ACE knockout mice—lessons for adult nephrology

Kenneth E. Bernstein

Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia, USA

Introduction

The creation of ‘knockout mice’ resulted from the convergence of two lines of research [1]. In the United States the research groups of Mario Capecchi and Oliver Smithies were independently studying DNA recombination [2,3]. They found that if DNA were inserted into cells, a small percentage of the cells would substitute the inserted DNA for the equivalent portion of DNA normally present within the genome of the cell. In England, Martin Evans discovered that cells taken from the inner cell mass of a mouse blastocyst could be cultured in vitro [4]. These cells (now termed ES cells) remain pluripotential in the sense that when reinjected into a blastocyst, ES cells will reassociate with the inner cell mass and give rise to a chimeric mouse composed of tissues derived from both the injected (ES) cells and the blastocyst. If the chimerism of a male mouse includes the testes, then some sperm will carry genetic material derived only from the ES cells. The use of targeted homologous recombination in embryonic stem cells is the basis for creating knockout mice; disruption of a gene in an ES cell leads to an animal with that same gene disrupted in all tissues.

In collaboration with Dr Mario Capecchi, my group studied mice lacking angiotensin-converting enzyme (ACE) [5]. The first strain of animals we prepared was completely null for ACE expression; ACE activity was absent from circulating plasma and from organs such as the lung and the kidney. Also a unique isozyme of ACE produced by male germ cells, testis ACE, was also not produced by these knockout mice.

In analysing ACE knockout mice, we compared these animals to wild-type mice (animals with two copies of the ACE gene (+/+)) and heterozygous mice (animals with one functioning ACE gene (+/−)). The ACE plasma activity of heterozygous animals is approximately 60% that of wild-type mice, but these animals are phenotypically indistinguishable from wild-type mice. In contrast, ACE knockout mice have a phenotype characterized by decreased blood pressure, renal abnormalities, and a deficit of male reproductive capacity.

Blood pressure

We assessed the blood pressure using a computerized instrument that accurately and reproducibly determines tail-cuff systolic blood pressure. Male and female wild-type mice (Figure 1, +/+ ) have systolic blood pressures of roughly 110 mmHg. In comparison, mice lacking ACE presented with systolic blood pressures of 73 mmHg (Figure 1, −/−). Mice are similar to humans in that the blood pressure of individual animals will vary around a group mean. The effect of eliminating ACE activity was such that no animal in the ACE knockout group presented with a blood pressure that

![Blood Pressure Graph](https://academic.oup.com/ndt/article-abstract/13/12/299/1808670/121206311198670/1)

Fig. 1. Systolic blood pressure. The systolic blood pressures of male and female wild-type (+/+), heterozygous (+/−) and knockout (−/−) mice were determined. Each data point represents the average for one mouse as determined by 120 separate tail-cuff pressure measurements over 4 consecutive days. Male and female wild-type mice averaged 110.1 ± 1.9 mmHg while knockout mice averaged 73.3 ± 1.7 mmHg.

Correspondence and offprint requests to: Kenneth E. Bernstein MD, Professor, Department of Pathology, Rm. 7107A WMB, Emory University, Atlanta, GA 30322, USA.
exceeded the lowest blood pressure recorded in the wild-type mice. Studies by other groups have demonstrated that mice lacking either angiotensinogen or receptors for angiotensin II present with an equally low systolic blood pressure [6-8]. The conclusion is that mice cannot compensate for the lack of a functional renin–angiotensin system; its absence results in a profound reduction of systolic blood pressure.

When our group first generated ACE knockout animals, we observed that these animals were unable to effectively concentrate urine. Histological examination of renal architecture demonstrated that ACE knockout animals lacked the normal development of the renal medulla and papilla, thus providing a histopathological explanation for the inability of the mice to concentrate. Surprisingly, these animals, with a profoundly low systolic blood pressure, demonstrated a marked thickening of the small arteries and arterioles within the kidney. This vascular pathology was strictly limited to renal blood vessels. The phenotype of renal medullary underdevelopment and renal vascular thickening has also been noted in mice unable to produce angiotensinogen or receptors for angiotensin II.

Mammals produce a unique isozyme of ACE, termed testis ACE, within developing male germ cells. This isozyme is fully catalytic but contains only one of the two catalytic domains present in the ACE isozyme made by somatic tissues. The ACE knockout animals were engineered so that they lacked testis ACE. While testis histology was normal and sperm motility was indistinguishable from that of wild-type mammals, the ACE knockout males demonstrated a very significant reduction of male fertility. When these animals were mated with superovulated, wild-type females, litter size was on average three pups. This compares to wild-type males, which under similar circumstances sired litters of about 10 offspring. Thus, while the physiological action of testis ACE is not fully understood, it appears necessary for full male reproductive capacity.

Tissue ACE

The majority of ACE enzymatic activity is found associated with tissues such as the lung and the kidney. The relative importance of tissue-bound ACE versus plasma ACE has been a subject of some debate. In part this issue was addressed through our analysis of a second line of ACE knockout mice termed ACE.2. Because of an unusual pattern of RNA splicing, these animals have approximately 34% normal ACE enzymatic activity within plasma [9]. However, the mice completely lacked tissue ACE activity. Thus, ACE.2 mice were a model to investigate the importance of tissue ACE. The results were very clear in that this second strain of knockout animals, despite having significant ACE activity within plasma, had a phenotype very similar to that of the knockout mice completely lacking all ACE. For example, ACE.2 mice presented with a systolic blood pressure that was indistinguishable from the very low systolic blood pressures observed in the complete ACE knockouts (Figure 2). The low systolic blood pressure was observed despite the ability of these animals to produce significant quantities of angiotensin II within plasma. These data strongly argue that the ACE enzyme activity bound to tissues is the critical participant in regulating the blood pressure of mammals.

The major difference between the ACE.2 mice and those mice completely lacking ACE activity was the renal pathology. The ACE.2 mice presented with a much milder form of the renal medullary and papillary underdevelopment. Indeed, in the majority of these animals, renal medullary development was approximately equivalent to that of wild-type mice. However, despite a relatively normal renal architecture, the ACE.2 mice were unable to effectively concentrate urine (Figure 3). In fact, when we correlated the ability to concentrate urine with renal pathology among individual animals, we observed examples of mice with perfectly normal renal medulas that were functionally equivalent to the ACE null mice in their inability to concentrate. A similar discordance between a preserved (normal) renal architecture and a concentrating defect was independently observed in mice lacking the AT1 receptor [10]. These studies imply that the ability to generate angiotensin II locally within the kidney plays

![Fig. 2. Systolic blood pressure of ACE null and ACE.2 mice. The systolic blood pressures of male and female wild-type (+/+), heterozygous (+/−) and knockout (−/−) mice were determined as in Figure 1. Data from ACE null mice are represented as triangles (△) while data from the ACE.2 mice are closed circles (○). The ACE.2 knockout mice have blood pressures that are virtually identical to mice lacking all ACE.](https://academic.oup.com/ndt/article-abstract/13/12/2991/1808670/1998)
osmolality shows that the ACE.2 knockout mice produce volumes vs heterozygous (A) and knockout (•) mice during 24 h. Water was unavailable for 30 h beginning 6 h before the collection. A plot of volume vs osmolality shows that the ACE.2 knockout mice produce large amounts of a dilute urine.

Fig. 3. ACE.2 Urinalysis. Urine was collected from wild-type (O), heterozygous (△) and knockout (•) mice during 24 h. Water was unavailable for 30 h beginning 6 h before the collection. A plot of volume vs osmolality shows that the ACE.2 knockout mice produce large amounts of a dilute urine.

a critical role in the ability of mice to properly concentrate urine.

Conclusion

The work from my laboratory is one part of an effort by several different research groups to use knockout technology to study individual components of the renin–angiotensin system [10–17]. Collectively, this work shows that mice lack the ability to compensate for a non-functional renin–angiotensin system. In the complete absence of angiotensinogen, ACE, or receptors for angiotensin II, mice present with systolic blood pressures approximately 35 mmHg below that observed in wild-type mice. Such an effect is profound and emphasizes the importance of the renin–angiotensin system in normal cardiovascular function and fluid balance. Surprisingly, the lack of a renin–angiotensin system also leads to a developmental defect in the kidney characterized by the underdevelopment or atrophy of the renal medulla and papilla. The exact pathophysiology of this lesion is not understood. Relevant potential aetiologies include renal abnormalities of blood flow, oxygen delivery, and urine haemodynamics. Another possible aetiology may be the necessity for the local generation of angiotensin II during the growth and development of the postpartum kidney. The role of the renin–angiotensin system within the kidney is not limited to developmental functions. It appears that even with normal renal histology, rodents must generate angiotensin II locally to concentrate effectively. Finally, the ACE knockout mice have demonstrated that the testis ACE isoyme, produced by developing male germ cells, is important to normal male reproductive function; animals lacking this isoyme produce fewer and far smaller litters than wild-type mice. In a sense, this is one of the most important functions of ACE in that a lack of testis ACE cripples the reproductive competitiveness of these animals.

The use of knockout technology allows us to investigate the phenotype of an extreme situation, the complete functional absence of a renin-angiotensin system. While this is very useful in demonstrating the effects of the renin–angiotensin system on fluid haemodynamics, our results must be judged with caveats concerning how conclusions are applied to human biology. For instance, what local role does angiotensin II play in the human concentrating mechanism? Also complex is the role that components of the renin–angiotensin system play in clinical human hypertension. [19] Why animals lacking ACE develop a lesion of the renal medulla is not known and is an active area for ongoing investigation. We wish to emphasize that this phenotype is found only with a complete dysfunction of the renin–angiotensin system. Finally, human males produce testis ACE with an expression pattern virtually identical to that of mice. It is reasonable to suppose that the critical functionality of this isoyme in mice is very relevant to its role in humans.

References

10. Itó M, Oliverio MI, Mannon PJ et al. Regulation of blood pressure by the type 1A angiotensin II receptor gene. Proc Natl Acad Sci USA 1995; 92: 3521–3525
Sodium proton exchanger (NHE) isoforms—potential relevance to hypertension and its complications

Leong L. Ng

Department of Medicine and Therapeutics, Leicester Royal Infirmary, Leicester, UK

Physiology and function of the Na⁺/H⁺ exchanger (NHE) isoforms

The Na⁺/H⁺ exchanger (NHE) family of membrane transporters utilize the extra- to intracellular Na⁺ gradient to drive H⁺ efflux from cells. Amongst the essential functions performed by this group of transporters are the regulation of cell volume and pH, a permissive role in cell proliferation and the trans-epithelial transport of Na⁺ [1]. Up to five different isoforms have so far been identified. NHE-1 is present in most types of tissue [2] and has a 'house-keeping' role in regulating cell pH and volume. Other isoforms such as NHE-2, 3 and 4 are present in Na⁺ reabsorbing epithelia such as gut and kidney [2]. In kidney epithelium, the presence of NHE-1 on the basolateral membrane and NHE-3 on the apical membrane is consistent with these proposed functions [3]. The distribution of these exchangers together with other transporters relevant to the reabsorption of Na⁺ are illustrated in Figure 1.

The Na⁺/H⁺ exchanger isoform 1 phenotype in hypertension and its complications

In hypertension, a number of defects in the membrane transport of Na⁺ has been described with a putative role in the genesis or maintenance of the raised blood pressure. Increased cellular NHE activity is one of the most consistent defects described, present in a wide variety of cells in both human essential hypertension and a genetic rat model of hypertension, the spontaneously hypertensive rat (SHR) [4–6]. These changes persist in culture and even in cells transformed into immortal lines [7,8]. The increased NHE-1 activity in these lines is associated with an increase in its phosphorylation [8] reflecting activation of upstream kinase pathways such as the mitogen-activated protein kinase (MAPK) pathway [9] which are crucial to the regulation of cell proliferation or hypertrophy. Such altered NHE-1 activity occurs concurrently with increased cellular proliferation [4,7–9] and increased sensitivity to agonist stimulation [10], leading to the conclusion that this particular phenotype may be related to an upregulation in the signalling pathways upstream of MAPK [9] and possibly involving pertussis toxin sensitive G proteins [11].

Increases in cellular NHE-1 activity have also been associated with left ventricular hypertrophy in hypertensive patients [12]. Furthermore, a link to diabetic nephropathy, particularly those with a family history of hypertension, has also been documented [13]. Such patients with diabetic nephropathy are susceptible to left ventricular hypertrophy. If this NHE-1 phenotype reflected an upregulation of upstream kinase signalling pathways such as the MAPK pathway, it may account for the hypertrophic (myocardial) or cell proliferative (glomerular mesangial) responses that are observed in some patients with hypertension or diabetes, leading to cardiovascular hypertrophy. The documentation of p90 ribosomal S6 kinase (p90rsk) as capable of phosphorylating NHE-1 [14] provides an explanation for the association of this NHE-1 phenotype with the increased proliferative responses, since p90rsk is immediately downstream of MAPK. Thus, this cluster of observations (including increased NHE-1 activity and phosphorylation, increased sensitivity to agonists and increased cell proliferation) may constitute a 'proliferative phenotype' conferring susceptibility to cardiovascular remodelling. Such a scheme is summarized in Figure 2 where the amplified step(s) would be upstream of MAPK.

Although increases in NHE-1 activity in the proximal tubule would not directly lead to altered Na⁺ reabsorption due to the predominant basolateral localization of NHE-1, this may have indirect effects on...