The Central Role of Angiotensin I-Converting Enzyme in Vertebrate Pathophysiology

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Abstract: Genomic epidemiologic data, increasingly supported by clinical outcomes results, strongly suggest that overactivity of angiotensin I-converting enzyme (ACE) may underlie most age-related diseases. Angiotensin II, the main product of ACE, is a pleiotropic hormone, capable of serving as a neurotransmitter, growth factor, angiogenesis factor, vasoconstrictor, pro-thrombotic agent, and cytokine. So it is perhaps not surprising that the ACE D/D genotype is associated with several major psychiatric diseases, most cancers except prostate cancer (where the D/D genotype is actually protective), most cardiovascular diseases, most autoimmune diseases, and even infectious diseases like tuberculosis and HIV. In a preliminary study, angiotensin II blockade appeared to hasten recovery from West Nile virus encephalitis; it may be equally useful in SARS. The ACE gene underwent duplication at the origin of Chordata, just before the "Cambrian Explosion" in the number of species. The ancestral, unduplicated form of ACE is still expressed during the terminal differentiation of human spermatocytes, suggesting a critical role in reproduction. The crystal structure of testicular ACE (tACE) was recently published. Computer modeling suggests that tACE may be activated by both mechanical forces and reducing agents. The duplicated form of ACE (somatic ACE, sACE) is expressed in areas of high fluid flow. sACE may auto-dimerize via a novel protein motif, the "disulfide zipper." The sACE dimer is predicted to have higher catalytic efficiency and redox resistance than tACE.

Key Words: Aging, redox, cancer, disulfide zipper, autodimer.

INTRODUCTION

It is a tenet of modern biochemistry that form dictates function. This review will therefore begin with conjecture about the structure of somatic ACE (sACE), which has yet to be solved crystallographically. Angiotensin II, the major product of ACE, activates protein kinase C and the AP-1 transcription factor, which are very widely used in signal transduction. Redox- and mechanical activation of ACE could explain the enzyme's central role in pathophysiology. Overactivity of ACE appears to drive most common age-related diseases in vertebrates. Since there are a number of ACE inhibitors and angiotensin II receptor blockers (ARB's) already available, this may be excellent news for public health.

TESTICULAR ACE: REDOX- AND MECHANO-SENSOR?

Testicular ACE (tACE), the ancestral form of the molecule with a single active site, is a type I membrane protein with seven highly conserved cysteines. Of these, six are linked by disulfide bridges in a nearest neighbor, *aabbcc*, pattern [1]. The crystal structure of tACE in the presence of chloride was recently published [2].

The active site of tACE resembles a small box [2,3]. Zinc-dependent peptide bond hydrolysis appears to occur within a hole in the floor of the box which can accommodate nothing larger than a dipeptide [2]. A flow-sensitive "flap" occluding the two active sites of sACE was predicted [4]; a mobile "lid" composed of two alpha helices not hydrogen-bonded to substrate (lisinopril) or the rest of the protein was observed in tACE [2]. This lid may allow the enzyme to function as a mechanosensor in areas of turbulent flow [4].

In tACE, cystines link the short beta sheets β1 (C^{183}) to β2 (C^{189}), β4 (C^{383}) to β5 (C^{401}), and the alpha helix α17 (C^{569}) to the 3_{10} helix H7 (C^{581}) (amino acid numbering as in the tACE precursor, Swiss-Prot P22966, with structural motifs numbered as in [2]). In sACE, the corresponding cystines of the N-terminal domain link C^{157} to C^{165}, C^{359} to C^{377}, and C^{545} to C^{557} (numbering as in sACE precursor, Swiss-Prot P12821). C^{503} is a free sulfhydryl group. In the C-terminal domain, the corresponding disulfide bonds are between C^{757} and C^{763}, C^{957} and C^{975}, and C^{1143} and C^{1155}. C^{1101} is free. C^{503} does not appear to engage in disulfide bonding with C^{1101} [1]. In the model proposed below, C^{503} and C^{1101} fail to interact because they are located on opposite sides of a sphere.

To emphasize the molecule's underlying homology, in this review C-terminal domain cysteines will be referred to according to their N-terminal homologues, e.g. $C^{359'}$ and $C^{377'}$ instead of C^{957} and C^{975}. For conceptual simplicity, amino acids will be numbered according to their position in sACE rather than tACE.

The cystine linking domains β4 and β5 (C^{359}-S-S-C^{377}) is close enough to the active site (Fig. (**1**)) that β4 and β5

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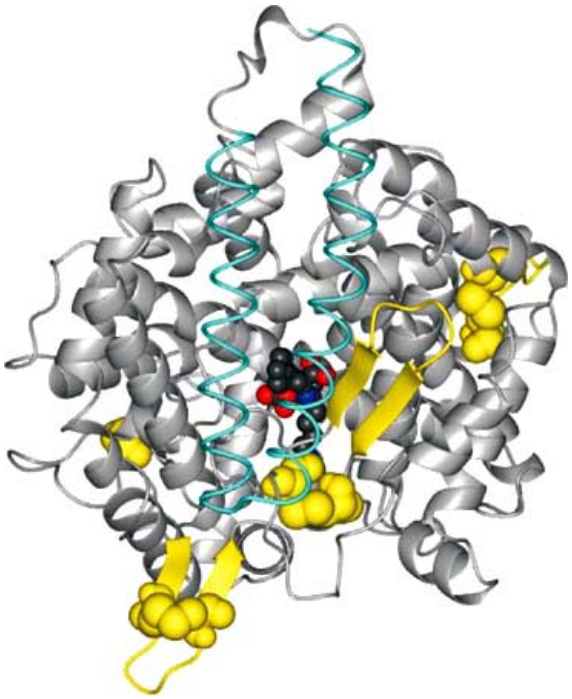

Fig. (1). Testicular ACE [2]. Ribbon model of tACE with an inhibitor, lisinopril, bound to the active site (space-filling red, blue and green atoms). The lid formed by helices α1 and α2 is indicated as a thin blue wire above the active site. The 7 evolutionarily conserved cysteines are indicated in yellow. The 2 cystines with their associated short β-sheets are visible. The cystine on the right of the figure links helix α17 to helix H7. The side wall formed by β4 and β5 is next to the active site, as indicated by lisinopril.

create a wall on one side of the active site. This side wall binds to substrate with at least one hydrogen bond [2]. Cystine C^{359}-S-S-C^{377} may act as a latch whose reduction may allow the side wall to swing open (Fig. (**2**)). This possibility is suggested by substitution of a single cysteine by alanine, and calculation of the resultant structure of tACE using GROMOS96, a free energy-minimization program (Fig.(**3**), [5]). Thus, substrate should be free to enter, and product to leave, the active site after reduction of C^{359}-S-S-C^{377}. *In silico* "reduction" of either of the two other cystines (C^{183}-S-S-C^{189} or C^{569}-S-S-C^{581}) separately did not alter the structure of tACE, but "reduction" of any two cystines dramatically altered the enzyme's tertiary structure (data not shown). The enzyme is therefore hypothesized to be activated by limited reduction, e.g. by glucose or other sugars, hypoxia, low pH, or homocysteine.

Experimentally, however, tACE is resistant to even exhaustive reduction. A 900-fold molar excess of dithiothreitol inhibited tACE's activity by only 78% [1]. The enzyme appears to be more sensitive to oxidation [6]. Besides "locking up" the side wall and preventing substrate entry, oxidation may also lead to formation of cysteine sulfinic acid [7], which cannot form a disulfide bond.

From the discussion above, it appears that tACE could already serve as a redox- and mechanosensor. tACE has a lid which could be opened by turbulent flow, and a key disulfide bridge at C^{383}-S-S-C^{401} which could be reduced by hypoxia, low pH, etc. Access to the active site, and hence activity, may be highest when both the lid and the side wall are open, i.e. for enzyme exposed to turbulent flow under reducing conditions.

Normoxia would keep the C^{383}-S-S-C^{401} cystine in its oxidized state, with the side wall shut. Perhaps only a swimming spermatid could mechanically activate tACE protruding from its plasma membrane [8]. This would have the benefit of reserving scarce fuel [9] for the sole use of motile sperm. Motion-activated tACE would generate a local, extracellular concentration gradient of angiotensin II for autocrine stimulation of the spermatid. Angiotensin II type 1 (AT1) receptors located in the tail of the spermatid [10] could efficiently transduce the mechanical signal to mitochondria located in the neck of the spermatid [11]. The result would be to increase fuel and oxygen consumption, energy production, and forward motility [10,12].

Once a spermatid reaches the more acidic and hypoxic region of the cervix and has to slow down [13], redox sensing rather than mechanosensing might take over to activate tACE. After fertilization, redox activation of tACE located in the surface membrane of the zygote, gently floating along the hypoxic and acidic Fallopian tube, could result in continued local production of angiotensin II. Angiotensin II may trigger the post-fertilization zygote to switch from slow meiotic to rapid mitotic divisions [14]. Redox activation of zygotic tACE with continued angiotensin II production may stimulate angiogensis and uterine smooth muscle cell proliferation after implantation [15].

If tACE can already sense flow and redox state, why did the gene undergo duplication, an event so successful that it was conserved in all species subsequent to the appearance of Chordata? How did sACE perhaps contribute to the Cambrian explosion [16]?

STRUCTURE OF SOMATIC ACE

Somatic ACE (sACE), the duplicated form of the enzyme with two active sites, is a type I membrane protein like tACE. sACE anchored in the plasma membrane of endothelial cells projects minimally into the vascular lumen [16]. Besides endothelial cells, sACE is also present on epithelia exposed to high flow, such as the brush border membrane of the kidney proximal tubule, jejunal microvilli, and the choroid plexus. Interestingly, these tissues share the unusual ability to undergo hypertrophy. Angiotensin II, the product of sACE, helps initiate compensatory renal growth [16].

The structure of the N-terminal domain of sACE is still unknown, although the C-terminal domain is expected to be identical to tACE. The N-terminal domain seems to be more resistant to denaturation by heat or thiols than the C-terminal domain [17-19], suggesting that there might be differences in the tertiary structure of the two domains.

Hydrophobic ACE inhibitors like ramipril inhibit nearly 100% of serum ACE activity, whereas hydrophilic ACE inhibitors like enalapril inhibit only about 50%. This 2:1 ratio suggests that hydrophobic ACE inhibitors may bind to

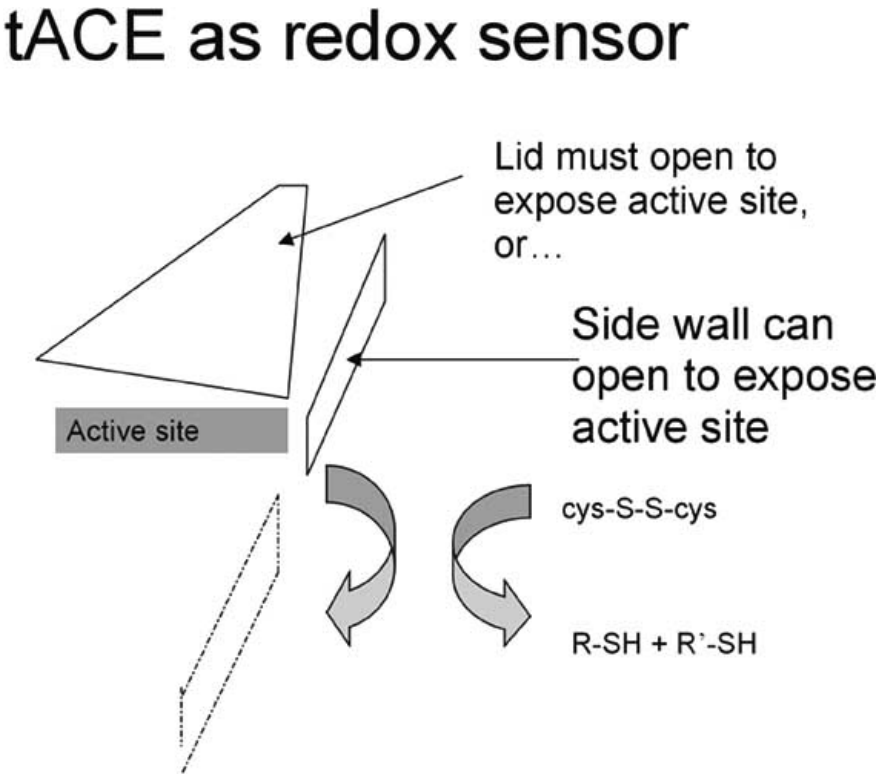

Fig. (2). Testicular ACE as redox- and mechanosensor.

both active sites of sACE, whereas hydrophilic ACE inhibitors bind only to one [20]. Clinically, hydrophobic ACE inhibitors appear to be more effective than hydrophilic ones [20-22] (Table **1**). For example, quinapril was more effective than ramipril at delaying the progression of chronic renal failure [20], and ramipril more effective at lowering pulmonary hypertension than enlapril [20,22]. Maximal inhibition of tissue ACE appears to be an appropriate clinical goal [25,26].

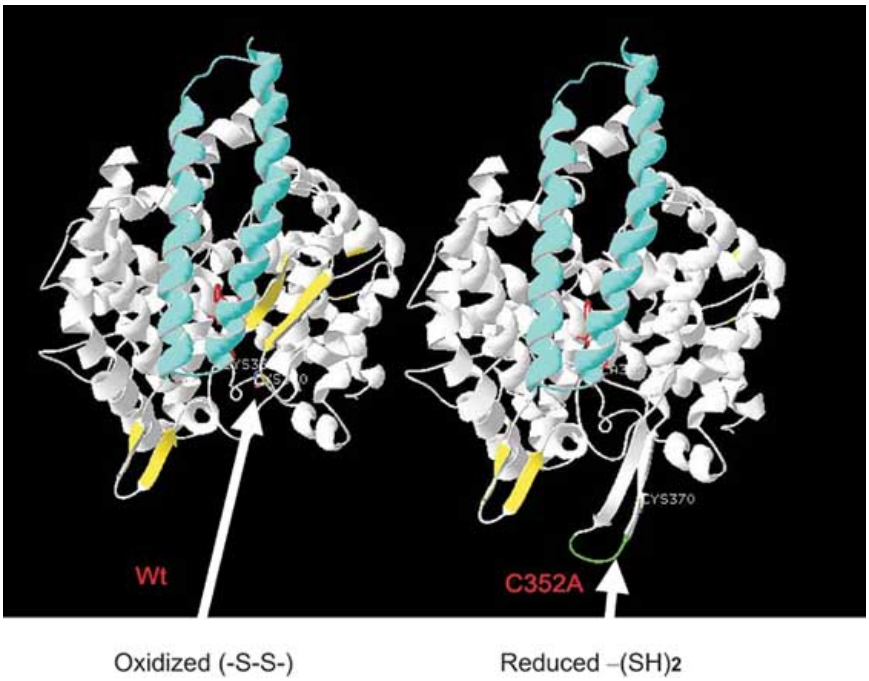

Fig. (3). *In silico* reduction of tACE: conversion of one cysteine to an alanine. "Reduction" of the middle cystine of tACE removes a side wall of the active site. C352 and C370 in this drawing correspond to C383 and C401, respectively, in the numbering scheme of the tACE precursor (SwissProt P22966).

Table 1. ACE Inhibitors, Listed in Decreasing Order of Hydrophobicity at pH 7.4. The Active Moiety, not the Pro-drug, is Considered. Data are Calculated from [23] or [24]. Discrepancies, which are Occasionally Marked, Reflect Different Assay Conditions.

	P(octanol:water)			
Trandolaprilat	10.47	[23]		
Quinaprilat	6.6	[23]		
Benazeprilat	3.0	[23]		
Fosinoprilat	---	---	0.33	[24]
Zofenoprilat	---	---	0.22	[24]
Ramiprilat	2.6	[23];	0.011	[24]
Cilazaprilat	1.7	[23]		
Enalaprilat	0.62	[23];	<0.001	[24]
Perindoprilat	0.44	[23]		
Lisinopril	0.05	[23];	<0.001	[24]
Captopril	0.01	[23];	0.004	[24]
Ceronapril	---	---	<0.001	[24]

More hydrophobic ACE inhibitors have a somewhat higher binding affinity. For example, captopril has an IC_{50} of 9.7 nM vs. 1.7 nM for zofenoprilat; enalaprilat has an IC_{50} of 2.8 nM vs. only 0.67 nM for ramiprilat [27]. The "off time" for hydrophobic ACE inhibitors like quinapril and ramipril is 24 hr vs. only 4 hr for a hydrophilic ACE inhibitor like enalapril [28-30]. This suggests that hydrophobic ACE inhibitors are able to gain access to a different kind of active site than hydrophilic ACE inhibitors. The simplest explanation for all these data is that the N-terminal active site is more hydrophobic than the C-terminal active site. Hydrophobic ACE inhibitors can gain access to both active sites of sACE, whereas hydrophilic ACE inhibitors bind only to the C-terminal domain active site. Having to displace a hydrophobic autoinhibitory tripeptide (FQP) from the N-terminal domain active site might explain why this active site can be accessed only by hydrophobic ACE inhibitors at the doses used clinically [4,31].

Strong sequence homology nevertheless suggests that the two domains are at least somewhat similar in structure. If so, then the two domains may auto-dimerize via a novel motif, a "disulfide zipper" (Fig. (**4**)). The three cystines from each domain can easily be interposed. Indeed, there appears to be a "tongue-in-groove" fit along a relatively flat surface at the bottom of each domain of the holoenzyme (Fig. (**4b**)).

The six disulfides might even form an extended electron transport chain (Fig. (**5**)), analogous to an iron-sulfur cluster without iron atoms [32]. Perhaps reducing equivalents interact with the free, conserved cysteine accessible to solvent (C^{503} or C^{1101}), present on opposite sides of the autodimer (Fig. (**4b**)). Electrons may tunnel from the surface of the homodimer through to the disulfide zipper located along the interior seam of the autodimer [33].

Reduction of one domain's cystine "latch" (C^{359}-S-S-C^{377} or C^{957}-S-S-C^{975}, the equivalent cystine in the C-terminal domain) might occur at the expense of the other domain's cystine through a disulfide isomerase reaction (Fig. (**6**)). Relative to tACE, sACE might gain an additional catalytic mechanism involving two "swinging gates" (Fig. (**7**)). A limiting amount of reductant could perhaps lead to a reciprocating or "ping-pong" mechanism whereby one cystine "latch" opened only after the other cystine "latch" closed. In theory, this could be set in motion by a single reducing equivalent.

No such "perpetual motion" mechanism could apply to a single domain enzyme like tACE. A single reducing equivalent (2 electrons or hydride ion) could start the side walls of both active sites of sACE flapping (Fig. (**7**)), whereas it would lead to only a single, non-repeated action by the side wall of tACE (Figs.(**2)** and (**3**)).

When ACE underwent gene duplication, a single-shot pistol may have become a machine gun. The effect may have been an enormous gain in sensitivity to reducing equivalents by sACE as compared to tACE. A single reducing equivalent would result in production of far more angiotensin II by sACE than by tACE, for a huge systems gain [34]. Furthermore, the two "latches" (cystines) participating in the "swinging gate" mechanism would be inaccessible to solvent, since they would be part of the disulfide zipper at the interior of the autodimer (Fig. (**4b**)).

The overall effect of gene duplication might therefore be a tremendous increase in catalytic efficiency for sACE relative to tACE, as well as increased resistance to redox inactivation [6]. This latter feature would be especially advantageous for an ectoenzyme on the surface of

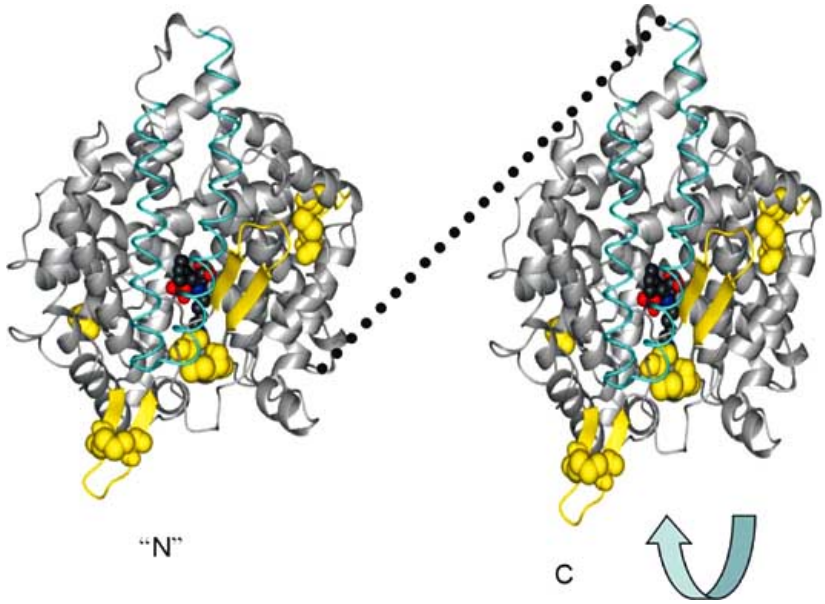

Fig. (4a). Somatic ACE (sACE).

macrophages whose product, angiotensin II, stimulates the respiratory oxidative burst via protein kinase C [35].

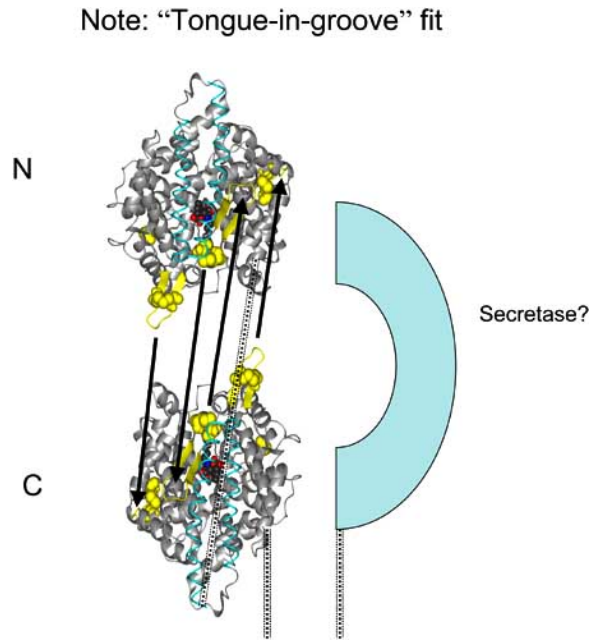

Fig. (4b). Somatic ACE showing complementarity of the N- and C-terminal domains. The secretase, an integral membrane protein associated with sACE, may have a chaperone-like function to hold the two domains of sACE together.

THE SECRETASE

An endo-proteolytic ectoenzyme ("secretase") cleaves sACE from its membrane-bound stalk to release soluble sACE [36,37]. The activity of the secretase is stimulated by protein kinase C (PKC) [38-40]. The soluble form of sACE does not appear to contribute to disease [41], although it may help maintain systemic blood pressure [42,43]. Given that the major product of sACE, angiotensin II, also stimulates PKC, the secretase may thus participate in a negative feedback loop to decrease tissue ACE activity. The secretase may have an additional role, however.

The specific activities of secretase-deficient forms of sACE, including tACE, secretase-cleaved soluble sACE, detergent-solubilized sACE, and recombinant sACE are all similar [44-46], but may not reflect the enzyme's activity *in situ*. Pulmonary sACE has over 30 times the specific activity of sACE in other organs [47], and contains the secretase [37].

Soluble sACE may not remain in the same autodimeric structure as postulated above for membrane-bound, secretase-associated sACE. Once cleaved by the secretase, soluble sACE may no longer be able to function as a "reciprocating enzyme" (Figs. (**6**) and (**7**)). The soluble enzyme may retain an active N- or C-terminal domain with a single reduced cystine "latch," but without the ability to regenerate activity in the other domain. This would occur if the disulfide zipper came apart, preventing disulfide isomerase activity. This might explain the striking negative cooperativity of soluble sACE observed by Kost and her colleagues [19].

The secretase which releases sACE from the plasma membrane might help hold the two domains of sACE together to form an autodimer (Fig. (**4b**)). The C-domain of sACE is glycosylated, although somewhat less so than the N-terminal domain (7 vs. 10 Asn's). The carbohydrate residues bound to the N-terminal domain appear to promote autodimerization and binding to the secretase [48,49]. In other words, the secretase may function as a chaperone to bring the two domains of sACE together. Binding of sACE by the secretase might explain why antibodies directed against membrane-bound sACE recognized only the N-terminal domain, and not the C-terminal domain [50].

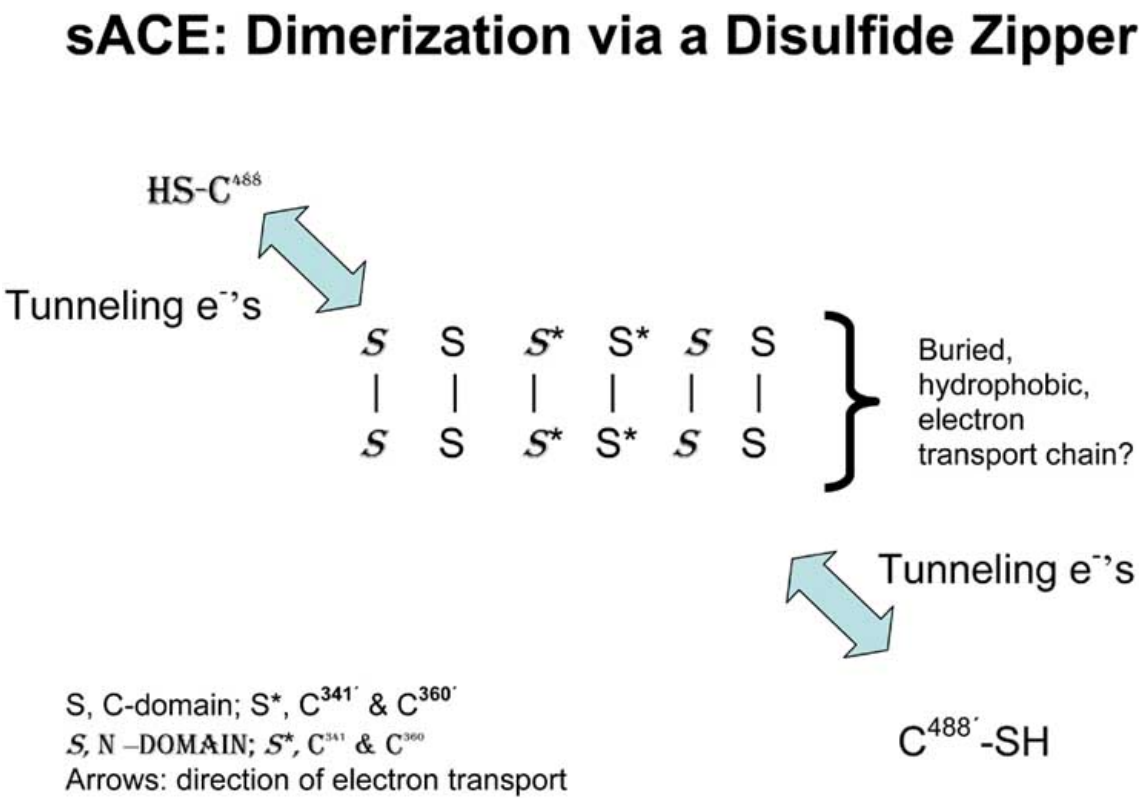

Fig. (5). The "disulfide zipper" at the heart of sACE: a potential electron transport chain.

sACE AND PHYSIOLOGY

Angiotensin II and ROS

The role of reactive oxygen species (ROS) in pathophysiology is receiving a lot of attention [51-55], especially the relevance of the redox state to aging [56-59]. Angiotensin II strongly stimulates the production of ROS [60]. Angiotensin II induces the transcription of several protein components of NADH oxidase and NAD(P)H oxidase present beneath the plasma membrane of endothelial cells [61,62], vascular smooth muscle cells [63,64], and adventitial fibroblasts [65]. Angiotensin II stimulates expression of the same enzymes located within the phagosomes of neutrophils [66]. Although not yet studied, the same is expected to be true for the phagosomes of macrophages.

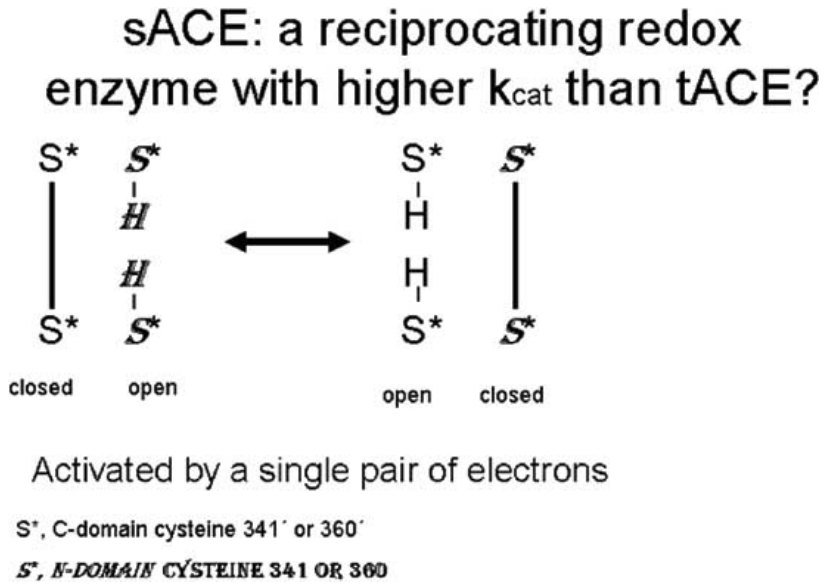

Fig. (6). The disulfide isomerase exchange reaction possible at the heart of the disulfide zipper which might convert sACE into a kind of "reciprocating" enzyme.

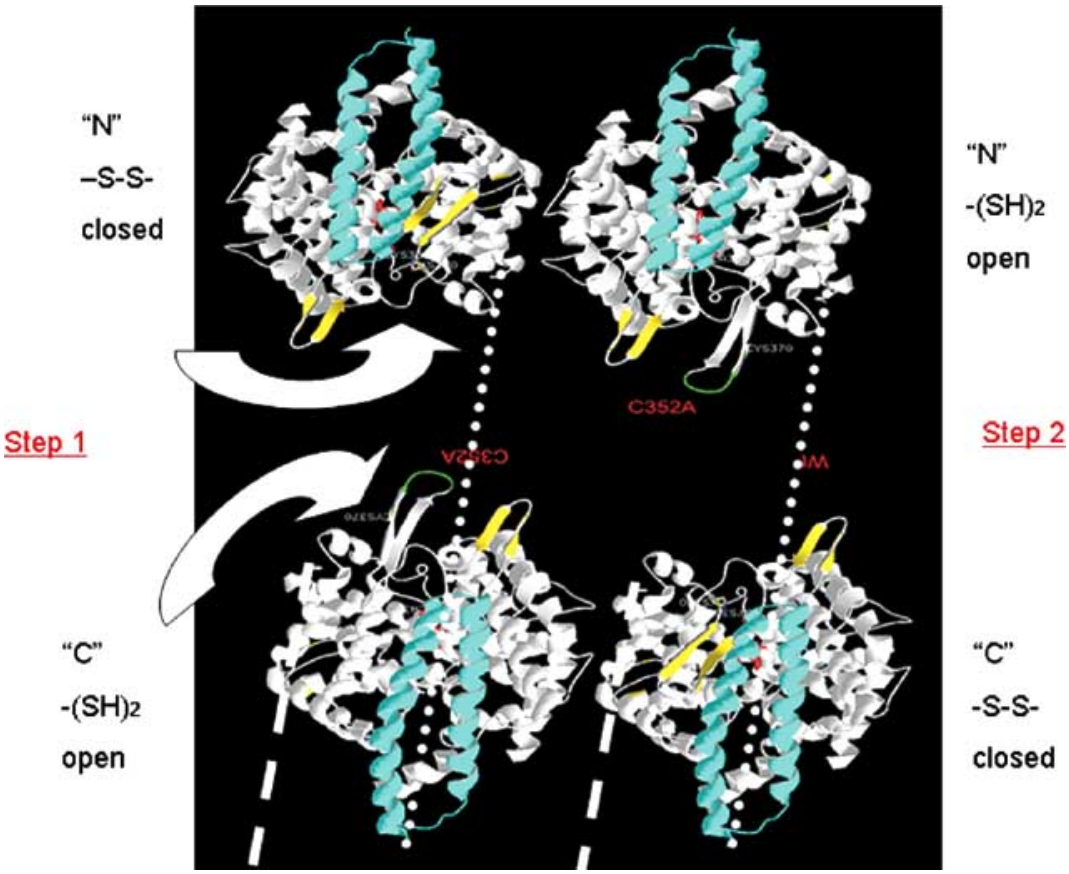

Fig. (7). The two steps in the catalytic cycle of the "reciprocating" enzyme, sACE.

Macrophages express sACE on their surface membrane when activated [67]. An important role of phagocytic cells such as macrophages and neutrophils is to degrade chemically supramolecular structures using ROS, e.g. $O_2^{\cdot-}$ and hydrogen peroxide. Targets of phagocytes include viruses, bacteria, and other large, supramolecular aggregates. The latter include lipoproteins in atheromatous plaques [68], β-amyloid peptide aggregates in Alzheimer's disease plaques [69,70], and aggregates of huntingtin in Huntington's disease [71]. As discussed above, sACE on macrophages (called "microglia" in the brain) may remain active during such an oxidant storm by having its redox-sensitive cystine "latches" buried in the interior of the autodimer, as part of the "disulfide zipper." Unfortunately, bystander cells, especially neurons, suffer ROS-mediated apoptosis [72].

Activation of sACE by reductants, as well as by mechanical turbulence [4], could explain the enzyme's central position in pathophysiology. As mentioned above for tACE, maximal activity of sACE *in situ* may be realized only when both the "lid" is opened by turbulent flow and the "side wall" is opened in response to reductants. sACE has not yet been assayed in such conditions.

Nevertheless, how sACE is activated *in situ* may help explain such diverse phenomena as erythropoetin production by the renal "critmeter" [73], ventilation/perfusion ("V/Q") matching in the lung [74], activation of the immune response during infection [75-78], production of myeloid cells in the bone marrow [79,80], and the vicious cycle of ischemia, thrombosis, endothelial cell apoptosis, and vascular collapse seen in conditions such as sickle cell crisis, malignant hypertension, and disseminated intravascular coagulation (DIC) [81]. In addition, sACE is an excellent candidate gene responsible for most age-related diseases as well as aging itself in all vertebrates [4,16,20,82].

Homocysteine

Normally, the extracellular milieu is oxidizing, while the intracellular milieu is reducing [51,52]. Homocysteine, which contains a free sulfhydryl group, may react extracellularly in a similar way to intracellular glutathione. Oxidation of homocysteine to the disulfide, homocystine, results in the generation of a reducing equivalent (hydride ion) which could reduce one of the "latch" cystines (C^{359}-S-S-C^{377} or $C^{359'}$-S-S-$C^{377'}$), activating sACE according to the scheme in Figs. (**2**), (**3**), (**6**), and (**7**). This could explain the association of high homocysteine levels with accelerated atherosclerosis and cancer [83,84], diseases also linked to overactivity of ACE [82].

Angiotensin II *vs.* NO

Angiotensin II defends the integrity of the vasculature [4] as a vasoconstrictor, pro-thrombotic agent, and vascular smooth muscle cell mitogen or angiogenesis factor [85]. Operating through its type 1 receptor, angiotensin II

stimulates expression of, or sensitivity to, potent pressors such as endothelin [86], thrombin [87-89], thromboxane [90], epinephrine [91], and EPO [92]. Angiotensin II stimulates thrombosis in a number of ways: by inducing expression of the thrombin receptor and potentiating the action of thrombin [87-89], by stimulating the release of platelet activating factor (PAF) [93], and by stimulating platelet aggregation and adhesion directly [94-97]. Finally, angiotensin II stimulates the expression of the potent angiogenic factors vascular endothelial growth factor (VEGF) [98] and epiregulin [99], and acts as a potent angiogenesis factor in its own right [100], stimulating endothelial cell proliferation through activation of NF-κB [101].

Angiotensin II type 2 receptors, on the other hand, mediate apoptosis of endothelial cells [102] and other cell types such as type II pneumocytes [103,104].

In contrast to angiotensin II, nitric oxide (NO) is the primary endothelium-derived vasodilator [105]. NO can sometimes remove cells from proliferation or apoptosis [106], cellular programs which angiotensin II initiates.

Angiotensin II and NO are biological antagonists involved in a complex balance [107,108]. For example, angiotensin II can stimulate expression of all three isoforms of nitric oxide synthase to increase NO [108]. Yet ROS generated by xanthine oxidase, NAD(P)H and NADH oxidases in response to angiotensin II degrade NO and diminish NO signaling [109,110]. This appears to be the mechanism for impaired vasodilation in patients with essential hypertension [111,112].

Below, we explore how NO may directly inactivate sACE, the rate-limiting step for angiotensin II production by endothelial cells.

REDOX SENSING BY sACE AND THE RENAL "CRITMETER" [73]

The body's degree of tissue oxygenation is sensed in the outer medulla of the kidney, where erythropoietin (EPO) is made [73]. The signal for transcription of the EPO gene appears to be angiotensin II [113-115]. Patients who lack renal function require exogenous EPO. But some patients, after receiving a kidney transplant, develop erythrocytosis with high endogenous EPO levels [116]. EPO levels and the hematocrit can be reduced with an ACE inhibitor [117], specifically, with an angiotensin II type 1 receptor antagonist [118].

The implication is that production of EPO by the renal "critmeter" is normally driven by angiotensin II through its type 1 receptor, but in some kidney transplant recipients a negative feedback loop fails to shut off angiotensin II production and therefore EPO production. We shall see in more detail below how oxygenation inactivates sACE, the rate-limiting step for production of angiotensin II.

Chronically elevated renal tissue levels of angiotensin II may be due to ongoing hypertrophy of the renal transplant [119]. The signal for renal hypertrophy appears to be production of angiotensin II by sACE in the proximal tubular brush border membrane, which can diffuse into the inner medulla [16].

Hypoxia inducible factor (HIF-1α and related proteins) is often claimed to be the trigger for EPO production [120]. But HIF is induced several-fold more by angiotensin II than by hypoxia [121,122]. Therefore, quantitatively speaking, HIF operates downstream from angiotensin II in the generation of EPO.

The oxygen sensor for EPO production is likely to be hemoglobin itself [123,124]. Stamler and colleagues have shown that oxygenation of hemoglobin can displace NO from the heme ring, where it is bound in the absence of oxygen [123]. NO then becomes bound to a free cysteine sulfhydryl group on hemoglobin ("protein S-nitrosylation") [125]. NO is then transferred through a series of free cysteine sulfhydryl groups from hemoglobin in the interior of the erythrocyte to the cell exterior via the anion exchanger AE1, an abundant red cell membrane-spanning protein whose cytoplasmic tail contains numerous cysteines and binds hemoglobin [126]. From AE1, the NO group could easily be transferred to albumin [127] (Fig. (**8**)), and thence to a free cysteine on sACE, preventing the ability of sACE to engage in redox reactions (Fig. (**9**)).

The possible involvement of albumin in the inactivation of sACE may explain the relative vasoconstriction and decreased effective intra-arterial volume seen in hypoalbuminemic states [128-130].

Nitrosylation of C^{488} on sACE may limit its ability to receive and tunnel reducing equivalents (Fig. (**9**)). If the disulfide zipper becomes undone, S-nitrosylation of cysteines 340 or 361, or of equivalent cysteines on the C-terminal domain (Fig. (**9**)) would inactivate sACE's "swinging gates" directly.

In the absence of sufficient oxygen, sACE escapes inactivation by NO. Instead, we hypothesize that sACE is activated by the reducing conditions of hypoxia. Assuming all reactions are at equilibrium, a four-fold decline in tissue oxygen concentration from 80 mm Hg to 20 mm Hg in the inner medulla of the kidney would be expected to increase the fraction $sACE_{red}/sACE_{ox}$ by a factor of 4. After production by endothelial or even proximal tubular brush border membrane sACE [16], angiotensin II could easily diffuse into the renal interstitium to activate the fibroblast-like cells which make EPO [120].

MYELOID CELL PRODUCTION

The ACE D/D genotype is associated with chronic leukemias and lymphomas, as well as myelofibrosis and myelodysplasia [82], suggesting that angiotensin II stimulates the proliferation of bone marrow-derived cells.

This hypothesis has not yet received much study. It is known that hematopoetic cell precursors are stimulated by angiotensin II, perhaps through an oxygen-sensing, NO-mediated sACE system as described above [79,80]. Angiotensin II stimulates maturation, proliferation, and migration of dendritic cells, which originate in the bone marrow [131-133]. Angiotensin II activates NF-κB in

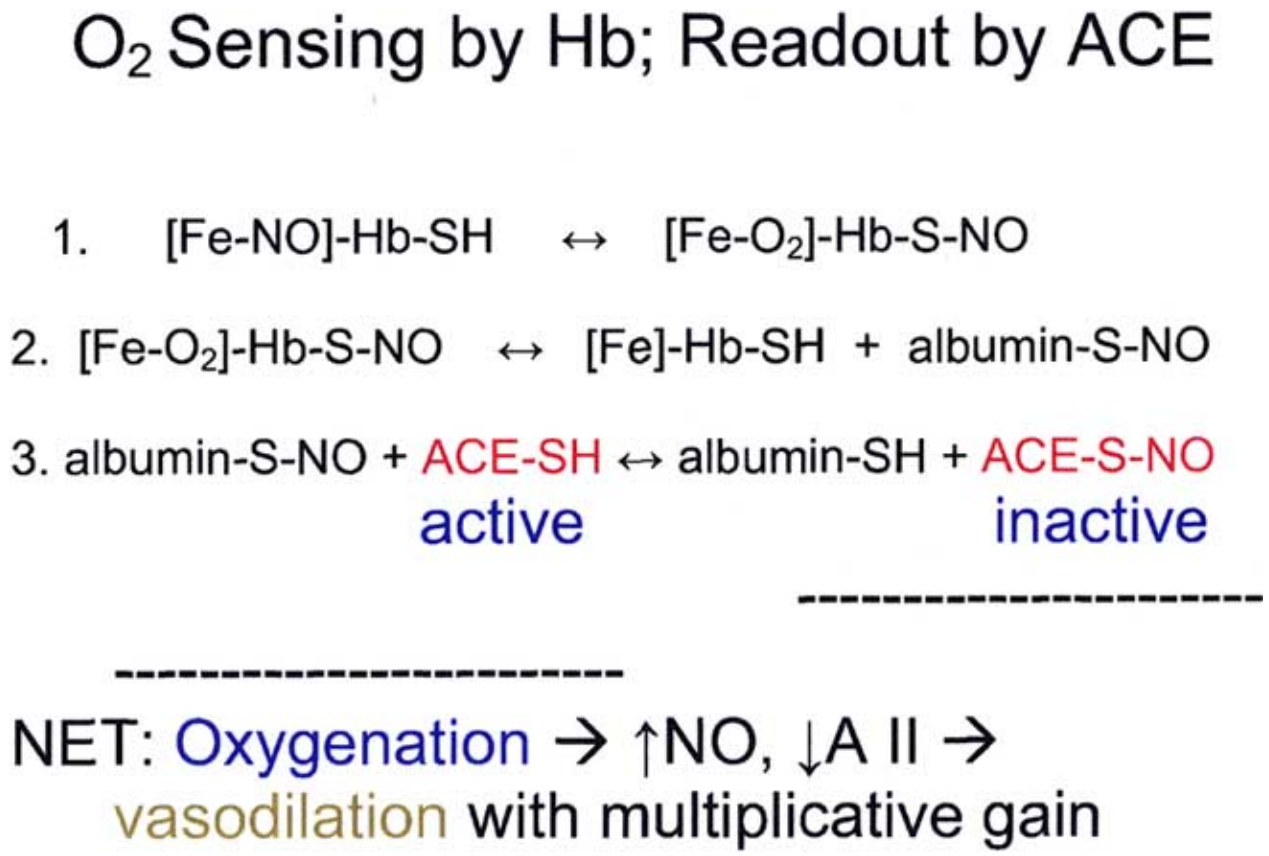

Fig. (8). Pathway for transfer of NO from oxygenated hemoglobin to sACE.

neutrophils [66] and monocyte/macrophages [134], and so may enhance proliferation of their myeloid precursors, since NF-κB is associated with immunocyte proliferation, maturation, and activation [135-138].

PULMONARY sACE: INVOLVED IN V/Q MATCHING?

Activation of sACE by reducing equivalents, and inhibition of sACE by NO *via* oxygenated hemoglobin as discussed above, could explain how the lung matches ventilation (V) to perfusion (Q). To maximize tissue oxygenation, the lung rewards only alveoli engaged in productive gas exchange with blood flow. Vessels supplying non-functional alveoli, in contrast, undergo vasoconstriction. The mechanism for matching Q to V has not been fully described, although depolarization of vascular smooth muscle cells via inhibition of voltage-gated K^+ channels appears to be involved [139]. Interestingly, this effect is mediated by PKC [139], so it may represent an event downstream of signaling by angiotensin II, as we shall now discuss.

sACE is present on endothelial cell membranes of pulmonary arterioles and capillaries. Pulmonary vessels are located in the interstitium, not more than a few cell widths away from the gaseous phase in neighboring alveoli. Oxygen diffuses from the alveolus to nearby blood vessels, is picked up by hemoglobin, and is pumped by the left ventricle to the rest of the body.

If the pulmonary capillary has no oxygen to pick up because the alveolus nearby is non-functional, then the oxygen tension in the interstitium surrounding that alveolus will fall, the carbon dioxide tension will rise, and the pH will fall. Both lower oxygen tension and higher CO_2 tension constitute reducing conditions, which, we postulate, should reduce the cystine bridges in sACE to free sulfhydryl groups. The side wall for each active site will fall apart, exposing the active site (Figs. (**2**), (**3**), (**6**) , and (**7**)).

Local angiotensin II production should increase, causing the vessel to constrict. In part, this may be mediated by depolarization of smooth muscle cells in response to PKC-mediated inhibition of ATP-sensitive, voltage-gated K^+ channels [139]. Turbulent blood flow may further activate sACE by causing the enzyme's "lids" to open.

When gas exchange improves, oxygen tension in the interstitium and at the plasma membrane of the endothelial cell should increase, the free cysteine sulfhydryl groups of sACE should become oxidized to cystine once again, and the side walls of sACE should get "locked" up and inactivated. Nitric oxide (NO) may also contribute significantly to the inactivation of sACE, as discussed above (Figs. (**8**) and (**9**)).

As a result, angiotensin II production should drop in a pulmonary capillary next to a functioning alveolus. The balance between vasoconstriction, mediated by angiotensin II and "downstream" vasoconstrictors whose expression is induced by angiotensin II, such as endothelin [86], and

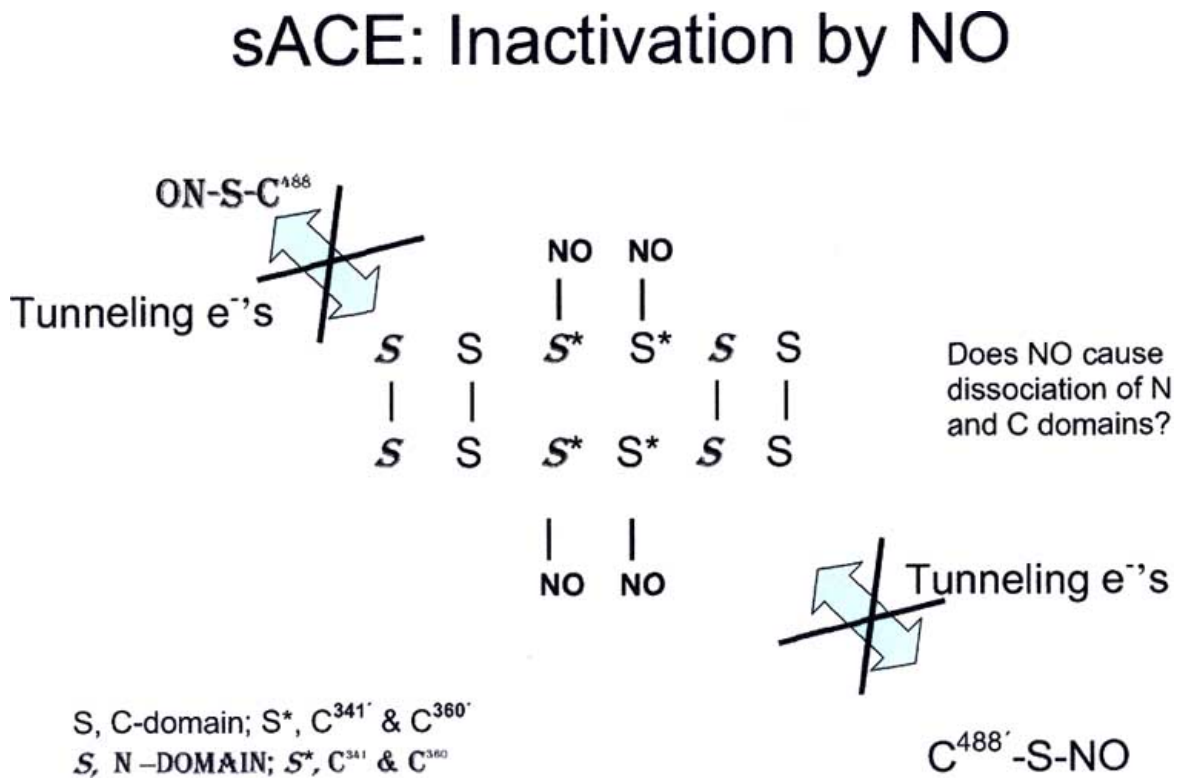

Fig. (9). NO inactivation of sACE: possible sites of S-nitrosylation.

vasodilation, mediated by NO, should shift in favor of vasodilation. Furthermore, the gain in the system will be multiplicative [34]. The pulmonary capillary will dilate, and blood flow will again resume to the functional alveolus.

If alveolar gas exchange is impaired for a long time (days), long-term effects of angiotensin II operating through AT1 receptors include hyperplasia of vascular smooth muscle cells leading to pulmonary hypertension, and elaboration of TGF-β with proliferation of interstitial fibroblasts, leading to pulmonary fibrosis [140]. Angiotensin II-mediated induction of sACE will amplify this positive feedback loop [141].

Over days, angiotensin II may stimulate apoptosis of alveolar epithelial cells [103,104] and loss of pulmonary parenchyma, the hallmark of emphysema [142]. Under the constant driving pressure of angiotensin II, some alveolar epithelial cells may escape from growth control (apoptosis) and become cancerous [82,143].

Because of sACE's key role in V/Q matching, effective inhibition of tissue ACE or antagonism of angiotensin II type 1 receptors by ARBs is expected to be useful for any pulmonary disease in which gas exchange is impaired. In addition to promoting vasoconstriction and pulmonary hypertension, as well as alveolar epithelial apoptosis, angiotensin II also appears to be a major cytokine (see below). Examples of diseases which are likely to benefit from angiotensin II blockade include emphysema [20], bronchiolitis obliterans especially after respiratory syncytial virus [144], cystic fibrosis [145], acute respiratory distress syndrome and smoke inhalation [146], severe acute respiratory syndrome (SARS) [147], radiation pneumonitis [148], and other forms of interstitial lung disease with pulmonary fibrosis. Lung cancers which may initially arise due to hypoxemia-induced production of angiotensin II might be delayed or perhaps prevented altogether with an ACE inhibitor or ARB [82].

The molecular mechanism described above fails to explain how hyperoxia, as in prolonged mechanical ventilation using an $F_IO2 \geq 0.5$, could result in pulmonary fibrosis. Indeed, both active sites of ACE should be "locked up" by hyperoxia-mediated oxidation of key cystines. Recent evidence suggests that hyperoxia mimics the action of angiotensin II by activating AP-1 directly [149], although the mechanism is unclear.

sACE IN PATHOLOGY

1. Diabetes

Diabetes, hypertension, and their complications could all result, at least in part, from activation of sACE [4,16,20,82]. In diabetes, sACE is activated by more than increased plasma osmolality, since hyperglycemia contributes relatively little (<5%) to plasma osmolality [4]. But sugars are potent reducing agents [150], and there is considerable evidence linking glucose concentration to the risk of developing diabetic complications [151].

According to the hypothesis presented here, tripling of the serum glucose concentration from 100 mg/dl to 300 mg/dl would result in tripling of the ratio of $sACE_{red}/sACE_{ox}$ where $sACE_{red}$ represents the reduced and fully activated form of sACE. If sACE normally exists in the ratio of 1:9 i.e.

10% reduced (constitutively active) and 90% oxidized (activated only by mechanical flow), then tripling the glucose concentration will change the ratio to 1:3, i.e. 25% reduced (constitutively active) and 75% oxidized (activated only by mechanical flow). The effect of hyperglycemia will thus be to increase the fraction of reduced, fully activated sACE from 10% to 25%, a 2.5-fold change. In the limit, the change in $sACE_{red}$ will be the same as the change in glucose concentration; e.g., for 1% $sACE_{red}$, or 1:99, tripling the glucose concentration will triple the fraction of $sACE_{red}$ to 1:33, or 3%.

Generation of angiotensin II could explain downstream events observed in diabetes, such as activation of protein kinase C and TGF-β [152,153]. In type II diabetes mellitus, angiotensin II appears to be involved in a positive feedback loop. Angiotensin II, operating through PKC, inhibits signaling by the insulin receptor by phosphorylation of one or more key serine residues [154]. Reduced responsiveness to insulin causes serum glucose levels to rise, which may further activate sACE through the redox mechanism postulated above. Angiotensin II levels rise, further activating protein kinase C and interfering with insulin sensitivity, establishing a vicious cycle.

Experimentally, ACE gene expression and activity is increased after the initiation of streptozotocin-induced diabetes [155,156]. As mentioned above, angiotensin II induces expression of sACE via the AT1 receptor and PKC [157], contributing to this positive feedback loop.

This may explain the "metabolic syndrome" (also called "syndrome X"), i.e. essential hypertension and insulin resistance [158]. As tissue angiotensin II levels rise, so will insulin resistance. The amplitude of the excursions in the plasma insulin concentration will therefore increase accordingly. Once β-islet cells begin undergoing apoptosis due to severe insulin overshoot and hypoglycemia, the metabolic syndrome is well on its way to becoming clinically overt type II diabetes mellitus [16].

2. Gout

Hyperuricemia and gout are features of the metabolic syndrome, and are associated with cardiovascular disease and type II diabetes [159]. These diseases are all associated with the ACE D/D genotype [82], a marker of excessive tissue ACE activity.

Uric acid is produced from xanthine and hypoxanthine by the enzyme xanthine oxidase (XO). Angiotensin II stimulates XO production by endothelial cells [160], perhaps in an autocrine/paracrine fashion. XO is expressed on the plasma membrane of endothelial cells in the same location as ACE. Uric acid can function as an anti-oxidant (reducing agent), and appears to activate ACE directly [161], creating the possibility of a vicious cycle: angiotensin II → ↑ XO → ↑ uric acid → ↑ sACE → ↑ angiotensin II.

XO can create uric acid through electron transfer to its molybdenum(VI) center, thence to an iron-sulfur protein, and thence to a flavin moiety. But XO can also create free oxygen radicals through its flavin center alone. These free oxygen radicals deplete NO by creating peroxynitrite (ONOO-) [109,110,159,161]. Thus, synthesis of XO appears to be yet another mechanism for the vasoconstrictor, prothrombotic, profibrotic, proapoptotic pathway initiated by angiotensin II to win the battle against the vasodilatory, antithrombotic, antiproliferative and antifibrotic pathway controlled by NO.

3. Vicious Cycles Leading to Vascular Collapse: Sepsis, DIC, Malignant Hypertension, Sickle Cell Disease, Pre-eclampsia

Reducing conditions (low oxygen tension, low pH) exist commonly in tissue vascular beds, such as liver and muscle, during conditions of hypoperfusion, e.g. in cardiogenic, hypovolemic, or septic shock; sickle cell disease; malignant hypertension; disseminated intravascular coagulation (DIC); and pre-eclampsia. In these diseases, sACE should be maximally activated by redox conditions and mechanical turbulence.

DIC often leads rapidly to death in patients with sepsis, shock, or malignant hypertension. The essence of DIC, hypercoagulation, could arise by a profound imbalance between angiotensin II, which is pro-thrombotic [162], and NO, which is anti-thrombotic [163]. In addition, endothelial cell ischemia and apoptosis expose pro-thrombotic tissue factors [164] and promote coagulation on the vascular wall.

Sickle cell "crisis" is similar to DIC since it involves a vicious cycle of vasoconstriction, hypoperfusion, local hypoxia and acidemia, and further sickling of red cells. Stiff, non-deformable sickled red cells scrape against the vascular wall [165,166], reducing the unstirred layer from 1 μm to perhaps 10 nm, the approximate dimension of the sACE molecule protruding from the endothelial cell plasma membrane [4].

In patients with sickle cell disease, sACE molecules on the surface of the endothelial cell are exposed to more shear stress than usual [165,166]. With a reduction in the thickness of the unstirred layer, sACE molecules in vessels usually exposed to laminar blood flow may be exposed to shear stress. If sACE is activated by mechanical flow, then endothelial sACE molecules in much of the vasculature will be activated. Increased local production of angiotensin II will result, leading to vasoconstriction, hypoxia, acidemia, and further sickling of erythrocytes.

Sickled cells release extracellular hemoglobin, which traps NO [167]. NO-mediated inactivation of sACE should decrease, with the angiotensin II-NO balance tilting further towards angiotensin II, promoting the vicious cycle.

Effective tissue ACE inhibition [20] or blockade of angiotensin II type 1 receptors, either orally or intravenously (the latter for patients who are vomiting and cannot keep pills down) is therefore proposed as prophylaxis against sickle cell crisis, as well as a treatment for it.

In malignant hypertension, shear stress is increased because of the abnormally high systemic blood pressure, not because of the scrubbing action of sickled erythrocytes. With higher blood velocity and shear stress, the unstirred layer is also reduced, leading to the same picture as sickle cell crisis

described above. ACE inhibition or angiotensin II blockade should therefore be of special effectiveness in the clinical management of malignant hypertension, and DIC.

Pre-eclampsia is another vaso-occlusive disease which appears to result from a vicious cycle favoring the production of angiotensin II over NO [168,169]. ACE inhibition or angiotensin II blockade should therefore also be effective in the clinical management of pre-eclampsia.

4. Role of sACE in Inflammation and Autoimmunity

Angiotensin II already functions as a cytokine in invertebrates [170]. In vertebrates, it is a pyrogen [171]. sACE appears, as CD143, on the plasma membrane of activated macrophages [172] and T lymphocytes [173]. T cells can stimulate the expression of sACE on monocytes in an MHC-restricted manner [174]. This apparently involves cell-cell contact (Fig. (**10**)) and induction of sACE *via* the AT1 receptor [157] and PKC [175].

Angiotensin II is a potent cytokine [176], capable of stimulating the synthesis of macrophage migration inhibition factor (MIF) [177], TNF-α [178], MCP-1 and TGF-β [179], among other cytokines.

Bacterial infection lowers tissue oxygen tension. Bacteria either consume oxygen themselves, or require an anerobic environment to replicate. Along with tissue hypoxia, bacteria produce lactic acid, lowering tissue pH. (An exception are the urease-producing bacteria in the urinary tract). Under such reducing conditions, sACE on the surface membrane of macrophages or T cells should become activated according to the hypothesis presented here.

Angiotensin II stimulates the production of the antiviral protein interferon-γ from T cells [180], which interferes with viral replication. Angiotensin II helps promote apoptosis [181], especially of virally infected cells [182], further limiting viral replication.

In addition, sACE may permit tight cellular interactions. Binding of the N-terminal domain of sACE on one cell (macrophage or T cell) with the C-terminal domain of sACE on another cell (macrophage, T cell, or endothelial cell, for example) may promote specific cell-cell binding (Fig. (**10**)).

Overactivity of ACE has been associated with autoimmune diseases such as rheumatoid arthritis [82,183], lupus [82,184], and fibromyalgia/chronic fatigue syndrome [185]. We have observed gratifying clinical responses to angiotensin II antagonism in patients with T-cell disorders such as psoriasis (Fig. (**12**)) and alopecia areata (manuscript in preparation), as well as viral diseases characterized by an overly exuberant host response such as West Nile virus encephalitis (Table **2**). A similar approach may work for SARS [147] as well as most other viruses.

HIV infectivity and progression to AIDS are also associated with the ACE D/D genotype [82]. This is perhaps not surprising considering that retroviruses require proliferating cells for their replication [186]. Angiotensin II stimulates proliferation of macrophages [138,176,187] where HIV replicates for its first several months in a human host [188], as well as T cells [173], HIV's eventual home [188].

Angiotensin II blockade may also be of benefit in the eradication of *Mycobacterium* species. Like HIV [82], *M. tuberculosis* [82] and *M. leprae* proliferate within activated monocytes and dendritic cells. These cells are activated by

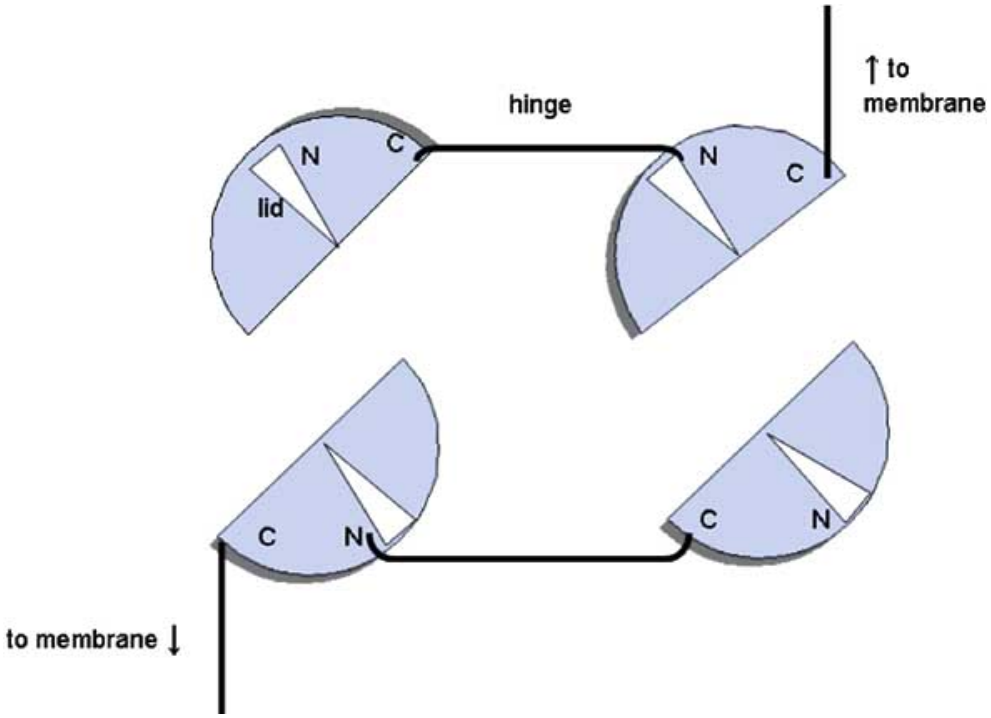

Fig. (10). sACE on one immunocyte (T cell, monocyte/macrophage, or endothelial cell) binding to sACE on another immunocyte so as to promote specific cell-cell interactions. White triangle, "lid" formed by alpha helices α1 and α2; N and C, termini of each domain. Bent black line, hinge region between N- and C-terminal domains. Straight black line, membrane anchoring segment.

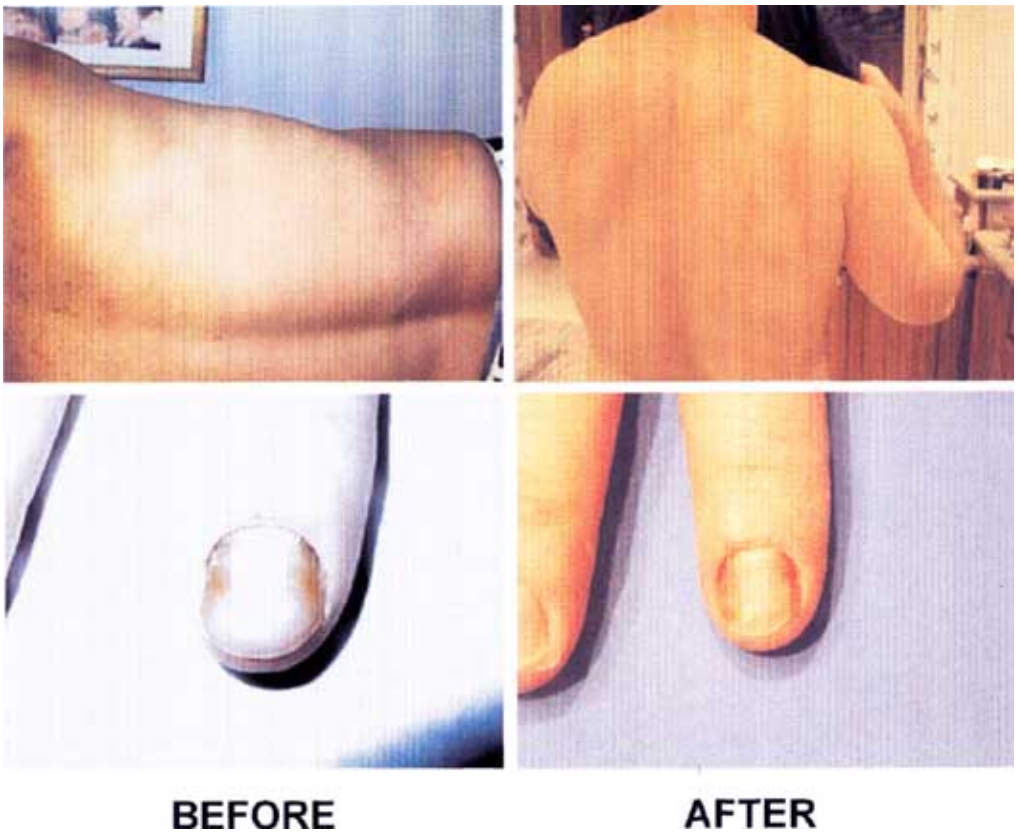

Fig. (11). Prompt response to angiotensin II receptor blockade in a 33 yr old white man with psoriasis before and after daily treatment with valsartan (80 mg) for 4 weeks. In a second case, a 62 yr old white man with chronic psoriasis, for which he took 75 mg methotrexate daily, was able to stop his methtrexate within 2 months of starting quinapril 200 mg/day.

angiotensin II [131,172].

Finally, angiotensin II blockade may be beneficial against hepatitis A and B [82]. Hepatic stellate cells have been implicated in hepatitis [189], and are specifically activated by angiotensin II [190-192]. The same approach may help in pancreatitis [193], especially for women [82].

5. Role of sACE in Cancer

Overactivity of sACE is associated with all solid and hematogenous cancers except prostate cancer in white men, in which sACE activity is actually protective [82]. Vascular signaling by sACE thus appears to drive most cancers. For example, many solid cancers, including colon, have recently been shown to be initiated by Wnt, which acts upstream of beta-catenin and APC [194]. Wnt is activated by PKC [195], so that angiotensin II production by vascular sACE operates upstream of Wnt.

Breast cancer has recently been associated with the ACE D/D genotype in Chinese women [196]. Since angiotensin II promotes cell proliferation and angiogenesis, ACE inhibitors or ARBs may be useful adjunctive treatment for these cancers [197,198].

Here we report a single case of a 67 year old white woman with unresectable pancreatic cancer who underwent a Whipple procedure, local radiation and chemotherapy with 5-fluorouracil (5-FU, 500 mg/m^2) and low dose naltrexone. Her weight dropped from 145 lb to 98 lb after the Whipple procedure. She initially responded to chemotherapy with dramatic lowering of her CA19. But by 9 months after diagnosis, her CA19 had risen to ~950. At that point, she was begun on quinapril for hypertension, which was eventually controlled by a dose of 60 mg twice a day (2.7 mg/kg/day).

Once she began taking quinapril, her appetite improved dramatically, and has remained excellent for an additional 12 months. Furthermore, her CA19 level dropped steadily to a nadir of 394 seven months after beginning quinapril (849 at 3 months after starting quinapril, 685 at 5 months, 420 at 6 months). However, ten months after starting quinapril, it rose to 534. Twelve months after beginning quinapril, she experienced tumor recurrence in her abdomen.

This patient is remarkable for two reasons. The first is that her tumor was chemosensitive, which is true in less than 10% of patients with pancreatic cancer. The second was her gratifying response to effective tissue ACE inhibition, with improvement in survival and quality of life. The median survival of similar patients is less than 4 months, and cachexia is common [199,200]. Presumably, quinapril inhibited TNF-α production by her monocytes [178].

Prostate cancer in white men is a notable exception. In black men, ACE overactivity is associated with prostate cancer and PSA level, as in other cancers [82,196]. In white

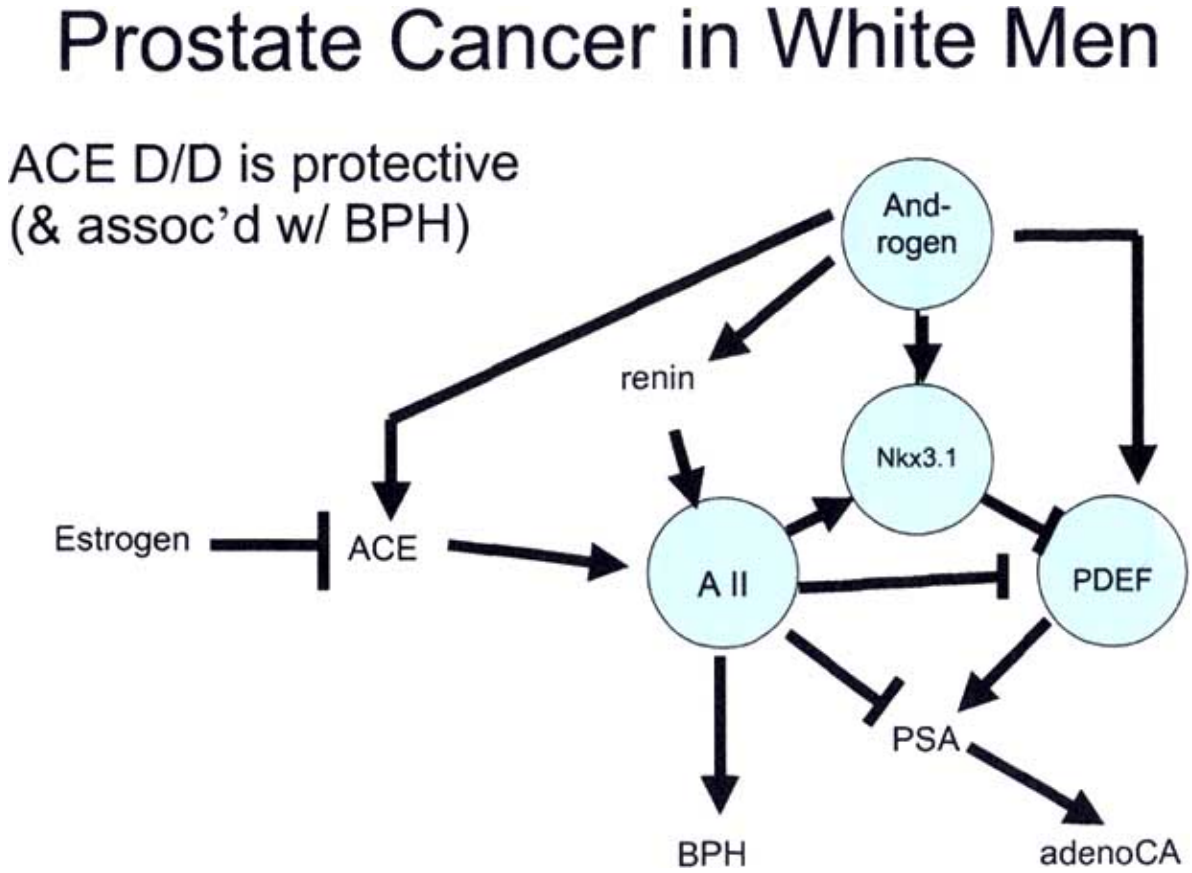

Fig. (12). Genetic pathway for initiation of benign prostatic hyperplasia and prostate cancer. PDEF, prostate-derived ets factor.

men, however, the ACE D/D genotype is associated with benign prostatic hyperplasia, but negatively associated with prostate cancer and PSA level [82], suggesting that angiotensin II promotes hyperplasia but guards against neoplasia of epithelial cells in white men.

sACE is highly expressed in the glandular epithelium of benign prostatic hyperplasia [201]. Patients at a predominantly white hospital who take an ACE inhibitor are at significantly higher risk of also having prostate cancer (Table **3**). The implication is that white men taking an ACE inhibitor should be followed closely with a PSA test.

The explanation for the unexpected protection against prostate cancer and the negative association of the ACE D/D genotype with PSA level in white men may lie with Nkx3.1, a prostate-specific inhibitor of prostate cancer [202]. The Nkx3.1 promoter (GenBank NM_006167) has a single TPA response element (TRE, *aatctacaatgattcaaaaga*) located 1.6 kb 5' to the translation start site [203]. This TRE could be activated by AP-1, acting downstream of angiotensin II, the angiotensin II type 1 receptor [204], and PKC. There are two additional TRE's located at -5.7 kb and -9 kb upstream, with the -9 kb site having two overlapping TRE's. However, these additional TRE's may be too far removed from the transcription start site to influence gene expression.

A possible genetic pathway for the initiation of BPH and prostate cancer in white men is presented in Fig. (**12**). Testosterone stimulates the production of both tACE [206] and sACE [207], renin [208], Nkx3.1 [209], the prostate tumor suppressor gene [210], and prostate-derived ets factor (PDEF) which promotes prostate cancer [211]. PDEF drives PSA production [212] as well as progression to cancer. PDEF expression is inhibited by Nkx3.1 [212]. Angiotensin II likely stimulates the expression of Nkx3.1 *via* PKC (discussed above), and inhibits the action of PDEF [213], which could explain the protective role of the ACE D/D genotype in prostate cancer. Angiotensin II inhibits expression of PSA via the AT1 receptor [213]. Finally, estrogen inhibits the expression of sACE [214], which might be expected to limit its efficacy in prostate cancer (Fig. (**12**), [215]).

6. Neurodegenerative Diseases

As mentioned above, neurons are sensitive to apoptotic signals such as intracellular redox imbalance and ROS [216]. It is unclear whether they are especially sensitive, or whether their cellular metabolism is higher than most other cell types, leaving little room for additional insults.

Neuronal apoptosis in response to ROS is thought to contribute to all neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS) [217,218], the retinal degeneration seen in age-related macular degeneration and retinitis pigmentosa [219], and diabetic neuropathy [220-223]. Diabetic neuropathy has already

Table 2. Response of Eight Patients with West Nile Virus Encephalitis to Angiotensin II Receptor Blockade. Seven Patients Showed an Unexpectedly Prompt Response, for an 88% Treatment Success Rate.

1.	A 50 yr old white man with past history of tremors was admitted with fever, stiff neck, severe confusion, and gross tremors to a hospital in Omaha, NE one night. His serum was positive for IgM reactive to West Nile virus. He was begun on olmesartan 20 mg daily. On the morning after admission, his tremors had lessened dramatically, and he was able to concentrate normally. He was discharged home on olmesartan 20 mg daily.
2.	A 50 yr old white woman with hypertension treated with a beta-blocker/thiazide combination drug, was admitted to a hospital in Omaha, NE with right leg paralysis and paresthesias and weakness in her left leg. Her serum was positive for IgM reactive to West Nile virus. She was begun on losartan 50 mg daily. The paresthesias and weakness in her left leg stopped within 1-2 days of starting losartan, but there was no change in her right leg paralysis.
3.	A 75 yr old white man with a history of seizures was admitted one night to a hospital in Omaha, NE with a fever and a grand mal seizure. He had been taking olmesartan 20 mg daily for mild hypertension. His serum was positive for IgM reactive to West Nile virus. He was given losartan 50 mg twice during the first 24 hr after admission. His usual dose of olmesartan 20 mg daily was resumed thereafter. After his presenting seizure, he had no more seizures and had a full recovery within 12 hr. He was able to use his son's lap-top computer the morning after admission.
4.	A 50 yr old white man was admitted with fever, stiff neck, and severe confusion to a hospital in Omaha, NE. His serum was positive for IgM antibodies reactive to West Nile virus. He was begun on losartan 50 mg daily. His meningoencephalitis disappeared within 48 hr.
5.	A 73 yr old white man was admitted with fever, stiff neck, and severe confusion to a hospital in Omaha, NE. His serum was positive for IgM antibodies reactive to West Nile virus. He was begun on losartan 50 mg daily. His meningoencephalitis disappeared within 48 hr.
6.	A 17 yr old white woman was admitted with fever, stiff neck, and severe confusion to a hospital in Pueblo, CO. Her serum was positive for IgM antibodies reactive to West Nile virus. She was begun on losartan 50 mg daily. Her meningoencephalitis disappeared within 24 hr.
7.	An 80 yr old white woman who had meningoencephalitis with positive IgM antibodies for West Nile virus in early August, 2003 was seen at a hospital in Pueblo, CO because of residual weakness and fatigue one month later. She was begun on losartan 50 mg daily, with disappearance of her weakness and fatigue within 24 hr.
8.	A 40 yr old white woman with chronic lymphocytic leukemia was admitted with fever and obtundation to a hospital in Pueblo, CO. Her serum was positive by EIA for West Nile virus antibodies. She was given intravenous enalapril on the night of admission followed by losartan 50 mg daily, but remained in coma. Head CT and MRI scans were negative.

shown improvement with ACE inhibitors [224,225], as expected [226].

7. sACE and Aging

Besides being associated with most common diseases of aging [82], overactivity of sACE is consistent with most current theories of aging [227,228,229].

For example, calorie restriction prolongs life-span in a number of species [230]. With less fuel consumption, mitochondrial electron transport and production of ROS are decreased [231]. sACE overactivity as a cause of aging is entirely consistent with this model, since angiotensin II stimulates mitochondrial electron transport, oxygen consumption [232], and production of ROS [233,234]. Chronic angiotensin II signaling leads to mitochondrial hypertrophy and proliferation [235]. Eventually, angiotensin II leads to mitochondrial dysfunction, with increased uncoupling of electron transport from ATP synthesis, and increased production of ROS. Inhibition of ACE in old animals restores mitochondrial function [236].

Mutations in the insulin-like receptor of *Drosophila* and daf-2 in *C. elegans* are associated with extended lifespan but small size [229,237]. As in rodents, growth and metabolism limit lifespan. Because they result in decreased fuel consumption, these mutations are equivalent to calorie restriction in rodents.

Werner's syndrome, characterized by accelerated aging, is due to mutations in a DNA helicase. DNA helicases like wrn are the cell's defense against DNA damage induced by ROS [238], consistent with the theory that senscence is promoted by ROS.

In vitro, telomerase activity is required for replicative competence of cells in culture [239]. Angiotensin II can repress telomerase activity indirectly through TGF-β and p53 [239,240], among other pro-apoptotic factors.

Osteoarthritis [82], skeletal muscle wasting and cachexia are features of old age. Skeletal muscle wasting can be due to angiotensin II, which opposes the action of IGF-1 [241]. Cachexia can result from a high circulating level of TNF-α [242], derived from monocyte/macrophages stimulated by angiotensin II [243].

In summary, population morbidity and mortality should be significantly reduced, and longevity enhanced, by widespread use of an ACE inhibitor or ARB. The only caveat is that white men taking an ACE inhibitor or ARB will need to check their PSA at least once a year.

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Table 3. Pharmacy Data from a Predominantly White Midwestern Veterans' Hospital Population. Goserelin Acetate, an LH-RH Agonist, was Chosen as a Marker Drug for Prostate Cancer, Since this is the Only Indication for the Drug's Use [205]. ACEI, ACE Inhibitor.

Goserelin					
		+	-	Subtotals	Total
	+	46	8,608	8,654	
ACEI's					23,699
	-	17	15,028	15,045	
Subtotals		63	23,636		
Total		23,699		(~70% white men, ~30% black men)	

Odds ratio = (46) (15,028)/(17) (15,028) = 4.72
95% Confidence Interval = [2.22-10.17]
χ^2 (1 df) = 34.74
$p < 0.00001$

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