SPECIAL COMMENTARY

Lessons from angiotensin-converting enzyme-deficient mice

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Abbreviations

ACE	angiotensin-converting enzyme
Ang	angiotensin
AT_1	angiotensin II type 1 (cell surface receptor)

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Introduction

Since the first description of renin by Tigerstedt and Bergman [1] in 1898, many papers have described the biochemistry and physiological roles of the renin–angiotensin system [2]. A critical component of this system is angiotensin-converting enzyme (ACE), a peptidase which cleaves the inactive peptide angiotensin (Ang) I to generate the potent vasoconstrictor Ang II. This enzyme also cleaves other peptides, including bradykinin. Its activity increases blood pressure, and ACE inhibitors have become a mainstay in the treatment of hypertension and congestive heart failure. It has recently become possible to create mice which lack ACE. These mice have low systolic blood pressure, striking renal defects and reduced male fertility, phenotypes which emphasize the familiar roles of ACE and provide insight into unexpected functions of this enzyme.

Angiotensin-converting enzyme

ACE is a zinc-dependent peptidase which is found as two isoforms in mammals: somatic ACE and testis ACE [3]. Somatic ACE is an ectoenzyme of M_r 150 000–180 000 that is abundantly expressed by vascular endothelium. Not surprisingly, the highest ACE levels are found in the lung. Somatic ACE is also produced by several other tissues including the renal proximal tubule, Leydig cells, activated macrophages, gut epithelia and brain [4,5]. Cleavage of tissue ACE releases a soluble enzyme which circulates in the blood and cerebral spinal fluid. Testis ACE is a protein of M_r 90 000–110 000, approximately half the size of the somatic isoform. In contrast to the wide tissue distribution of somatic ACE, testis ACE is expressed exclusively by elongating spermatids, and the testis has a very high level of ACE activity [6].

Molecular cloning of somatic ACE has revealed that the enzyme consists of two homologous protein domains, each of which has an active catalytic site [7–9]. Both active sites have similar affinities for Ang I, but show greater differences for other peptide substrates. Testis ACE is identical to the carboxyl-terminal half of somatic ACE, except for the amino-terminal 66 amino acids which are unique to this isozyme [10–12]. Testis ACE therefore contains only a single catalytic site.

The best established physiological role for ACE is in the renin–angiotensin system. In this system renin is produced by juxtaglomerular cells in response to reductions in renal blood flow or blood pressure. Renin cleaves the circulating protein angiotensinogen, releasing the peptide Ang I, which contains 10 amino acids. This peptide is rapidly converted to Ang II by ACE. Ang II is a potent vasoconstrictor and has other effects, including release of aldosterone, reabsorption of salt and water by the gut, stimulation of proximal tubular sodium reabsorption, stimulation of thirst and potentiation of sympathetic activity [13]. These effects act co-ordinately to elevate blood pressure. The renin–angiotensin system can be modeled as a biological machine in which the kidney acts as a sensor of hemodynamic status [14]. During environmental stress the renin–angiotensin system maintains homeostasis of blood volume, blood pressure and body electrolyte composition.

Although Ang I is the best known ACE substrate, the enzyme can cleave other peptides. ACE has long been known to degrade the vasodilatory peptide bradykinin, and ACE inhibition increases the effects of bradykinin. It has been suggested [15] that increased levels of this peptide are responsible for the cough associated with therapy with ACE inhibitors. Recent evidence [16] suggests that ACE is physiologically active in degrading the stem cell growth-arresting peptide *N*-acetyl-Ser–Asp–Lys–Pro. Interestingly, this is one of the few peptides known to be cleaved primarily by the amino-terminal active site. ACE also has in-vitro activity against several substrates, including enkephalins, gastrin, substance P and luteinizing hormone-releasing hormone [17]. There is, however, no

definitive evidence that ACE plays a role in the metabolism of any of these peptides *in vivo*.

Creation of angiotensin-converting enzyme deficient mice

Technology exists which allows the creation of mice with defined genetic modifications [18,19]. The first step is the targeting and modification of a particular genetic locus in cultured embryonic stem cells. Injection of these targeted cells into a mouse blastocyst results in a chimeric mouse with tissues derived from both the blastocyst and the injected cells. Selective breeding of the chimeric mice produces offspring which are homozygous for the modified locus. Several groups, including our group, have used these techniques to generate mouse lines that lack ACE [20,21]. Mouse lines have also been created which lack other components of the renin–angiotensin system, including angiotensinogen and a subtype of the Ang II receptor [22–25].

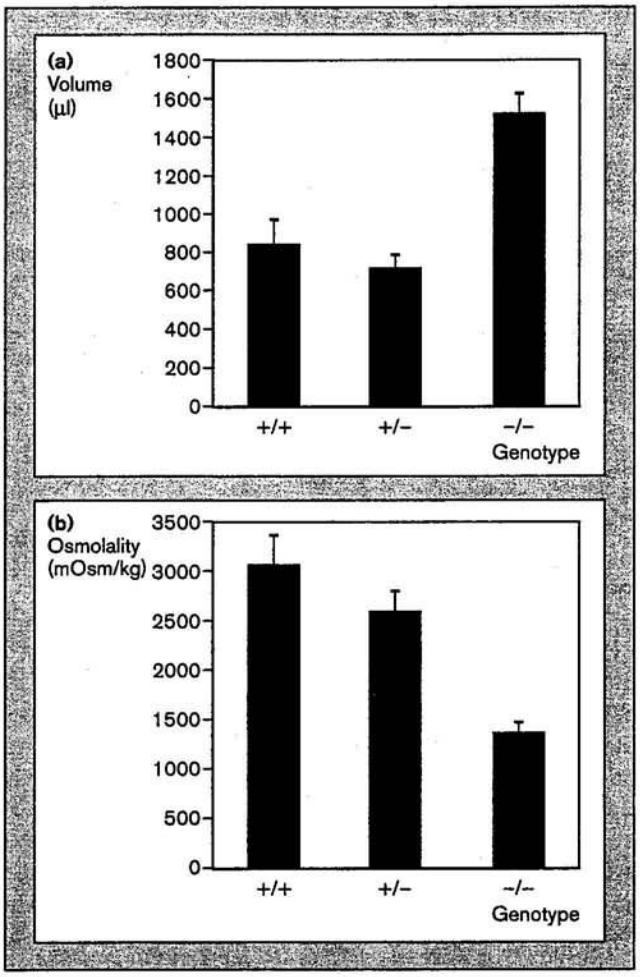
Blood pressure

Mice lacking ACE have profoundly reduced systolic blood pressures. The average systolic blood pressure is 70 mmHg, approximately 40 mmHg lower than in normal, wild-type mice. This is a much greater change than the reduction of 10 mmHg in systolic blood pressure which occurs when healthy human subjects or rodents are treated with ACE inhibitors [26]. This discrepancy suggests that the complete lack of ACE, achieved through genetic means, results in a greater effect on blood pressure than can be achieved with pharmacological blockade. Studies with ACE inhibitors may therefore underestimate the role of the renin–angiotensin system in maintaining normal blood pressure. The reduction in blood pressure observed in ACE-deficient animals is also greater than that found in mice which lack angiotensinogen. This implies that ACE substrates other than Ang I (e.g. bradykinin) are also important in the control of blood pressure.

Kidney development

Compared with control mice, mice which lack ACE produce a large volume of relatively dilute urine (Fig. 1). This urinary concentrating defect can be explained partly by the unusual renal histology observed in these mice. ACE-deficient mice have a marked thinning of both the renal medulla and the renal papilla. In extreme cases these mice have a significant expansion of the caliceal system, which represents nearly complete atrophy of the renal medulla with small renal papilla (Fig. 2). Surprisingly, ACE-deficient mice present with medial hyperplasia of the intrarenal arteries, despite their low blood pressure. Renal vessels often show a marked perivascular mixed lymphocytic infiltrate. Lymphocytic vasculitis is occasionally observed. Vascular and organ structural defects are both limited to the kidneys in these mice.

Figure 1



Analysis of (a) total volume and (b) osmolality of 24-h urine collected from wild-type (+/+), heterozygous (+/−) and angiotensin converting enzyme knockout (−/−) mice deprived of water for 6 h before collection. Under these conditions, the knockout mice produced a twofold larger volume of urine that was less than half as concentrated. Adapted with permission [21].

The defect in the ability to concentrate urine is consistent with the renal pathology, and indicates that the lesion is not secondary to urinary obstruction. Interestingly, the magnitude of the defect does not correlate with the severity of the renal lesion. This suggests that the renin–angiotensin system might play a physiological role in urinary concentrating mechanisms.

Several lines of evidence indicate that the maldevelopment of the renal medulla is a direct result of the lack of Ang II generation within the kidneys of these mice. Mice which lack angiotensinogen have renal pathology nearly identical to that in ACE-deficient mice clearly implicating Ang II [22,23]. Furthermore, neonatal rats treated with either an ACE inhibitor or an inhibitor of the Ang II type 1 (AT_1) receptor develop a similar renal lesion [27]. The medullary defect is unlikely to be an indirect effect of the

Figure 2

Typical histological features of a kidney from an angiotensin-converting enzyme knockout mouse (×25). The kidneys from knockout mice often have a thinned medulla with papillary atresia and dilated renal calyces. Regions of inflammatory infiltrate are evident at the cortical medullary junction. Reproduced with permission [21].

inability to concentrate urine or the low blood pressure. Mice with diabetes insipidus cannot concentrate urine but have no renal pathology and similarly, mice have been created which have low blood pressure but normal renal histology [25]. Ang II thus appears to be necessary for proper renal medullary development.

Angiotensin II as a growth factor

The role of Ang II in promoting the proper development of the kidney is only the latest addition to a growing set of data which suggests that Ang II can act as a growth factor. For example, chronic in-vivo infusion of low-dose Ang II leads to a vascular hypertrophic response in blood vessels which is caused partly by non-pressor mechanisms [28]. Infusion of Ang II also markedly exacerbates the myoproliferative lesions caused by balloon catheterization [29]. ACE inhibitors have, conversely, been shown to decrease neointimal proliferation after vascular carotid injury [30]. In-vitro, Ang II increases protein synthesis in rat smooth muscle cells by 45% and DNA synthesis by 56% during a 24-h incubation [31]. Growth factor properties of Ang II have been demonstrated in fibroblasts, adrenal cortical cells, cardiac myocytes, renal proximal tubular cells and tumor cells [32]. The hemodynamic effects of Ang II are known in some detail, but its biochemical actions leading to cell growth are less clear.

Recent studies [33–37] in our laboratory have begun to clarify the possible mechanisms by which Ang II can have growth-promoting effects. These studies concern the intracellular signalling pathways initiated when Ang II binds to its cell surface receptor (now called the AT_1 receptor). There are two known subtypes of receptors for Ang II, but virtually all of the known physiological effects are mediated through the AT_1 subtype [32]. This receptor is a protein with seven transmembrane domains which has the classic structure of receptors believed to signal via heterotrimeric G-proteins [38,39].

The classic paradigm of growth factor action involves stimulation of cell surface receptors which in turn, lead to increased tyrosine phosphorylation of important signaling intermediaries. For example, platelet-derived growth factor causes tyrosine phosphorylation, stimulation of the *Ras* gene and activation of downstream signaling events [40]. In one sense, a growth factor uses tyrosine phosphorylation as the first step in a cascade of information from the cell surface into the cell cytoplasm and nucleus. This information flow leads to cellular responses which include cell proliferation.

The cell surface receptor for Ang II is markedly different from that for growth factors. The AT_1 receptor lacks an intrinsic ability to phosphorylate other proteins on tyrosine. Despite this, our studies have clearly shown that tyrosine phosphorylation is an important intracellular signaling response initiated by Ang II. Both in vascular smooth muscle cells and in rat renal mesangial cells, Ang II leads to the tyrosine phosphorylation of phospholipase C-γ1 [33,34]. This, in turn, is responsible for an increase in the intracellular calcium concentration and, further downstream, signaling events. The intracellular tyrosine kinase Src is critically important in this signaling pathway. Neutralization of this important signaling molecule with anti-Src antibodies interferes with the ability of Ang II to induce tyrosine phosphorylation [35]. Ang II binding to its cell surface receptor thus stimulates Src activity in some manner.

Ang II also induces tyrosine phosphorylation of other classes of intracellular tyrosine kinases. Binding of Ang II to the AT_1 receptor causes tyrosine phosphorylation and activation of the Jak kinases [36]. These kinases stimulate the Signal Transducers and Activators of Transcription (STAT) family of transcription factors, which provide a second important signaling pathway to convey information from the cell surface into the nucleus. Ang II also stimulates Ras activation [37]. Ras acts as an important control switch within all cells. When bound to GDP, Ras is inactive. In contrast, Ras–GTP is fully active and participates in events often associated with cell proliferation. Recent studies from our laboratory have demonstrated that Ang II, acting through the AT_1 receptor,

stimulates the conversion of Ras–GDP to Ras–GTP. This pathway is also critically dependent on the intracellular function of Src kinases.

Our studies of cell signaling provide an important link between the effects of Ang II on individual cells and its known growth-promoting effects. This, in turn, helps to explain the renal defect observed in mice that are deficient in Ang II production because of a lack of ACE or angiotensinogen. These studies clearly suggest that Ang II promotes the development of individual medullary cells. This hypothesis is supported by the observation that the renal medulla contains abundant AT_1 receptors.

Male fertility

Of all the cells which express ACE, male germ cells are unique in producing an isozyme with only a single catalytic domain. This occurs because these cells begin transcription of the ACE gene far removed from the transcription start site used by somatic tissues. Male germ cells recognize a testis-specific promoter located in the middle of the ACE gene [41,42]. By understanding how developing male germ cells are unique in recognizing testis ACE, a small window is opened on an important area of tissue differentiation.

The testis *ACE* promoter is unusual because it drives the highest levels of ACE transcription but is active in a very restricted cell type. This combination of strong transcription and high specificity suggests a complicated genetic element, but a study from our laboratory has shown that only the 91 base-pairs upstream of the testis ACE transcription start site are required to target testis-specific expression of a reporter gene in transgenic animals [43].

The 91 bp testis *ACE* promoter contains a sequence similar to the consensus cAMP response element. It is now believed that the testis-specific, cAMP-dependent transcription factor CREM-τ binds to this site to induce testis ACE transcription [44]. CREM-τ is produced specifically by male germ cells as they make the transition from diploid to haploid cells, consistent with the developmental stage in which testis ACE transcription is observed. This finding has not, however, been confirmed *in vivo*, partly because mice which lack CREM-τ have severe defects in spermatogenesis and do not produce mature male germ cells [45,46].

The role of testis ACE is more complicated than that of CREM-τ. The morphology of the testis is unremarkable in mice which lack testis ACE. These mice produce mature sperm which appear to be normal in number, morphology and motility. When male mice which lack ACE are mated to normal females, however, they sire litters that contain fewer pups than those sired by wild-type males. Specifically, mice which lack ACE sire litters that are on average one-third the size of normal litters. ACE-deficient mice thus have a functional defect in male fertility despite sperm that appears normal.

Interestingly, the defect in male fertility appears to be the only phenotype of ACE-deficient mice that is not related to the renin–angiotensin system. Angiotensinogen-deficient mice seem to have normal male fertility despite lacking Ang II [22]. ACE is known to cleave a variety of peptides, and some of these such as kinins and substance P, are known to influence sperm physiology [47,48]. We therefore speculate that one of these alternative peptides must be cleaved, inactivated or perhaps activated by ACE to achieve normal male fertility.

Conclusion

Mice which lack ACE have low systolic blood pressure, reduced male fertility and a renal abnormality characterized by medullary hypoplasia and the inability to concentrate urine. The diverse phenotypes caused by inactivation of a single gene emphasize the many functional roles of ACE and the renin–angiotensin system.

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