New approaches to genetic manipulation of mice: tissue-specific expression of ACE

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Cole, Justin M., Hong Xiao, Jonathan W. Adams, Kevin M. Disher, Hui Zhao, and Kenneth E. Bernstein. New approaches to genetic manipulation of mice: tissue-specific expression of ACE. Am J Physiol Renal Physiol 284: F599–F607, 2003; 10.1152/ajprenal.00308.2002.—The renin-angiotensin system (RAS) plays a central role in body physiology, controlling blood pressure and blood electrolyte composition. ACE.1 (null) mice are null for all expression of angiotensin-converting enzyme (ACE). These mice have low blood pressure, the inability to concentrate urine, and a maldevelopment of the kidney. In contrast, ACE.2 (tissue null) mice produce one-third normal plasma ACE but no tissue ACE. They also have low blood pressure and cannot concentrate urine, but they have normal indices of renal function. These mice, while very informative, show that the null approach to creating knockout mice has intrinsic limitations given the many different physiological systems that no longer operate in an animal without a functioning RAS. To investigate the fine control of body physiology by the RAS, we developed a novel promoter swapping approach to generate a more selective tissue knockout of ACE expression. We used this to create ACE.3 (liver ACE) mice that selectively express ACE in the liver but lack all ACE within the vasculature. Evaluation of these mice shows that endothelial expression of ACE is not required for blood pressure control or normal renal function. Targeted homologous recombination has the power to create new strains of mice expressing the RAS in selected subsets of tissues. Not only will these new genetic models be useful for studying blood pressure regulation but also they show great promise for the investigation of the function of the RAS in complicated disease models.

angiotensin-converting enzyme; blood pressure; angiotensin II; liver; renin-angiotensin system

ROUGHLY ONE-HALF OF ALL AMERICANS die from cardiovascular disease. Put differently, more Americans die of cardiovascular disease than the next seven leading causes of death combined (1). To understand cardiovascular disease, one must understand how the body regulates blood pressure, a complex process governed by many different components, including multiple vasoconstrictors and vasodilators. Among these is the renin-angiotensin system (RAS), which produces the vasoconstrictor angiotensin II. The basic physiology by which angiotensinogen is degraded to angiotensin II has been known for many years, with the importance of this system underscored by the enormous clinical utility of pharmaceuticals that interrupt angiotensin II formation or action. So with all that is known about the RAS, what could conceivably be new? In fact, the use of targeted homologous recombination in mouse embryonic stem (ES) cells has produced a startling series of insights into the operation and functional importance of the RAS. This progress has been so rapid and dramatic as to implicate the RAS as easily the most important regulator of cardiovascular function as measured through blood pressure control.

The use of homologous recombination to modify genes relies on the properties of cultured ES cells (4). Originally derived from the inner cell mass of a mouse embryo, these cells are pluripotent; when reinjected back into a mouse blastocyst, the ES cells contribute to the formation of all the organs of the recipient. Thus the recipient mouse is chimeric in that its tissues are derived from both wild-type cells and ES cells. Knockout mouse technology depends on homologous recombination to introduce specific changes into the DNA of cultured ES cells. Once these cells are used to create a chimeric mouse, simple breeding ultimately results in homozygous, heterozygous, and wild-type mice for the modified genetic locus. Although the typical strategy is to eliminate a particular gene, knockout technology...
can produce virtually any conceivable genetic change, including gene duplication and the selective mutation of amino acid composition. Indeed, as discussed below, the selective modification of a gene (as opposed to its elimination) holds great promise in creating novel genetic models.

Similar to all techniques, the use of targeted homologous recombination in ES cells has both conceptual advantages and disadvantages. The greatest advantage is the plasticity of the technology, allowing a precise change to be introduced into the mouse genome. Also, when a gene is altered, the level of functional modification is complete and unvarying from the conception of the animal until the death of that animal. Another advantage of this technology is that mouse lines bearing defined mutations of genes on separate chromosomes can be crossed to generate offspring with combined mutations. The knockout approach also has potential disadvantages. For example, the technology seems far easier in mice than in other animals, even other rodents. Another potential disadvantage is that in models of gene inactivation, a mouse has the entire embryonic period to develop compensatory mechanisms. Thus a mouse that has never produced a protein is different from an animal in which that same protein is inhibited as an adult. This problem can be partially offset through the creation of conditional and/or tissue-specific knockouts in which gene inactivation only occurs in the presence (or absence) of specific drugs or in certain tissues (31). Finally, knockout technology creates a permanent change in the animal's genome. This is different from pharmacological inhibitors, which can be administered in varying doses and then withdrawn. Despite the potential limitations of the knockout technique, its use in creating unique genetic models has made this technology one of the most important in modern biological research.

Since 1995, targeted homologous recombination has been used to create knockout mice that are null for the individual components of the RAS (6, 7, 17, 20, 22, 23, 25, 27, 36, 37, 44, 48, 51, 53, 54, 58). Several recent reviews have cataloged the individual mouse lines and discussed the phenotype of these animals. In this review, we will focus on recent work from our laboratory that made use of homologous recombination to selectively inactivate angiotensin-converting enzyme (ACE). We develop the hypothesis that the creation of mouse models bearing a functional RAS in only a small subset of tissues is a powerful approach to gain insight into the organ-specific function of the RAS.

**ACE.1: MICE NULL FOR ACE**

ACE is a carboxypeptidase that converts angiotensin I into angiotensin II. ACE has other substrates such as bradykinin and acetyl N-acetyl-seryl-aspartyl-lysylproline (a putative bone marrow suppressor), but the physiological role of ACE in the regulation of these peptides is unclear. There are two ACE isozymes. Somatic ACE is made by endothelium and other somatic tissues and is the form of ACE responsible for blood pressure regulation. This protein contains two separate catalytic sites (termed NH2-terminal and COOH-terminal sites) that are capable of hydrolyzing angiotensin I (3, 50, 56). Most somatic ACE is bound to tissues via a hydrophobic COOH-terminal domain (2). Endothelium expresses abundant ACE; thus the lung, an organ rich in endothelium, contains a high concentration of ACE. ACE protein is also produced by renal proximal tubular epithelium, vascular adventitia, areas of the gut, activated macrophages, and selected portions of the brain (5, 49, 52). A second ACE isozyme is produced by developing male germ cells (10). This protein, termed testis ACE, is the result of a tissue-specific promoter located within intron 12 of the ACE gene (21). Testis ACE corresponds to the COOH-terminal half of somatic ACE and contains only the COOH-terminal catalytic domain (15, 29, 30). As described below, studies in knockout mice have proven that testis ACE plays a critical role in male fertility.

Both our group and the laboratory of Dr. Oliver Smithies created mice null for all ACE expression (17, 27). The animals made by our group are called ACE.1 (null) and produce neither somatic nor testis ACE (Fig. 1). ACE null mice have a profound reduction of blood pressure. When blood pressure is assessed with an automated tail-cuff device, a normal mouse will average 105–110 mmHg. In contrast, littermate mice null for ACE have a systolic blood pressure of 73 mmHg. The magnitude of the blood pressure reduction (~30 mmHg) is striking, and, to our knowledge, mice lacking ACE have a lower blood pressure than any other animal model investigated to date. Maybe not surprisingly, the one exception is mice lacking angiotensinogen (25, 43, 53), both AT1 receptors (AT1A and AT1B) (44, 54), or renin (36, 58), which also present with a reduction in systolic blood pressure of ~30 mmHg. Despite having a lifetime to regulate the many other physiological systems that influence blood pressure, a mouse lacking a functional RAS presents with a profound reduction of blood pressure. Thus no physiological system or combination of systems can compensate for the lack of angiotensin II. The RAS is the central regulator of blood pressure, an observation that gives insight into the remarkable clinical efficacy of RAS inhibitors.

In addition to low blood pressure, ACE.1 (null) mice have other components to their phenotype. Perhaps most surprising was a maldevelopment of the kidney characterized by underdevelopment of the renal medulla and papilla. The origins of this lesion are not known, although elegant work by Miyazaki et al. (39, 40) suggests improper function of the ureter as a potential cause.

ACE.1 (null) mice also give insight into the role of testis ACE. Male mice homozygous for the ACE.1 (null) gene do not reproduce well; they produce few litters and each litter is far smaller than that produced by wild-type male mice. Several additional studies have definitively shown that it is the lack of testis ACE that is responsible for the reproductive phenotype. For example, elegant experiments by Ramaraj et al. (46) used...
transgenic technology to reinstate testis ACE expression onto the background of ACE null mice. The restitution of testis ACE expression restored normal fertility, even in the presence of a reduced blood pressure due to the continued lack of somatic ACE. In contrast to male knockout mice, female ACE.1 (null) mice appear to have relatively normal fertility.

ACE.2: NO TISSUE ACE EXPRESSION

In creating the ACE.2 (tissue null) model, we hoped to engineer a line of mice that would express somatic ACE in the absence of testis ACE (Fig. 1) (18). Unfortunately, our manipulation of the mouse genome resulted in a stop codon being introduced after exon 12 of the somatic ACE gene. The resulting animal produced a truncated ACE protein roughly one-half the size of the wild-type mouse. This protein retained the NH2-terminal signal sequence (responsible for protein export from cells) and the NH2-terminal catalytic region.

What this protein lacked was the COOH-terminal catalytic domain and, even more importantly, the COOH-terminal hydrophobic tail of the protein normally responsible for anchoring the protein to cell membranes. In summary, ACE.2 (tissue null) mice have a truncated ACE protein that circulates in the plasma but no measurable ACE activity associated with tissues such as the lung or the kidney. The phenotype of ACE.2 (tissue null) mice was similar to ACE.1 (null) knockout animals; systolic blood pressure was in the mid-70s and the mice were unable to concentrate urine.

A comparison of ACE.1 (null) and ACE.2 (tissue null) illustrates several different aspects of blood pressure control. First, the ACE.2 (tissue null) mice give insight into the concept of "total body load" of ACE. The RAS has sufficient homeostatic capacity to function across a wide range of ACE body load. For example, Krege et al. (28) have demonstrated that mice with one, three, and even four copies of the ACE gene maintain normal...
blood pressure. However, in a normal mouse, most ACE is bound to tissues. Although the ACE.2 (tissue null) mice have roughly one-third of normal levels of circulating plasma ACE activity, they have no tissue ACE. Thus these animals have a marked deficiency of total body ACE. This is reflected in very low levels of plasma angiotensin II that are similar to the levels seen in ACE.1 (null) mice (8). A hypothesis developed from these two strains was that total quantities of body ACE (as opposed to the precise localization of ACE activity) may be the critical factor in determining adequacy of ACE activity.

There is another way to view the ACE.2 (tissue null) mice. These animals are also deficient in specific tissue beds of ACE. Recent theories of the RAS have proposed important physiological (and pathophysiological) roles for angiotensin II generated locally by tissues such as the vascular endothelium (11, 14, 41). ACE.2 (tissue null) mice lack endothelial ACE, and the low blood pressure in these animals may be due to a specific inability to generate vascular angiotensin II. The relative importance of these two hypotheses (total body ACE vs. precise patterns of tissue expression) is discussed below in the analysis of the ACE.3 (liver ACE) mice.

A major difference between the ACE.1 (null) and ACE.2 (tissue null) strains of mice is in renal development. As previously noted, ACE.1 (null) mice have an underdevelopment of the renal medulla and papilla. In contrast, most ACE.2 (tissue null) mice do not have this defect (18). Because the blood pressures of these two strains are virtually indistinguishable, this implies that the renal malformation is attributable to other factors besides the absolute reduction of blood pressure. Also, the difference in kidney phenotype between the ACE.2 (tissue null) and ACE.1 (null) mice suggests that among the several different facets of the phenotype found in the ACE null animals, renal development is the most sensitive to amelioration by the small increases of ACE and angiotensin II present in ACE.2 (tissue null) knockout mice compared with truly null animals. The precise biochemistry of this is not at all understood.

That ACE.2 (tissue null) knockout mice have a very low blood pressure but retain normal renal function creates a mouse model of exceptional utility. Specifically, although ACE.1 (null) animals have abnormal measures of renal function, as indicated by an elevation of serum creatinine and a reduction of creatinine clearance, ACE.2 (tissue null) mice have a normal serum creatinine and creatinine clearance (8). Despite this, ACE.2 (tissue null) knockout mice are still unable to concentrate urine. Thus in ACE.2 (tissue null) mice, the abnormal physiology must be the result of the lack of ACE and angiotensin II, as opposed to renal insufficiency. To understand the molecular basis for the inability to concentrate, we determined the abundance of several different transport proteins and ion channels typically associated with renal concentrating ability (26) (Fig. 2). Within the inner medulla, ACE.2 (tissue null) knockout mice had a reduction of the 117-kDa urea transporter UT-A1 (28 ± 5% of normal), the chloride channel CIC-K1 (6 ± 6% of normal), and aquaporin 1 (39 ± 5% of normal). In contrast, the protein abundance of the water channel aquaporin-2 and the urea transporter UT-B were equivalent to levels found in wild-type mice. Within the outer medulla, there was a modest reduction of Na-K-Cl cotransporter isoform 1/type 1 bumetanide-sensitive Na-K-Cl cotransporter (56 ± 11% of normal) as well as a more marked reduction of the water channel aquaporin-1 (29 ± 6% of normal). Quite unexpectedly, there was also a sevenfold increase in the apical potassium channel ROMK (711 ± 187% of wild type). To our knowledge, this is the first observation of an animal with upregulation of ROMK. One explanation for this unusual finding is as a response to the hyperkalemia that is typical in ACE knockout mice.

In evaluating the ACE.2 (tissue null) data, the marked decrease in UT-A1 and, in particular, CIC-K1 would be consistent with an inability to concentrate urine. The reduction of UT-A1 may reduce the amount of urea transported from the terminal inner medullary collecting duct. However, almost certainly, the profound reduction of CIC-K1 would reduce NaCl reabsorption from the thick ascending limb. Knockout mice that lack CIC-K1 (Clcnk1 mice) also present with a marked inability to concentrate urine (35). Why mice with a substantial reduction of angiotensin II generation have a defect in the protein concentration of UT-A1 and CIC-K1 is not understood. However, these data illustrate how the creation and analysis of knockout mice have already yielded new insights into angiotensin II-mediated regulation of renal function. Another new approach in the study of the concentrating defect in ACE.2 (tissue null) mice is through micropuncture, and studies of this type are currently under investigation.

**ANGIOTENSIN II AND ERYTHROPOIESIS**

Both ACE.1 (null) and ACE.2 (tissue null) knockout mice present with an ~20% reduction of both hemocrit and hemoglobin levels (8). Although one might envision that ACE.1 (null) mice are anemic partly due to renal insufficiency, ACE.2 (tissue null) mice are equally anemic despite having normal chemical measures of renal function. The anemia in these strains is not due to iron deficiency or apparent hemolysis. Furthermore, both ACE.1 (null) and ACE.2 (tissue null) knockout mice have elevated serum erythropoietin levels. The anemia in these mice is real in that a donor erythrocyte infusion using 51Cr-labeled red blood cells demonstrated a reduction of red cell mass associated with normal total blood volume. Perhaps even more informative was a study in which ACE.2 (tissue null) knockout mice received angiotensin II during a 2-wk period by subcutaneous osmotic minipump. These mice remained deficient in ACE, despite the restoration of angiotensin II. Thus this experiment directly assessed the physiological role of angiotensin II in the anemia, as opposed to other peptides that may be ACE sub-
strates. The results were clear: restitution of angiotensin II elevated both systolic blood pressure and hematocrit to near normal levels. Although we have not eliminated the possibility that blood pressure itself played a role in erythrocyte formation, these studies compliment a host of clinical investigations suggesting a direct role of the RAS in erythropoiesis (16, 19, 33, 55). It is well known that erythropoietin is dependent on the intracellular signaling of the Jak-STAT system for efficacy. Erythropoietin knockout, erythropoietin receptor knockout, and Jak 2 knockout mice all present with the phenotype of embryonic lethality due to severe anemia (24, 32, 42, 57). Angiotensin II activates Jak-STAT signaling (34). Whether this somehow provides the link by which angiotensin II influences erythropoiesis is under investigation.

ACE.3: SELECTIVE HEPATIC EXPRESSION OF ACE

To summarize, the evaluation of ACE null mice revealed a complex phenotype involving changes in blood pressure, electrolyte balance, renal function, renal development, reproduction, and hematological parameters. Perhaps this was not surprising given the many different organ systems possessing receptors for angiotensin II. In fact, it is important to realize that an ACE null mouse is very different from a wild-type animal. Put another way, the typical approach of knocking out a gene induces a major change in the physiology of that animal. Such an approach is highly informative but not at all subtle. Indeed, the null animal approach has intrinsic limitations given the many different physiological systems that no longer operate in the knockout mouse.

Our group has developed an approach for creating a more selective knockout of protein expression. The basis of the approach is a simplistic view of gene structure: the ACE gene, as with any gene, can be modeled as two separate parts. There are the exonic portions of the gene encoding amino acid sequence and the promoter portion of the gene regulating the temporal and tissue-specific expression of the protein. In principle, a modification of the promoter is capable of changing the tissue-specific patterns of gene expression. Specifically, we reason that if the endogenous ACE promoter is substituted with a highly tissue-specific promoter, then ACE expression would only be present in the limited subset of tissues recognizing this new promoter. Such a mouse would be null for ACE

Fig. 2. Altered medullary transport proteins in ACE.2 (tissue null) mice. Left: Western blot analysis of CIC-K1 (A; arrow, 80-kDa protein band for CIC-K1) and ROMK (B, arrow, 45-kDa protein band for ROMK) expression within the renal medulla of wild-type (WT) and ACE.2 (tissue null) knockout mice (KO). Right: densitometric data averaged from several mice (*P < 0.03). Knockout mice express 66 and 711% of CIC-K1 and ROMK levels, respectively, found in WT mice.
expression in other tissues not recognizing the new tissue-specific promoter. This is similar to creating a transgenic mouse and breeding it onto a knockout background, an approach that has been previously used to investigate the tissue-specific expression of angiotensinogen (13). Technically, we believed that our approach would be simpler for the study of ACE due to the reduced fertility present in the ACE knockout. Thus the goal of the ACE.3 (liver ACE) mouse was to investigate such an approach by placing the coding portions of the ACE gene under the control of the albumin promoter (9).

The ACE.3 (liver ACE) mouse was engineered to contain two DNA cassettes separating the endogenous somatic ACE promoter from the translation start site of the gene (Fig. 1). A neomycin cassette was inserted immediately 3' of the endogenous somatic ACE promoter and acted as a transcriptional barrier to promoter activity. We inserted an albumin promoter cassette 3' to the neomycin cassette, containing both enhancer and core promoter sequences sufficient to initiate transcription.

Evaluation of tissue ACE activity revealed a marked difference among wild-type, ACE.3 (liver ACE) heterozygote, and ACE.3 (liver ACE) homozygote mice (Fig. 3). Although a wild-type mouse expresses virtually no ACE within the liver, the ACE.3 (liver ACE) homozygous mouse makes abundant hepatic ACE that is localized on the cell membranes of hepatocytes. In contrast, a wild-type mouse expresses abundant ACE in the lung but the ACE.3 (liver ACE) homozygous mouse makes abundant hepatic ACE that is localized on the cell membranes of hepatocytes. In fact, we were unable to detect any endothelial ACE expression in any tissue of the ACE.3 (liver ACE) mice. ACE.3 (liver ACE) mice express no ACE in the vascular adventitia, the GI tract, the spleen, or the heart. Only in the kidney is residual ACE activity observed, but even there, the level is only 15% that of wild-type mice. Histological examination of the kidney revealed no ACE expression by renal endothelial cells. Rather, the residual ACE is expressed by renal tubular epithelium. Finally, ACE.3 (liver ACE) mice express ACE in two other locations, circulating ACE in plasma presumably due to enzymatic release from hepatocytes and normal testis ACE expression, because the testis ACE promoter remained unaltered in the ACE.3 (liver ACE) construction. In summary, by changing promoter control of the ACE gene, we created a mouse strain that produces no endothelial or adventitial ACE but rather makes abundant ACE in the liver, an organ that normally has none. Such an approach allowed us to directly examine the physiological role of vascular ACE. Specifically, we asked whether such a mouse could adequately regulate blood pressure. What we observed was that the ACE.3 (liver ACE) mouse was in all ways normal. Its systolic blood pressure was indistinguishable from littermate controls. Renal development was typical. The ACE.3 (liver ACE) concentrated urine equivalently to a wild-type mouse. Serum electrolyte values were unremarkable, and the animal had both a normal hematocrit and a normal reproductive function. Indeed, evaluation of angiotensin II peptide levels within the plasma shows that the ACE.3 (liver ACE) mouse had equivalent levels to those of a wild-type mouse.

What are the implications of this analysis? First, vascular ACE expression is not obligatory for normal blood pressure control. Indeed, with sufficient ACE in the liver, the intrinsic regulatory capacity of the kidney can maintain homeostasis. Such a result again emphasizes the concept of total ACE body load. We hypothesize that sufficient ACE in any location within the body, coupled with the normal renal homeostatic mechanisms, is sufficient to result in normal blood pressure control. Second, ACE.3 (liver ACE) mice produced a concentrated urine despite having only 15% of normal renal ACE. Although we cannot conclude that renal ACE has no intrinsic function, it seems clear that the

![Fig. 3. Tissue-specific ACE expression in the ACE.3 (liver ACE) strain. ACE.3 (liver ACE) wild-type (+/+), heterozygote (+/-), and knockout (-/-) mice were killed, and ACE activity was measured in organ homogenates. Total organ ACE activity was calculated for the liver, lungs, and kidneys of each mouse and normalized for the mass of the mouse. The livers of ACE.3 (liver ACE) -/- mice have abundant total ACE activity, due in part to the large size of this organ. In contrast, ACE.3 (liver ACE) -/- mice have no detectable ACE activity in lung. ACE.3 (liver ACE) -/- mice have ~14% of the renal ACE found in wild-type mice. Values are group means ± SE.](http://ajprenal.physiology.org/Downloaded_from)
kidney can operate normally with even small amounts of renal ACE. Finally, the ACE.3 (liver ACE) mouse is valuable in establishing the validity of promoter substitution in creating strains of mice with very selected expression of ACE.

The ACE.3 (liver ACE) mouse also showed some of the potential drawbacks of the promoter substitution approach. First, the residual ACE expression in the kidney was probably due to the intrinsic properties of the albumin promoter used in the construct. Mice transgenic for similar promoter constructs also showed slight renal expression of a reporter protein (45). Also, the principal site of ACE expression in the ACE.3 (liver ACE) mouse was liver hepatocytes, an organ and tissue that does not normally produce the protein. It is conceivable that such ectopic expression of ACE could influence the phenotype of the animal. Another drawback of the ACE.3 (liver ACE) mice was substantial plasma ACE, which was 80% of normal. We believe this protein originates in a fashion similar to the naturally circulating ACE present in wild-type mice, namely, as a result of proteolytic cleavage of ACE from the surface of cells (2). However, we would like to find a means of reducing both the circulating ACE levels and the breakthrough expression of ACE in the kidney. One possibility is to create a compound heterozygous animal in which one ACE allele will be from the ACE.1 (null) mouse while the second ACE allele will be ACE.3 (liver ACE). In theory, such a compound heterozygous animal should produce liver ACE with reduced ACE activity in both the plasma and the kidney.

ACE.4: KIDNEY ANDROGEN-REGULATED PROTEIN PROMOTER DRIVING ACE

A strategic goal is to create an animal with ACE expression limited to the kidney. Such an animal would be informative about specific renal functions of the RAS vs. systemic effects. One animal created for this purpose is termed ACE.4 [kidney androgen-regulated protein (KAP) ACE] and has the KAP promoter-driving somatic ACE expression (Fig. 1). Normally, KAP is highly responsive to androgen and is found in proximal tubular epithelium and the uterus (38, 47). Although the KAP promoter was satisfactory in some transgenic experiments (12), it proved inadequate to target ACE expression in the ACE.4 (KAP ACE) mouse. Specifically, in the absence of exogenous androgens, virtually no ACE expression was measured in the tissues of either male or female ACE.4 (KAP ACE) mice. With exogenous androgens, renal ACE expression remained <2% of normal values. Thus ACE.4 (KAP ACE) mice had a profound reduction of somatic ACE and presented with a phenotype nearly identical to that of the ACE.1 (null) mouse. For example, the blood pressure of seven ACE.4 (KAP ACE) homozygotes averaged 70.0 ± 3.3 mmHg, a value very similar to that observed in the ACE.1 (null) and ACE.2 (tissue null) knockouts. The essential characteristics of the ACE.1–4 strains are summarized in Table 1. The results with ACE.4 (KAP ACE) mice indicate that the promoter substitution approach is highly dependent on the characteristics of individual promoter constructs. As opposed to transgenic mice, only a single copy of the tissue-specific promoter is inserted immediately upstream of the ACE gene. In the case of the KAP promoter, this proved unable to drive ACE expression.

SUMMARY

Targeted homologous recombination has the power to create new strains of mice expressing the RAS in selected subsets of tissues. Not only will these new genetic models be useful for the study of blood pressure regulation, but they also have great promise in the investigation of the function of the RAS in complicated disease models. For example, the ACE.3 (liver ACE) mouse has a normal blood pressure in the absence of endothelial or adventitial expression of ACE. One can envision experiments in which this animal is used to derive insight into the function of the RAS in disease processes such as atherosclerosis or diabetes. By choosing which tissues of the mouse will express a functional RAS, we can perform subtle yet elegant experiments to evaluate individual functions of a system that has many different physiological roles. Such an approach may reveal yet additional subtle actions of the RAS in the control of both normal and aberrant physiology.

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Table 1. Phenotypic characteristics of ACE knockout mice

<table>
<thead>
<tr>
<th>Tissue ACE</th>
<th>ACE.1 –/– (Null)</th>
<th>ACE.2 –/– (Tissue Null)</th>
<th>ACE.3 –/– (Liver ACE)</th>
<th>ACE.4 –/– (KAP ACE)</th>
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<tr>
<td>Plasma ACE</td>
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<td>Null</td>
<td>Liver, ↓ ↓ kidney</td>
<td>Kidney &lt;1% normal</td>
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<tr>
<td>Plasma ANG II</td>
<td>Null</td>
<td>~1/3 Normal</td>
<td>Normal</td>
<td>ND</td>
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<td>Plasma ANG I</td>
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<td>Blood pressure</td>
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<td>Serum creatinine</td>
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<td>Hematocrit</td>
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All comparisons are to littermate wild-type mice. ACE, angiotensin-converting enzyme; KAP, kidney androgen-regulated protein; ND, no data.
REFERENCES


