et al., 1986). Evidence that ACE plays a role in its degradation in vivo is 3-fold. First, ACE colocalizes with SP to a striatonigral pathway (Strittmatter et al., 1984; Strittmatter & Snyder, 1987) in which there is no evidence for AII (Brownfield et al., 1982), AII receptors (Mendelsohn et al., 1984), or BK (Correa et al., 1979). Although the rat striatal enzyme is smaller than its lung counterpart and has been termed an ACE isozyme analogous to the testicular form (Strittmatter et al., 1985b; Strittmatter & Snyder, 1987), this difference is likely the result of differential glycosylation rather than separate isozymes (Hooper & Turner, 1987). Second, ACE inhibitors increase SP-induced salivation in rats (Cascieri et al., 1984) and also lead to an increase in SP-like immunoreactivity in the substantia nigra and trigeminal nucleus when administered intraventricularly (Hanson & Lovenberg, 1980). Third, cough, an increasingly recognized side effect of ACE inhibitor therapy (Morice et al., 1987), may be due to SP accumulation (Morice et al., 1987), since both the bronchoconstrictor response to intravenously administered SP and its plasma level were increased by the ACE inhibitor captopril (Shore et al., 1988). In addition, recent studies with rodents have shown that ACE inhibitors enhance short-term memory and alleviate the amnesia induced by the muscarinic receptor antagonist scopolamine (Usinger et al., 1988; Costall et al., 1988). Although the basis for these effects is unknown, it could involve the metabolism of acetylcholine, which is thought to be regulated by both SP and Met-enkephalin (Sastry & Tayeb, 1982). Taken together, these various lines of evidence suggest that ACE may, at least in part, be involved in the metabolism of SP, and therefore ACE may play a role in neurotransmission.

ACE Inhibition

The development of captopril (D-3-mercapto-2-methylpropanoyl-L-proline) (Cushman et al., 1977) and the two carboxyalkyl dipeptides enalaprilat {*N*-[(*S*)-1-carboxy-3-phenylpropyl]-L-alanyl-L-proline} and lisinopril {*N*-[(*S*)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline} (Patchett et al., 1980), among others, has revolutionized the treatment of hypertension and congestive cardiac failure (Edwards & Padfield, 1985; Johnston, 1988). These compounds are active-site-directed, zinc-coordinating, slow- and tight-binding competitive inhibitors with subnanomolar K_i values ($K_i \sim 10^{-10}$–10^{-11} M) (Shapiro & Riordan, 1984a,b; Bull et al., 1985). A detailed kinetic analysis of their mechanism of action revealed it to be complex and substrate dependent (Shapiro & Riordan, 1984a,b). Inhibitor binding appears to follow a two-step mechanism with the initial EI complex undergoing slow isomerization to a more tightly bound EI* complex (Shapiro & Riordan, 1984a,b; Bull et al., 1985), and this slow step is chloride dependent (Shapiro & Riordan, 1984b). It is worth noting that, in the absence of structural information, interpretation of the complex kinetic data remains difficult, as the inhibition is both substrate and anion dependent, and may even be species dependent, since it was recently shown that the hydrolysis of AI by human ACE shows some differences from that by the rabbit enzyme (Ehlers & Kirsch, 1988) (all kinetic data on ACE inhibition have, to date, been obtained with the rabbit enzyme).

In view of the widespread use of these inhibitors in clinical practice, it is becoming increasingly important to understand the catalytic mechanism of the enzyme in molecular terms, permitting the rational design and synthesis of newer agents with specific properties, as well as to understand the diverse physiological roles of the enzyme, facilitating the anticipation and evaluation of specific inhibitor-related adverse reactions. In the context of the RAS, ACE, as discussed earlier, is involved in blood pressure and fluid and electrolyte homeostasis

in most tissues. Thus, inhibition of endothelial ACE, in both the lungs and peripheral vasculature, and inhibition of ACE in the kidneys, heart, adrenal, and the circumventricular organs in the brain is, in all these various tissues, likely to result in a hypotensive effect (Dzau, 1988; Unger et al., 1988). Yet what of ACE in those loci where, although a component of the RAS, it participates in functions other than fluid and pressure balance and/or where there is no evidence of an independent RAS? In a recent study (Sakaguchi et al., 1988) tissue ACE was assessed by quantitative in vitro autoradiography using the ACE inhibitor [^{125}I]351A as a ligand: following oral administration of lisinopril, ACE activity was markedly inhibited in kidney, adrenal, duodenum, and lung but was not altered in testis, an organ considered to be protected by a blood–testis barrier (Waites & Gladwell, 1982). Similarly, ACE in the choroid plexus and the basal ganglia of the brain was not inhibited, while that in the circumventricular organs, which are considered to lie outside the blood–brain barrier, was inhibited (Sakaguchi et al., 1988). Captopril has also been shown not to cross the blood–brain barrier readily when administered peripherally (Vollmer & Boccagno, 1977), but it does seem to have an effect on cognitive processes in laboratory animals (Costall et al., 1988). Nevertheless, the ACE present in at least two sites where it may have important functions unrelated to blood pressure control, a striatonigral pathway and the testis, may be largely unaffected by the present generation of ACE inhibitors under ordinary circumstances.

On the other hand, intestinal ACE, which is present in high concentration on the microvilli (Defendini et al., 1983; Ward et al., 1980) and may, by the generation of AII, be involved in fluid and sodium absorption (Crocker & Munday, 1970), or function as a digestive peptidase (Yoshioka et al., 1987), is vulnerable to inhibition by orally active agents (Sakaguchi et al., 1988; Stevens et al., 1988), although this does not appear to have any pathophysiological consequences (Gavras & Gavras, 1988). Where ACE and the RAS are associated with the immune system, the administration of ACE inhibitors results in suppression of granulomatous inflammation, which was beneficial when associated with a nonreplicating antigen such as schistosoma eggs (Weinstock & Boros, 1981) or BCG inoculum (Schrier et al., 1982), but worsened the severity of acute infection with the yeast *Histoplasma capsulatum* (Deepe et al., 1985). Lastly, ovarian ACE may be inhibited by orally active ACE inhibitors, since in the ovary there is no barrier comparable to the blood–testis barrier (Richards, 1980). This may have a significant effect on ovarian function since it was recently shown that ovulation is blocked by an AII antagonist (Pellicer et al., 1988).

From these considerations, and from the paucity of side effects specifically related to the inhibition of ACE (rather than to nonspecific effects resulting from the chemical structure of the inhibitor) (Gavras & Gavras, 1988), it seems likely that the bulk of mammalian ACE forms part of the circulating and tissue RAS and is primarily concerned with blood pressure and fluid and electrolyte homeostasis. In those loci where it performs a different, more specialized function, either the enzyme is inaccessible to current inhibitors or its function is not rate limiting and thus its inhibition is not pathophysiologically important. Despite the apparent effectiveness and specificity of current ACE inhibitors, it nevertheless remains desirable to develop new generations of compounds, for two reasons. First, drugs with similar mechanisms of action as the current agents but with different physical properties allowing their penetration across the blood–brain and blood–testis barriers would be of interest; compounds with much higher lipophilicity than captopril, enalapril, or lisinopril have already been synthesized (Ondetti, 1988). Second, once the substrate-binding and catalytic mechanisms of ACE are understood in molecular terms, it may be possible to design inhibitors that preferentially inhibit hydrolysis of some substrates more than others. Thus inhibitors that do not disable the catalytic zinc or other moieties indispensable for the catalysis of all substrates, but instead bind only to substrate-binding residues in the active site, may displace some substrates but not others. This is feasible since the ACE active site has been hypothesized to form an extended linear trench with both obligatory and auxilliary binding sites (Cushman et al., 1981). Binding to the latter may explain the good inhibitory activity of compounds like teprotide, which have a C-terminal tripeptide sequence with poor affinity for the obligatory binding sites (Ondetti, 1988), as well as the finding that the binding of substrates is significantly influenced by the N-terminal residues distant from the site of hydrolysis (Gaynes et al., 1978). Further, binding of different components of substrates to the various binding sites may be synergistic, and the ACE active site may be dependent upon an induced-fit mechanism for optimal catalytic activity (Pfuetzner & Chan, 1988). The synthesis of inhibitors that, for example, minimally retard the hydrolysis of AI but significantly interfere with the breakdown of either BK or SP could therefore be envisaged.

Conclusion

Great strides have been made in our understanding of the biological role of ACE. Considerable insights have been provided by the use of ACE inhibitors, and these have confirmed the importance of this enzyme in the RAS and of the latter's role in blood pressure regulation and hypertension. Further, recent advances in our understanding of the RAS have led to newly evolving concepts, including an expansion of its role as a purely endocrine system to one having important additional paracrine/autocrine effects; the probability of an entirely intracellular RAS; and the participation of the RAS in functions unrelated to blood pressure regulation, namely, reproduction and immunity. The introduction of molecular biologic techniques has provided important additional information, such as the primary structure of this enzyme, and should lay the groundwork for an eventual elucidation of its catalytic mechanism in molecular terms. In addition, the use of cDNA probes that are now available will no doubt provide an insight into the expression and regulation of ACE at the tissue and cellular level.

Registry No. ACE, 9015-82-1; angiotensin, 1407-47-2; renin, 9015-94-5.

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Accelerated Publications

Downregulation of Cell Growth and Cell Cycle Regulated Genes during Chick Osteoblast Differentiation with the Reciprocal Expression of Histone Gene Variants†

Victoria Shalhoub,‡ Louis C. Gerstenfeld,§ David Collart,‡ Jane B. Lian,*,‡ and Gary S. Stein*,‡

Department of Cell Biology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655, and Children's Hospital, Boston, Massachusetts 02115

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ABSTRACT: Expression of cell cycle (core and H1 histone) and cell growth (c-myc and c-fos) regulated genes was examined in primary cultures of chick calvarial osteoblasts during a developmental sequence associated with the progressive maturation of the osteoblast in a bonelike mineralized extracellular matrix. We have identified a transition point early in the developmental sequence which occurs when proliferation ceases and expression of genes related to the differentiated phenotype of osteoblasts is initiated. During this transition period, cellular levels of RNA transcripts from core and H1 histone genes and the c-myc and c-fos protooncogenes decrease in a parallel and coordinate manner. The decline in histone gene transcription that accompanies the loss of accumulated histone mRNA indicates that the downregulation of histone gene expression is at least, in part, transcriptionally mediated. In contrast, persistence of c-myc and c-fos transcription following completion of the proliferation period, when the mRNAs are no longer present at detectable levels, suggests that the initial downregulation of protooncogene expression is controlled at the level of messenger RNA stability. Thus, two types of signaling mechanisms are operative in the downregulation of cell proliferation genes during osteoblast differentiation—one that impinges on regulatory sequences that influence the interactions of transcription factors with cis-acting promoter elements and a second that modulates messenger RNA turnover. Of significance, downregulation of the cell cycle regulated histone genes is accompanied by a reciprocal increase in the expression of a structurally distinct subset of the histone genes that are not coupled with DNA replication during the period of expression of osteoblast phenotype markers. A similar increase in expression of these histone genes that encode high molecular weight, poly(A+) transcripts was also observed during the shutdown of proliferation and onset of differentiation in promyelocytic leukemia cells. Thus, expression of this subset of histone genes serves as a marker for the onset of differentiation. Involvement of these genes may relate to modifications of the structural and transcriptional properties of chromatin that occur at the initiation of tissue-specific gene expression.

Development of the osteoblast phenotype is associated with a complex and interdependent sequence of events reflected by modifications at the cellular, biochemical, and molecular levels. Recently, the development of osteoblast culture systems of normal diploid cells isolated from embryonic chick (Aubin et al., 1982; Gerstenfeld et al., 1987) and fetal rat (Bellows et al., 1986; Ecarot-Charrier et al., 1983) calvaria has provided an experimental system to address control of events related to the onset and progressive maturation and differentiation of proliferating osteoblasts into fully mature, nonproliferating osteocytic cells in a tissuelike mineralized matrix (Stein et al., 1988a,b). A temporal expression of proteins that are phenotypically unique to the osteoblast occurs in these cultured cells. The biological relevance of this in vitro developmental sequence leading to a bonelike matrix is supported by a similar developmental sequence during fetal formation of the calvarium in the intact animal (Yoon et al., 1987).

In situ, several osteoblast populations that include proliferating preosteoblasts and nondividing osteoblast cells at various developmental and maturational stages are identified by morphological criteria and tissue organization (Nijweide et al., 1986). We have identified in vitro (Stein et al., 1988b) a key transition point in the ordered developmental sequence associated with osteoblast differentiation that occurs early in the process when cell proliferation ceases and expression of genes associated with extracellular matrix maturation and mineralization is initiated. An understanding of molecular mechanisms operative in the downregulation of cell growth

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‡ University of Massachusetts Medical Center.
§ Children's Hospital.

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