Hypertension, Kidney, and Transgenics: A Fresh Perspective

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I. Introduction 710
II. Control of Blood Pressure by the Kidney 710
   A. Role of the kidney in hypertension 710
   B. Impaired renal sodium excretion as a risk for hypertension 710
   C. Vascular-dependent hypertension 711
III. Additional Hypertension Risk Factors 712
   A. Environmental 712
   B. Ontogenic 712
   C. Prenatal programming 712
   D. Genetic 713
IV. Sodium Balance 714
   A. Sodium transport 714
   B. Tubuloglomerular feedback 715
   C. TGF and the renin-angiotensin-aldosterone system 715
V. Evaluating Candidate Genes 716
   A. Transgenesis by microinjection 716
   B. Gene targeting 717
   C. RNA interference 718
VI. Mendelian Hypertension 718
   A. Mineralocorticoid hormone 718
   B. Mineralocorticoid receptor mutations 719
   C. Mutations altering renal transport proteins 720
VII. Essential or Multigenic Hypertension 723
   A. Renin-angiotensin system 723
   B. Natriuretic peptides and receptors 727
   C. Endothelin system 728
   D. Nitric oxide signaling pathways 729
   E. Kallikrein-kinin system 730
   F. The dopaminergic system 732
   G. Cyclooxygenase pathway of arachidonic acid metabolism 732
   H. Other potential contributing factors 733
VIII. Perspective 734

Mullins, Linda J., Matthew A. Bailey, and John J. Mullins. Hypertension, Kidney, and Transgenics: A Fresh Perspective. Physiol Rev 86: 709–746, 2006; doi:10.1152/physrev.00016.2005.—In this review, we outline the application and contribution of transgenic technology to establishing the genetic basis of blood pressure regulation and its dysfunction. Apart from a small number of examples where high blood pressure is the result of single gene mutation, essential hypertension is the sum of interactions between multiple environmental and genetic factors. Candidate genes can be identified by a variety of means including linkage analysis, quantitative trait locus analysis, association studies, and genome-wide scans. To test the validity of candidate genes, it is valuable to model hypertension in laboratory animals. Animal models generated through selective breeding strategies are often complex, and the underlying mechanism of hypertension is not clear. A complementary strategy has been the use of transgenic technology. Here one gene can be selectively, tissue specifically, or developmentally overexpressed, knocked down, or knocked out. Although resulting phenotypes may still be complicated, the underlying genetic perturbation is a starting point for identifying interactions that lead to hypertension. We recognize that the development and maintenance of hypertension may involve many systems including the vascular, cardiac, and central nervous systems. However, given the central role of the kidney in normal and abnormal blood pressure regulation, we intend to limit our review to models with a broadly renal perspective.
I. INTRODUCTION

Primary or essential hypertension is defined as high blood pressure where no obvious secondary causes, such as renal disease, adrenal tumor, drug therapy, or diabetes, have been identified. Given the large number of environmental, behavioral, and genetic factors that can potentially influence blood pressure, essential hypertension covers a wide range of underlying causes, and although it would be extremely useful to subdivide essential hypertension for the purposes of treatment, the parameters for doing so are not clear-cut (41). Given the polygenic nature of blood pressure homeostasis, any change due to mutation is likely to be redressed by feedback, complementary action, or change in some other control mechanism, in an effort to return blood pressure to normal. The genetic complement of an individual may determine his/her ability to respond to such change, or to impinging environmental factors. It is only when the balance is sufficiently disturbed, when checks and balances fail to counteract the perturbation, that essential hypertension results. Thus the presenting picture can often give little clue to the true underlying cause or causes of the hypertension.

II. CONTROL OF BLOOD PRESSURE BY THE KIDNEY

Long-term regulation of mean arterial blood pressure (MABP) is intimately associated with extracellular fluid volume (ECFV) homeostasis, which itself is determined by sodium content. Sodium balance, i.e., the equalizing of sodium intake by sodium output, is critical to ECFV, and the kidneys, as the principal route through which sodium is eliminated from the body, are therefore central to the long-term stability of MABP. Guyton’s “renal-body fluid feedback” hypothesis used a systems analysis approach to demonstrate the primary importance of the kidney. Systemic vasoconstriction could not induce sustained increases in MABP if kidney function was normal (102). Kidney perfusion studies, exemplified in renal function curves (Fig. 1; see Ref. 100 for review), show that a rise in MABP (or renal perfusion pressure) is matched by increased renal excretion of sodium, or pressure natriuresis, which reduces ECFV and cardiac output, and returns MABP to normal. (Fig. 1, point A). In other words, the kidney strives to protect against perturbation from the equilibrium set point, and sodium balance is thus restored by a feedback system displaying infinite gain (101). Likewise, if MABP falls below the equilibrium point, the resulting antinatriuresis increases ECFV and MABP.

In vivo, the renal function curves can be modulated by both neuronal and endocrine factors, one of the most powerful being the renin-angiotensin system (RAS). The role of volume and pressure natriuresis and the impact of the RAS on these responses have recently been reviewed (32).

A. Role of the Kidney in Hypertension

If the Guyton hypothesis is valid, hypertension results from either a failure to increase sodium output in response to an increase in intake (i.e., a failure to shift the renal function curve to the left to produce a higher level of excretion at any given pressure; Fig. 1, point B) or a shift in the renal function curve to the right so that a higher equilibrium pressure is required to match sodium output to intake (Fig. 1, point C). All forms of hypertension are predicted to be a consequence of abnormal pressure natriuresis responses (107); blood pressure homeostasis is sacrificed to preserve sodium balance.

In several forms of hypertension the underlying cause of impaired renal natriuretic function is easily identified. Structural changes to the kidney, such as loss of functional renal mass, or a narrowing of the renal arteries (stenosis), can impede salt excretion and increase the risk of hypertension, especially when combined with increased salt intake, as demonstrated by the one-kidney, deoxycorticosterone acetate rat model and by Goldblatt’s two-kidney, one-clip model (18). In some cases, a clear underlying genetic defect can be identified. All of the Mendelian hypertensive disorders arise from mutations in genes encoding proteins directly or indirectly involved with renal sodium handling [see review by Lifton et al. (192) and below].

B. Impaired Renal Sodium Excretion as a Risk for Hypertension

The kidney is usually histologically normal in the early stages of essential hypertension. Nevertheless, a
wealth of data, obtained from both humans and experimental models, suggest that an inadequacy in terms of sodium excretion is a risk factor for essential hypertension.

A variety of approaches have found that an inability to excrete sodium leads to increased blood pressure in humans and experimental animals (382). On intravenous infusion of saline, renal sodium excretion is markedly blunted in patients with essential hypertension (209). In a subset of essential hypertensive patients, the “salt retention” is associated with impaired pressure natriuresis response (208). Evidence for the causal link between high salt intake and high blood pressure has been reviewed elsewhere (225).

Cross-transplantation of kidneys between normotensives and hypertensives have provided strong evidence that the kidney plays a key role in primary hypertension (98). Studies in humans show a normalization of blood pressure in six hypertensive patients who, following bilateral nephrectomy, received kidney transplants from normotensive cadaver donors (63). These patients, in whom high blood pressure was resistant to a four-drug antihypertensive treatment, showed a prolonged (~4 yr) lowering of MABP without the need for therapeutic intervention. Conversely, it is noted that the incidence of hypertension in transplant recipients correlated strongly with the familial incidence of hypertension in the donor’s family (99).

Several independent groups performed rodent cross-transplantation studies in the 1970s. Dahl’s original findings (65), confirmed later in a number of studies (119, 227, 279, 313), found that on a 0.3% salt diet, blood pressure was “determined by the genotype of the donor kidney rather than by the genotype of the recipient.” Interestingly, the insertion of a control kidney into a DS rat did not prevent blood pressure increases, evoked by a high-salt diet (8%), indicating that extrarenal factors also exert a significant influence on MABP. One possible criticism of these experiments is that they demonstrate the effect of transplanting a kidney already damaged by exposure to sustained hypertension. This issue was addressed in young, Milan hypertensive (MH) rats, studied before the onset of hypertension. Insertion of a normotensive control kidney into a bilaterally nephrectomized MH rat prevented development of hypertension, whereas insertion of an MH kidney into a control rat induced chronically elevated MABP (83). Likewise, cross-transplantation of kidneys from spontaneously hypertensive (SHR) rats, given life-long antihypertensive therapy by angiotensin converting enzyme (ACE) inhibition, and never therefore exposed to high perfusion pressure, conferred hypertension on the genetically normotensive recipient (278).

The studies described above suggest that 1) blood pressure can be set by the kidney and 2) the renal defect is genetically determined. Congenic approaches have been used to localize the genomic region responsible for setting of blood pressure by the kidney. For example, congenic SHR rats carrying a segment on chromosome 1 from the normotensive Brown-Norway rat have markedly lower blood pressures than noncongenic SHR rats (55). Elegant cross-transplantation studies between progenitor SHR rats and the congenic strain revealed that the Brown-Norway fragment of chromosome 1 lowered blood pressure. It is important to note that the hypotensive effect was observed whether the fragments were present renally or extrarenally, indicating again that other factors exert powerful influences on MABP (57).

C. Vascular-Dependent Hypertension

It can be difficult to envisage a central role for the kidney in the onset of hypertension, since gross renal abnormalities are mostly absent in the early stages of the disease. Moreover, volume expansion and increased cardiac output would be expected if blunted natriuretic capability plays a primary role in essential hypertension, but neither of these are cardinal features. Guyton’s hypothesis argues that the period during which blood pressure is volume-dependent may only be transitory (104), since elevation of MABP would increase renal salt excretion to restore sodium balance. Failure to return blood pressure to normal is attributed to autoregulatory vasoconstriction in the peripheral vascular beds, triggered locally in response to prolonged exposure to high perfusion pressure. Despite the fact that chronic hypertension, under this model, is maintained by the vasculature, impaired renal sodium excretion remains the initiating event. Data in support of this hypothesis, such as studies showing that prevention of volume expansion following salt loading in DS rats prevents the development of hypertension (95), are reviewed elsewhere (105).

Nevertheless, other studies in salt-sensitive hypertensive models do not find volume expansion to be a key hypertensive event (168, 273). It is known, for example, that an increase in sympathetic nervous system (SNS) activity is often observed in the early stages of hypertension (146). It has been proposed (143) that this increase in sympathetic drive is the initiating hypertensive event. These data suggest that repeated intermittent bouts of sympathetic hyperactivity cause renal vasoconstriction and promote subclinical changes to the renal structure, particularly the afferent arteriole, which in turn leads to altered salt handling (141). Impaired renal sodium excretion persists as a key feature for hypertension but is no longer the initiating event. Instead, the hypertension becomes Guytonian only after the kidney is subjected to repeated ischemic episodes following vasoconstriction and reduced renal plasma flow (142). Moreover, this may be a vicious circle in that small increases in plasma sodium concentration can exert a central pressor effect via activation of both the RAS and SNS (69). For the purposes of the present review, it is unnecessary to reconcile these
remains central to the misregulation of MABP.

III. ADDITIONAL HYPERTENSION
RISK FACTORS

A. Environmental

Apart from age and gender, environmental factors contributing to persistent or pathological changes in blood pressure include poor diet, lack of exercise, increased body weight, stress, and smoking or alcohol intake. Dietary factors thought to have adverse effects on blood pressure include high sodium, especially in combination with low potassium (383). Also, higher caloric intake, reflected in weight gain and increased adipose tissue, correlates strongly with increase in blood pressure. It has been estimated that 60–70% of hypertension is attributable to adiposity, with 5 mmHg increase in systolic blood pressure (SBP) for every 5 kg weight gain (152).

B. Ontogenic

There are several distinct developmental windows during which certain stimuli affect long-term development of the cardiovascular phenotype. For example, short-term treatment of genetically hypertensive rats with antihypertensive drugs, at 5–9 wk of age, has a long-term beneficial effect (118). If hypertension is deferred, then blood pressure elevation, cardiovascular hypertrophy, and end-organ damage are considerably attenuated, compared with rats developing hypertension during the critical period in development (118, 354). Immature rats are highly susceptible to increased salt intake, whilst dietary calcium has antihypertensive effects. Dietary protein intake also affects blood pressure in juvenile rats (395). An in depth review of dietary salt and development of salt sensitivity has been published elsewhere (225).

It is important to study animals in the transitory phase, when blood pressure is developing, in addition to analyzing the established hypertensive phase. Genetic alterations that occur in the latter phase are likely to reflect mechanisms of target organ damage such as left ventricular hypertrophy, rather than the hypertension per se. In this regard, the inducible hypertensive rat model (153), which places time course and extent of hypertensive damage in the hands of the investigator, should prove invaluable.

The recent description of a mitochondrial tRNA mutation being linked to hypomagnesemia, hypertension, and hypercholesterolemia, each of which showed variable penetrance, poses the interesting possibility that loss of mitochondrial function with age might also contribute to age-related increase in blood pressure (379).

C. Prenatal Programming

There has been much debate regarding birth weight as a predictor of future hypertension, stemming from observations that lower birth weight was associated with higher death rate from coronary heart disease and stroke (19). There seems to be a small but consistent inverse relationship between birth weight and later blood pressure: a 1-kg increase in birth weight correlates to a 1–2-mmHg decrease in SBP (78).

In rats, severe sodium restriction during the last week of gestation causes intrauterine growth retardation and subsequently leads to high blood pressure and renal dysfunction in adulthood (21). Treatment of rats with dexamethasone (a poorly metabolized corticosteroid), during the last week of pregnancy, also causes a reduction in birth weight, glucose intolerance, and increased adult blood pressure. Additionally, carbamoxolone (an inhibitor of 11β-hydroxysteroid dehydrogenase type 2, Hsd11b2) treatment in the last trimester leads to postnatal hyperglycemia, increased blood pressure in adult progeny, and tissue-specific increases in glucocorticoid receptor density leading to increased corticosteroid sensitivity (29, 308). Glucocorticoids affect glomerular number and kidney maturation (178) but may also affect blood pressure by altering renal and vasculature catecholamine receptor levels, by inducing growth factors such as insulin-like growth factor (IGF), by indirect effects on carbohydrate and fat metabolism, or through increased vasooconstriction via regulation of catecholamines, nitric oxide, and angiotensin synthesis (30).

The level of protein intake in pregnant rats is inversely proportional to blood pressure in adult offspring (207), although blood pressure can be normalized by treatment with ACE inhibitors at 8 wk of age. Protein restriction causes placental enlargement and is characterized by decreased Hsd11b2 activity, and thus reduced placental protection of the fetus against maternal corticosteroids (29). Exposure of the fetus to high glucocorticoid levels may adversely affect the fetal hypothalamo-pituitary-adrenal (HPA) feedback system that regulates adrenal output. This is likely to have an immediate effect on fetal growth but may also reset the fetal HPA axis, with effects persisting into adulthood. Current evidence suggests that key targets for programming include the HPA axis, the glucocorticoid receptor gene, and Hsd11b2 gene expression (30).

In 1988, Brenner et al. (38) suggested that nephron number was inversely related to MABP. This was based on the observation that inbred hypertensive rats (SHR for example) tended to have smaller kidneys and fewer nephrons than normotensive controls. Similarly, a reduction in nephron number has been found in adults with essential hypertension; whether this is cause or effect remains unknown. Low nephron number does not initially manifest as a reduction in whole kidney glomerular filtration rate, since hyperfiltration of individual glomeruli...
compensates. Unfortunately, glomerular hyperfiltration is associated with accelerated loss of renal function, and a reduction in nephron number reduces the renal reserve, i.e., the capacity of the kidney to cope with injury, etc. Brenner’s hypothesis is that a congenital reduction in nephron number is prenatally programmed, possibly as a consequence of low birth weight or protein content of the maternal diet (200), and this increases the likelihood of developing hypertension in adulthood, especially in the setting of elevated salt intake. Protein restriction in the latter half of pregnancy, when nephrogenesis normally occurs in the rat, has been found to reduce the number of nephrons in the developing kidney by almost half. Both male and female progeny developed salt-sensitive hypertension, although females were less severely affected (381).

D. Genetic

As already mentioned, blood pressure is influenced by a wide variety of physiological systems, that have pleiotropic effects, and interact in complex ways. These include the baroreceptors, which detect acute changes in blood pressure, but are not yet defined at the molecular level; the renin-angiotensin-aldosterone and kinin-kallikrein systems, which influence sodium retention in the kidney and vascular tone; natriuretic peptides produced in response to local increased pressure in the heart and brain; the adrenergic receptor system, which affects cardiac contraction and vascular tone; dopamine receptors, which affect natriuresis; vasodilators such as nitric oxide; and vasoconstrictors such as endothelin. The distinction between primary alteration and secondary adaptive response contributing to long-term high blood pressure may be extremely difficult to discern.

Candidate genes are identified in a number of ways. In some cases, a simple Mendelian form of hypertension (or hypotension) can be identified by pedigree analysis. Detailed linkage analysis may then reveal the chromosome, the subchromosomal region, or even the gene most likely to be involved. Once variants that cosegregate with the hypertensive phenotype have been identified, then detailed sequence analysis may identify the causative genetic mutation. Examples of this include Liddle’s syndrome, caused by a mutation in the β- or γ-subunits of epithelial Na+ channel (ENaC) (110, 315).

When hypertension is the result of a complex genetic trait, the power of linkage analysis is more limited. Hypertension may be the result of small increments in blood pressure due to a number of contributing gene variants. Each of these may vary not only in phenotype but may also be affected by polymorphic allele frequencies, low penetrance, and the epistatic effects of other genes. As a consequence, the positions of the quantitative trait loci may fall below detectable LOD (logarithmic odds) scores, or at best identify very large chromosomal regions.

Genome-wide linkage analyses, which systematically evaluate the human genome for segments containing genes that influence blood pressure, have to be carefully designed (68) and may identify different regions in different populations (172, 385) or even fail to identify any contributing regions (155). These studies suggest that different sets of genes contribute to hypertension in different ethnic groups and that precise analysis is difficult in humans because of their heterogeneous genetic background. Such analyses have been improved by increasing the density of markers, restriction fragment length polymorphisms (RFLPs), single nucleotide polymorphisms (SNPs), and microsatellites, throughout the genome. Recently developed microsatellite databases for the human (58) and the mouse (352) greatly aid whole genome scans.

With the complete analyses of human (221), mouse (248), rat (90), and now chimpanzee (53a) genomes being published, conserved synteny for blood pressure quantitative trait loci (QTLs) will hopefully refine the chromosomal location of blood pressure regulating loci (396). Moreover, comparisons of whole genomes (60) are beginning to reveal highly conserved functional sequences outside the coding sequence of the genes themselves (243). These are short- and long-range cis-regulatory sequences controlling the spatial and temporal expression patterns of the gene or the entire locus (242). Variation in these regions can have as dramatic an effect on gene expression as a mutation within the coding region.

An additional source of candidate genes arises from the study of defects underlying the numerous animal models selected for hypertensive phenotype (276). These include the SHR, the Dahl salt-sensitive rat, and the Milan rat. A wealth of knowledge has been, and continues to be, accumulated for these models, which often prove to be highly complicated. In fact, the selective breeding strategies that generated the models are likely to have “fixed” many allele variants and modifier genes affecting blood pressure homeostasis so that the underlying etiology may not be at all clear. [It should be noted that breeding of strains at different ethnic groups and that precise analysis is difficult in humans because of their heterogeneous genetic background.]

Nevertheless, QTL analyses in animals can be highly informative, because of the large numbers of progeny that can be screened. This phenotype-driven approach (332) capitalizes on natural variation among inbred strains (214, 289). By using crosses between two inbred mouse strains (screening F2 progeny), QTLs associated with blood pressure, heart rate, and heart weight were identified (331).

The recombinant inbred strain platform, generated between the SHR and the Brown-Norway rat (270, 272), has been useful for identifying QTLs for numerous cardiovascular phenotypes including arterial pressure. The development of a new framework marker-based linkage map, which clearly defines the strain distribution patterns (137), will greatly enhance the utility of these recombinant inbred (RI)
strains. An equally useful resource is provided by the panel of consomic rat strains (61), in which an entire chromosome from one inbred strain is introgressed onto the background of a second inbred strain. These can be used to assign traits and QTLs to any given chromosome.

Congenic strains can be rapidly developed using the marker-assisted speed congenic approach (135) to locate the QTL more accurately over narrow regions of the chromosome. Arguably this may disrupt the very genotype-phenotype relationships being sought, but consomics with multiple chromosome substitutions, and double congenic strains, can be used to investigate such gene-gene interactions in the future (217). It must be stressed that the identification of any candidate gene in an animal model does not automatically imply its involvement in hypertension in humans. This may be due to species specificity, a topic that will be revisited later in the review, or simply to a lack of genetic variation in some species.

IV. SODIUM BALANCE

Sodium balance is key to the homeostatic control of blood pressure, and a brief overview of the mechanisms and enzymes involved in renal salt reabsorption is pertinent in relation to Mendelian and essential forms of hypertension, and to put transgenic models in context.

A. Sodium Transport

Sodium is freely filtered at the glomerulus, with ~99% of the filtered load being reabsorbed along the nephron, by an integrated system of ion channels, ion exchangers, and ion transporters (Fig. 2A). In the proximal convoluted tubule, ~50% of filtered sodium is reabsorbed. Although there are ~20 different sodium transporters in the apical membrane, most of these couple to “substrates” (such as amino acids and carbohydrates), and collectively they mediate only ~10% of the proximal tubule sodium reabsorption. The sodium-hydrogen exchanger, NHE3, mediates the majority of Na⁺ reabsorption (Fig. 2B).

The loop of Henle as a whole reabsorbs considerable amounts of sodium (30–40% of the filtered load). It is a heterogeneous nephron segment, consisting of the straight portion of the proximal tubule (pars recta), the descending and ascending thin limbs, and the thick as-

Fig. 2. A: percentage sodium reabsorption over the length of the nephron. Principal mechanisms of sodium reabsorption are shown in the proximal tubule (B), the thick ascending loop of Henle (C), the distal convoluted tubule (D), and the collecting duct (E).

Physiol Rev • VOL 86 • APRIL 2006 • www.prv.org
cending limb (TAL). In the TAL, sodium is reabsorbed (~20% of the filtered load) but water is not, thereby creating a steep osmotic gradient in the medullary interstitium, which permits vasopressin-dependent water reabsorption in the collecting duct. In the TAL, almost all sodium transport results directly or indirectly from Na\(^+\)/H\(^+\)-K\(^+\)-2Cl\(^-\)cotransport (96). Efficient operating of this transporter (NKCC2) requires K\(^+\) to recycle across the apical membrane through a K\(^+\) channel (ROMK) and chloride to exit basolaterally through a chloride channel (CLCNKB; Fig. 2C). Potassium recycling creates a potential difference, which drives the reabsorption of cations through the paracellular pathway. This is especially important for divalent ions as shown by the hypercalciuria of Bartter’s disease.

Sodium reabsorption in the early distal tubule (DCT1 and DCT2) is mediated by the thiazide-sensitive NaCl cotransporter (NCC) (Fig. 2D) and also, to a lesser extent, by sodium-hydrogen exchange (NHE2). The remaining reabsorption is achieved in the collecting tubule and cortical collecting duct via ENaC, and it is this segment in which the fine-tuning of sodium reabsorption occurs, under the control of aldosterone (Fig. 2E).

B. Tubuloglomerular Feedback

The early distal tubule is the site of the macula densa (MD), a collection of specialized epithelial cells able both to “sense” the NaCl concentration of the tubular fluid and to influence filtration at the glomerulus of origin (Fig. 3). This mechanism, called tubuloglomerular feedback (TGF), rapidly stabilizes acute increases in Na\(^+\) delivery by vasoconstriction of the afferent arteriole and reduction in single-nephron glomerular filtration rate (SNGFR): delivery of sodium to the MD is thus held within narrow boundaries, ensuring that the distal tubule and cortical collecting duct (CCD) are not overloaded. Equally important is the increase in SNGFR following a drop in distal Na\(^+\) delivery, which serves to restore Na\(^+\) concentration to levels compatible with the maintenance of K\(^+\) and H\(^+\) secretion. TGF responses are rapid, and the sensitivity of response is dictated by afferent arteriolar tone. Thus TGF is modulated by agents such as angiotensin II (ANG II), nitric oxide (NO), and the eicosanoids and can be reset during chronic perturbations in MD sodium delivery and altered volume status (299). Moreover, studies in inbred hypertensive rat strains show that TGF is inappropriately sensitized during the development of hypertension, i.e., GFR, and thus filtered sodium load, is lower for a given delivery of sodium to the MD than normal, reducing the ability of the kidney to shed sodium (265).

The basolateral membrane of the MD cells is physically separated from the afferent arteriole by the extraglomerular mesangium. Because no direct cellular contact has been demonstrated between them, it is hypothesized that the first event in juxtaglomerular apparatus (JGA) communication is paracrine, with controlled, altered NaCl concentration at the apical membrane of the MD evoking release of a signaling substance from the basolateral side. Recent studies, using the isolated perfused JGA, found channel-mediated release of ATP from the basolateral membrane correlating with luminal NaCl concentration in the physiological range (24, 166). Furthermore, the concentration of ATP in the cortical interstitium responds predictably to inhibition or activation of TGF in vivo (240). Because the afferent arteriole constricts in response to ATP, an effect mediated by P2X1 receptors, it has been suggested that ATP is the mediator of TGF [see Unwin et al. (353) for review]. Preliminary data, however, find that P2X1 receptor knockout mice display normal TGF responses (297), despite having impaired pressure-induced autoregulation in the afferent arteriole (133). Gene targeting experiments support the notion that P1 (adenosine) rather than P2 receptors mediate TGF; in vivo responses are absent in the majority of tubules from mice lacking A1 receptors (333). It is thus proposed that hydrolysis of extracellular ATP to adenosine is a prerequisite for TGF, since pharmacological blockade of hydrolysis (277) or genetic ablation of 5’-eectonucleotidase (46) attenuates the TGF response.

C. TGF and the Renin-Angiotensin-Aldosterone System

The intimate connection between TGF and the RAS is shown by cessation of TGF response in mice lacking angiotensin AT\(_{1A}\) receptors (300). The RAS can certainly...
modulate TGF but represents a distinct mechanism for regulating sodium excretion depending on the requirements of systemic sodium balance. Thus, if enhanced sodium delivery to the MD is maintained, then renin secretion is reduced, local ANG II levels fall, and SNGFR rises to promote sodium excretion. Likewise, reduced salt levels in the TAL lead to release of renin. Increased renin levels lead to increased production of ANG II, which acts on the adrenal glomerulosa cells, via a G protein-coupled receptor, to increase aldosterone synthase activity, and thus aldosterone secretion. Aldosterone targets the mineralocorticoid receptors in the distal nephron, and ultimately causes an increase in sodium reabsorption and potassium secretion. Any mutation that causes sustained salt retention concomitantly increases water retention and will thus raise blood pressure. Likewise, any mutation that causes salt wasting will lead to hypotension.

V. EVALUATING CANDIDATE GENES

Before discussing the identities of numerous candidates, we first overview the strategies used to assess their probable contributions (91). Initially, circumstantial evidence, such as the association of altered protein expression or function of naturally occurring variants with altered phenotype, may provide strong support. High-throughput gene expression profiling has emerged as a powerful tool in this respect. For example, microarray analysis of a rat strain (derived from Wistar-Kyoto and stroke-prone SHR rats), congenic for a region of chromosome 2, revealed significant reduction in glutathione-S-transferase mu-type 2, a gene involved in oxidative stress defense, which parallels the observed decrease in systolic and diastolic blood pressures (216).

Ultimately it is desirable to alter the expression of a candidate gene, to ascertain or confirm its mode of action, and the pleitropic effects, including hemodynamic parameters, which might result from altered expression. Detailed analysis of transgenic models may reveal the presence of previously unidentified modifier loci, which attenuate or exacerbate the phenotype, on different genetic backgrounds.

Genetic modification can be achieved in a number of ways, including microinjection and homologous recombination. Each strategy is detailed in the following sections.

A. Transgenesis by Microinjection

Transgenesis by microinjection generally leads to overexpression of the transgene. A transgene construct is microinjected into the pronucleus of a fertilized oocyte (see Fig. 4), which is then placed in the oviduct of a pseudopregnant host and allowed to develop to term. Resultant pups are screened for potential founders, from which transgenic lines can be derived. The transgene integrates at random in the genome; therefore, every transgenic line is unique. The researcher has no control over the number of copies inserted, which are present in addition to the endogenous copies of the gene. The site of insertion can have a profound effect on expression of the transgene (114). If it inserts in a silent region of the chromosome, then expression may be completely suppressed. On the other hand, insertion near a strong enhancer may result in aberrant patterns of expression. Integration within a gene may insertionally inactive it, causing a completely unrelated and unexpected phenotype.

Despite these caveats, transgenesis by microinjection is an attractive option. Effects from sequences flanking...
the insertion site can be largely overcome by increasing the size of the transgene construct. Bacterial artificial chromosomes (BACs) and P1 artificial chromosomes (PACs), vectors capable of carrying inserts up to 300 kb, are used routinely to generate transgenic animals. The long segments of DNA upstream and downstream of the gene of interest act as a buffer against effects from flanking sequences. As a bonus, the transgene construct is more likely to include both short-range and long-range control elements, making correct tissue-specific and developmentally specific transgene expression more likely. The BAC or PAC may also include other genes, and the researcher must be aware of any interactions between these genes, or their control elements, and the gene of interest, or again unexpected phenotypes may occur (233, 241).

With the development of homologous recombination systems in Escherichia coli (184, 236), BACs and PACs can be easily engineered to carry reporter genes, or to place the transgene under the control of inducible or conditional promoters. There are sophisticated systems for conditionally turning genes on or off by dietary changes (153), or antibiotic induction or suppression [e.g., using tetracycline/doxycycline (394)]. Strategies using the Cre-lox recombination system (237) or FRT-flp technology (281) have proven particularly useful. A detailed discussion is beyond the scope of this review, but it is worth pointing out that many of these strategies require the generation of two independent transgenic lines. For example, one line might carry the transgene of interest, flanked by loxP sites (floxed) to allow conditional knockout, or have an upstream floxed stop cassette to allow conditional knock-in. The second line would carry tissue-specifically or developmentally expressed Cre recombinase. Crossing the two lines would result in conditional Cre-mediated recombination yielding the desired alteration in gene expression.

Reporter strains, which express β-galactosidase (325) or enhanced green fluorescent protein (156, 293) following Cre recombinase activation, indicate the tissue specificity and extent of recombination for any given Cre strain. This should be tested, since Cre expression may be leaky, ectopic, or mosaic. The latter observation has been used to advantage, recently, for generating Cre-mediated germline mosaicism (124, 187). It is also worth noting that the loxP footprint left behind after Cre recombination may affect gene expression. (See detailed reviews of conditional transgenic technologies in Refs. 35 and 290.)

B. Gene Targeting

Gene targeting and gene knockout is achieved when a modified copy of the target gene is introduced into embryonic stem (ES) cells (Fig. 5). Under appropriate selective pressure, the transgene replaces the endogenous gene by homologous recombination. The targeted ES cells are introduced into blastocysts, where they have the potential to contribute to all tissues of the developing embryo. The resultant chimeric pups are bred to screen for germline transmission of the transgene. This technique can be used not only for loss-of-function analysis, but also for replacement of the targeted gene with a reporter, such that the developmental and tissue-specific expression patterns of the endogenous gene can be visualized. An obvious potential drawback to loss-of-function targeting is the possibility of embryonic lethality due to a requirement for gene expression early in development. To overcome this, sophisticated strategies for making transgene expression

![Gene Targeting Flowchart](http://phystrev.physiology.org/)
conditional are available. With the advent of BAC homologous technology, much larger targeting constructs can be engineered, and multiple alterations can be achieved in one round of ES cell recombination (344). High-throughput engineering technology is now established, which can replace precisely any given gene of interest with a reporter, or generate point mutations or deletions (355). Coupled with high-resolution expression analysis, this will be a powerful tool in functional genomics.

C. RNA Interference

RNA interference, using small interfering RNAs (siRNAs), is a powerful new tool for analyzing gene knockdown phenotypes. RNA interference is an evolutionarily conserved mechanism mediated by double-stranded microRNA (miRNA), which targets mRNA for degradation by cellular enzymes, effectively limiting translation by binding to sites within introns, or in the 3′-untranslated region (139). The dsRNA is processed into small duplex RNA molecules (20–25 nucleotides) by an RNase III enzyme called dicer. The siRNAs interact with an RNA-induced silencing complex, leading to sequence-specific binding and cleavage of the target mRNA. Introduction of siRNA molecules directly into somatic mammalian cells circumvents the nonspecific response against larger dsRNA molecules (53).

At present, knockout technology is not available for the rat, since rat ES cells have proven technically difficult to produce. Chemically synthesized siRNAs and short hairpin RNAs (shRNAs) have recently been shown to transiently or stably knock-down gene expression in transgenic mice and, significantly, in rats (113, 189, 218, 257). This technology, together with others discussed in future perspectives, promises to widen the application of transgenic technology to the rat and other species.

VI. MENDELIAN HYPERTENSION

As already noted, where hypertension can be shown to result from mutations in a single gene, exhibiting classic Mendelian inheritance, it has been found to affect, without exception, net renal salt reabsorption (192). Recently, mutation in a mitochondrial tRNA, exhibiting maternal (i.e., non-Mendelian) inheritance, was shown to be the cause of hypomagnesemia, hypertension, and hypercholesterolemia in a large Caucasian kindred (379). The Mendelian forms of hypertension fall broadly into three classes: those that affect levels of circulating mineralocorticoids, those that affect the mineralocorticoid receptor, and those affecting renal ion transport (see Fig. 6). (For a detailed review, see Ref. 192).

A. Mineralocorticoid Hormone

1. Aldosterone synthase

Aldosterone synthase deficiency, arising through either point mutation or microdeletion (261), results in

![Diagram of aldosterone synthase pathway]

**FIG. 6.** The action of aldosterone on the mineralocorticoid receptor leading to salt reabsorption by ENaC. Mendelian forms of hypertension and hypotension arising from mutations in components of the pathway are noted.
severe impairment of salt reabsorption, leading to reduced plasma volume and severe hypotension, and impaired secretion of K⁺ and H⁺ leading to hyperkalemia and acidosis. Mice completely lacking aldosterone synthase (Cyp11b2) have been reported to have a broadly similar phenotype (185): hypotension, abnormally electrolyte homeostasis, and increased urine production, together with high levels of circulating renin and ANG II. They also showed increased cyclooxygenase-2 (COX-2) expression in the macula densa, which was somewhat enlarged (206). Interestingly, all the abnormalities, except for low blood pressure, were substantially corrected on administration of a high-salt diet.

Glucocorticoid remedial aldosteronism (GRA; Ref. 261) occurs when unequal crossing over between aldosterone synthase and the closely related gene 11β-hydroxylase puts aldosterone synthase under the control of adrenocorticotropic hormone (ACTH), which normally controls adrenal 11β-hydroxylase expression and cortisol secretion. Constitutive aldosterone secretion causes sodium retention, increased plasma volume and blood pressure, and increased secretion of K⁺ and H⁺, leading to hypokalemia and alkalosis. As the name suggests, exogenous glucocorticoids completely suppress the aldosterone secretion, through negative feedback of ACTH production.

2. 11β-Hydroxylase deficiency

The complementary hybrid gene resulting from unequal crossover between 11β-hydroxylase and aldosterone synthase places the chimeric gene under the aldosterone synthase promoter (108, 267). The gene is induced by ANG II and K⁺, and its expression is restricted to the zona glomerulosa, leading to 11β-hydroxylase deficiency, hypertension, and congenital adrenal hyperplasia (CAH).

The most common cause of CAH (accounting for 90% of cases) is 21-hydroxylase deficiency, while up to 8% of classic CAH is caused by 11β-hydroxylase deficiency (375). Decreased or absent cortisol secretion stimulates ACTH secretion, which results in accumulation of steroid precursors and increased androgen levels. Patients suffer from masculinization and short adult stature due to precocious pubertal development, and increased levels of deoxycorticosteroid may cause hypertension because of its mineralocorticoid activity. The nonclassic form of 11β-hydroxylase deficiency has milder symptoms and is characterized by missense mutations, which have been shown to affect enzyme activity in vitro (138). Severity of symptoms appears to be determined by the nature of the point mutation or deletion in the gene (171). No transgenic models of 11β-hydroxylase (Cyp11b1) deficiency have been reported to date.

3. Syndrome of apparent mineralocorticoid excess

The syndrome of apparent mineralocorticoid excess (SAME) is autosomal recessive and occurs when the enzyme HSD11B2 is defective. This enzyme normally converts active cortisol (or corticosterone in rodents), which can act at the mineralocorticoid receptor (MR) to inactive cortisone (or 11-dehydrocorticosterone), effectively protecting the receptor from the high circulating levels of cortisol. In SAME, physiological levels of glucocorticoids illicitly activate the MR in the distal tubule. This leads to sodium retention, early-onset hypertension, hypokalemia, and alkalosis, but additionally, suppressed plasma renin and absence of circulating aldosterone are observed due to feedback inhibition. Interestingly, glycyrrhetinic acid, the active ingredient of licorice, inhibits HSD11B2 and induces hypertension, presenting as pseudohyperaldosteronism (358).

A mouse transgenic model, in which Hsd11b2 was knocked out (169), developed early-onset hypertension and demonstrated major features of SAME, including polynephrosis and hypokalemia. Homozygous mutants appear normal at birth, but 50% show motor weakness and reduced suckling, and die within 48 h, presumably related to the severity of hypokalemia. Surviving null animals exhibit enlarged kidneys, associated with distal tubule enlargement due to hyperplasia and hypertrophy of epithelia. Administration of dexamethasone (the synthetic glucocorticoid, which suppresses the HPA axis and hence corticosterone production), which is not a ligand for MR, increases the urinary Na⁺/K⁺ ratios to wild-type levels (see Fig. 7), while electrolyte abnormality is recreated following corticosterone administration (123).

B. Mineralocorticoid Receptor Mutations

1. Pseudohypoaldosteronism type I

Pseudohypoaldosteronism type I (PHA-1) is an autosomal dominant condition caused by loss-of-function mutations in the mineralocorticoid receptor (89). Despite elevated levels of aldosterone, affected patients have severe neonatal salt wasting and hypotension, with hyperkalemia and metabolic acidosis. The MR deficiency is potentially lethal, suggesting that two copies of functional MR are required by the neonate. All cases improve with age, and by adulthood, a normal-salt diet is asymptomatic.

Mineralocorticoid receptor (Nr3c2)-deficient mice die around day 9 or 10 after birth. By day 8, the animals show symptoms of pseudohypoaldosteronism, with high levels of plasma renin, ANG II, and aldosterone, high renal salt wasting, hyponatremia, and hyperkalemia (27, 127). They also exhibit histological changes in the kidney, with enlargement of the macula densa segment of the distal tubule and recruitment of renin-producing cells along the afferent arteriole. Daily subcutaneous injections of isotonic NaCl solution until weaning, followed by continued oral NaCl, rescue the pups (34). Under these conditions, plasma Na⁺ concentration is normal, but the animals...
remain hyperkalemic, their fractional renal excretion of Na\(^+\) (FE\(_{Na}\)) is still enhanced and plasma renin and aldosterone levels remain high. Interestingly, the mRNA abundance of \(\alpha\)-ENaC is reduced by 30% in the kidney (though that of the \(\beta\)- and \(\gamma\)-ENaC subunits is unaltered) and ENaC activity is almost absent. Recently, it has been shown that glucocorticoid treatment restores plasma K\(^+\) and almost normalizes FE\(_{Na}\) (304). This is achieved by increasing the mRNA abundance of \(\alpha\)-ENaC in the kidney.

Interestingly, cardiac-specific, conditional expression of an antisense mRNA of murine MR caused cardiac fibrosis and heart failure in the absence of chronic hypertension or chronic aldosteronism (22). Antisense mRNA could be up- or downregulated by Tet or Dox, respectively, the latter leading to rapid improvement. This model demonstrates the potential to interrogate gene function in specific cell types, rather than by constitutive alteration, which has both systemic and local effects.

As further evidence for the involvement of MR in hypertension, a missense mutation in the mineralocorticoid receptor ligand-binding domain has been shown to cause an autosomal dominant form of hypertension accelerated by pregnancy. The missense mutation renders the receptor activatable by steroids that would not normally bind to MR, including progesterone. Because progesterone levels increase 100-fold during pregnancy, a rapid acceleration of hypertension associated with complete suppression of the RAS is observed (88).

Overexpression of the human mineralocorticoid receptor in mice (directed by its P1 promoter, which is transcriptionally active in all MR-expressing tissues and importantly directs appropriate transgene expression in the distal nephron) leads to alterations in cardiac and renal functions. These mice have a decreased urinary sodium/potassium concentration ratio, consistent with an increased aldosterone action in the connecting tubule and collecting duct, and this is exacerbated on sodium depletion (186).

C. Mutations Altering Renal Transport Proteins

Animals with null mutations in renal NaCl and water transporter genes provide a powerful tool for studying the physiological balance between glomerular filtration rate and tubular reabsorption (296). In general, all Na\(^+\) transport deficiencies cause a permanent reduction in extracellular fluid volume, with symptoms such as increased renin expression and aldosterone synthesis, and a tendency to hypotension. This leads to compensatory activation of Na\(^+\) conserving mechanisms, such as the TGF. If Na\(^+\) balance can be reinstated, then the volume depletion is self-limiting and a steady state is achieved. When sodium balance cannot be achieved, salt wasting and volume depletion would lead to death.

1. Proximal convoluted tubule

The Na\(^+\)/H\(^+\) exchanger, NHE3, is the major pathway for sodium reabsorption in the proximal tubule (see Fig. 2B), and as such, knockout of the exchanger would be expected to markedly impair tubule function and result in renal salt loss. However, despite a significant (50–60%) reduction in proximal tubule fluid reabsorption, NHE3 (Slc9a3) knockouts have only mild volume depletion and hypotension (301, 374). A major compensatory mechanism is the reduction in SNGFR that reduces filtered sodium load to match impaired proximal reabsorption capability or capacity, to the extent that delivery to the end of the tubule segment is comparable to that in controls (197). SNGFR is reduced through the action of TGF. Because sodium delivery to the macula densa is not increased in Slc9a3\(-/-\) mice, chronic volume depletion must cause resetting of the TGF response.

A “targeted proteomics” approach (semiquantitative immunoblotting), profiling sodium transporter expression along the entire renal tubule (40) demonstrated upregulation of both the proximal tubule Na\(^+\)-phosphate co-transporter, and the collecting duct \(\gamma\)-ENaC subunit. Upregulation of NHE2 in the early distal tubule has also been demonstrated (16). Together with the reduced GFR, changes in transporter expression (which may relate to the increased aldosterone and renin levels, Ref. 301) compensate for the loss of NHE3 so efficiently that absolute...
urinary sodium excretion is significantly lower in knockouts than controls (16, 40). The volume depletion and hypotension may relate to salt/fluid loss attributed to an intestinal sodium reabsorption defect, since the knockouts have pronounced diarrhea. In support of this, partial transgenic rescue through restoration of NHE3 expression in the small intestine improves the volume-pressure defects (244).

Aquaporin-1 (AQP1) is a water channel highly expressed in the proximal tubule, thin limbs of Henle, and the vasa recta (363). Aqp1 knockout mice are polydipsic and have moderate hypotension. Like the Slc9a3 knockout mouse, Aqp1 deficiency results in a marked deficit in proximal tubule reabsorption which is compensated for by activation of TGF (298). Loss of Aqp1 in the thin limb of Henle and in the vasa recta disrupts the countercurrent multiplication system and thus knockout mice are unable to concentrate urine in response to water deprivation, even during administration of vasopressin (363).

From these two examples, it is apparent that impaired proximal tubule function results in modest hypotension, despite the quantitative importance of the proximal tubule in renal sodium reabsorption. This is because the TGF loop provides a powerful compensatory mechanism, which limits the severity of the genetic defect. One would predict that an inoperative TGF, coupled with a proximal tubule disorder, would result in severe salt wasting. To investigate this hypothesis, the Aqp1 −/− mouse was crossed with the adenosine A1a receptor knockout mouse, which has a defective TGF. Surprisingly, the double knockouts were able to maintain salt balance, despite having a higher GFR and lower proximal reabsorption than Aqp1 −/− mice (112). This demonstrates the remarkable redundancy of renal sodium transport and illustrates the compensatory power of distal sodium reabsorption.

2. TAL

Mutations in the Na\(^+\)-K\(^+\)-2Cl\(^−\) cotransporter, NKCC2, or any of the genes encoding ion channels required for its operation (ROMK, CLCNKB) cause Bartter’s syndrome (see Fig. 8), characterized by severe polyuria, low plasma potassium, high blood pH, hypercalciuria, proteinuria, and low blood pressure (116). The importance of Nkcc2 (Slc12a1) is demonstrated by the fact that homozygous knockout mice die within 2 wk of birth from severe volume depletion (335). [Animals heterozygous for the mutation show no phenotype (334).] Indomethacin (a potent nonselective COX inhibitor), administered from birth, rescues the phenotype, implicating prostaglandins in the regulation of renal salt excretion. Surviving adults exhibit all the features of Bartter’s syndrome and develop severe hydronephrosis.

Null mutations in the ROMK gene cause type II Bartter’s syndrome in humans, and mouse Kcnj1 knockouts faithfully model this disorder (195, 198). Although the majority of null mutants die within 3 wk of age, survivors have reduced NaCl absorption in the TAL and a marked renal sodium loss despite the reduction in GFR.

![Fig. 8. Syndromes characterized by reduced sodium reabsorption across the latter sections of the nephron.](image-url)
This lack of compensation, which contrasts with proximal tubule mutants described above, may result from impaired TGF. K⁺ channels are required for efficient sensing of luminal sodium by MD cells (356).

Loss-of-function mutations in CLCNkb or its associated protein Barttin produce Bartter’s syndrome types III and IV, respectively (116). Both proteins (Clcnkb being the mouse ortholog) are localized to the mouse TAL, but transgenic models have not yet been reported.

3. Distal convoluted tube

Sodium reabsorption in the DCT occurs via the apical thiazide-sensitive NaCl cotransporter (NCC), mutations of which cause Gitelman’s disease (see Fig. 8). Patients with Gitelman’s syndrome often present at adolescence with hypokalemia, metabolic alkalosis, and mild hypotension (320). In contrast to Bartter’s syndrome, hypocalciuria is observed as a consequence of an increased driving force for Ca²⁺ reabsorption in the DCT. Mice lacking NCC (Slc12a3) have no overt salt wasting phenotype unless sodium restricted (302). A targeted proteomics approach (Slc12a3)-ENaC in targeted deletion of the α-subunit (Scnn1a) die from failure to clear their lungs (128). Mice expressing an α-ENaC transgene on an Scnn1a knockout background have 50% perinatal mortality, and salt wasting, but can compensate for the deficiency as adults (129). Animals surviving to adulthood develop compensated pseudohypoaldosteronism, though aldosterone levels are sixfold higher than normal (371). These observations suggest that Na⁺ malabsorption in the terminal nephron causes severe salt wasting and that compensatory mechanisms for severe volume depletion in the newborn are relatively inefficient under these conditions. Interestingly, when α-ENaC was specifically inactivated in the collecting duct only, knockout animals were completely viable. This points to the connecting tubule as being critical for achieving sodium and potassium balance (288).

When the Scnn1b gene is “knocked down” and mRNA expression is reduced to 1% of wild-type levels in mouse kidney and lung (268), homozygous mutants develop normally on a normal-salt diet. Adults exhibit significantly reduced ENaC activity in the colon and increased levels of plasma aldosterone, but they have normal plasma Na⁺ and K⁺ concentrations, normal blood pressure, and no weight loss. On a low-salt diet, however, the mice develop acute pseudohypoaldosteronism type 1, indicating that β-ENaC is important for Na⁺ conservation during salt deprivation.

Liddle’s syndrome is characterized by early-onset hypertension, hypokalemic alkalosis, suppressed plasma renin activity, and low plasma aldosterone levels. The autosomal dominant syndrome is caused by mutations at the conserved PY motif in either the β- or γ-subunit of ENaC, which delete or modify their cytoplasmic COOH termini, resulting in increased ENaC activity (294), and increased water and salt reabsorption in the renal collecting tubules. The number of channels in the membrane is effectively increased due to their reduced clearance from the cell surface. Normally, a ubiquitin-protein ligase, Nedd4, binds to the PY motif of ENaC subunits leading to ubiquitination and degradation. In cells derived from the mouse collecting duct, it has been shown that Nedd4–2 is the isoform responsible for binding to the ENaC complex and negatively regulating it (150, 151; see Fig. 6). No knockout models of Nedd4l have as yet been published, but in vitro analysis has shown that Nedd4–2 specifically increases amiloride-sensitive Na⁺ current (324), whilst the mutation associated with Liddle’s syndrome (β566X) abolishes the effect of the siRNA.

A mouse model for Liddle’s syndrome has been generated by Cre/loxP-mediated recombination (269). Animals heterozygous for the β-ENaC-mutated allele contain-
ing the floxed neo gene were crossed with hemizygous mice ubiquitously expressing the EIIa-Cre transgene. Mice showing complete excision of the neo gene were then used to generate new mouse lines. Under normal-salt diet, mice heterozygous (L/+ ) and homozygous (L/L) for the Liddle mutation (L) develop normally during the first 3 mo of life. Blood pressure is normal, despite increased sodium reabsorption in the distal colon and low plasma aldosterone, suggesting chronic hypervolemia. Under high salt intake, the Liddle mice develop high blood pressure, metabolic alkalosis, and hypokalemia accompanied by cardiac and renal hypertrophy. This animal model reproduces to a large extent the human form of salt-sensitive hypertension and establishes a causal relationship between dietary salt, ENaC expression, and hypertension.

The earliest effect of aldosterone is to increase ENaC activity without increasing its mRNA or protein levels, suggesting the presence of an ENaC regulator. To identify aldosterone-stimulated gene products that modulate ENaC activity, a subtracted cDNA library was generated from a renal distal nephron-derived cell line (50). With the use of a coexpression assay, a serum and glucocorticoid-regulated kinase, sgk, was found to stimulate ENaC activity sevenfold in Xenopus laevis oocytes. Induction of sgk mRNA by aldosterone was detected in kidney cortex and medulla as early as 30 min after hormonal application, and independent of de novo protein synthesis, suggesting that the response is mediated by occupancy of mineralocorticoid receptor (314). Sequence comparison has revealed a homologous serine-threonine kinase in rats and frogs, suggesting an ancient

VII. ESSENTIAL OR MULTIGENIC HYPERTENSION

Essential hypertension is characterized by high blood pressure without any obvious cause. Instead of single genes showing simple Mendelian inheritance, the pattern of inheritance is not clear-cut, despite a 30% genetic contribution, and suggests that essential hypertension is determined by the actions of multiple genes and results from the unfortunate, random combination of small genetic variations that alone might not be considered harmful. Subdivision of the hypertensive population into more homogeneous genetic subgroups, or intermediate phenotypes, should restrict the number of genes involved in each subgroup. For example, “nonmodulating” hypertension is characterized by inappropriate adrenal and renal vascular responses to ANG II infusion (377), with reduced renal blood flow during high salt intake and reduced aldosterone response during low salt intake. Such subdivision would also allow more appropriate or relevant treatment to be administered.

We have already considered the many strategies for confirming candidate genes in animal models of mono- genic hypertension. We will now review some of the transgenic experiments, which have identified potential contenders for involvement in dysregulation of blood pressure. These are considered within their specific control systems and pathways, but it must be stressed that the latter do not occur in isolation. Interactions between the different pathways are alluded to where pertinent. We have limited our discussions to transgenes that show clear renal versus systemic actions.

A. Renin-Angiotensin System

The aspartyl protease renin specifically cleaves angiotensinogen to produce the decapeptide ANG I. This is cleaved by ACE to yield the active octapeptide ANG II (see Fig. 9A). ANG II acts through angiotensin receptors AT₁ and AT₂ to effect aldosterone production in the zona glomerulosa of the adrenal gland, to affect vascular tone, and to feedback on the juxtaglomerular cells of the kidney, to control renin production. The involvement of components of the RAS in hypertension is undisputed, despite the lack of strong QTL association with primary hypertension, or families with severe mutations. In high-renin hypertension and nonmodulating hypertension, the RAS is inappropriately active, and the effectiveness of ACE inhibitors and AT₁ blockers as antihypertensive agents points to its key role in determining blood pressure. Recently, mutations in RAS genes have been associated with autosomal recessive renal tubular dysgenesis, highlighting their crucial role in kidney development (97), and a strong association between renin intronic dimorphism and essential hypertension has been identified in United Arab Emirate populations (4).

1. Renin transgenics

In the human and the rat, there is only one renin gene. Some strains of mice, such as C57BL/6, also contain
one renin gene (Ren1<sup>c</sup>), whereas others, such as DBA2/J and 129, contain two (Rend<sup>c</sup> and Ren2<sup>d</sup>). The cardiovascular and renal phenotypes of one and two renin gene strains have been extensively studied (199), as have their differential patterns of gene expression (317). The renin locus exemplifies natural variation among inbred strains (214, 289).

The Ren2 transgene was cloned from the 129/Ola strain of mouse, and the first direct demonstration of its effect on blood pressure was its introduction into the rat, which developed fulminant hypertension by 8 wk of age (232). This was surprising, given that the same transgene introduced into a single renin-gene mouse (Ren1<sup>c</sup>) did not cause hypertension and is a classic example of species specificity. The specificity may arise from differences in the kinetics of angiotensin cleavage by renin in the two species (287, 347). In addition to end-organ damage (in heart, mesentery, kidney, and brain), the hypertension is characterized by unchanged or suppressed plasma concentrations of active renin, ANG I, ANG II, and angiotensinogen, but plasma prorenin levels are greatly increased. Although kidney renin is suppressed, mouse Ren2 is expressed at high levels in the adrenal gland, suggesting the involvement of extrarenal RAS in this model. Treatment with ACE inhibitors and AT<sub>1</sub> blockers efficiently reduces the hypertension, suggesting that it is mediated through ANG II.

This rat strain, together with an inducible-Ren2 rat (153), has been extensively studied, to determine the potential pleiotropic effects of the mouse renin transgene. When the transgene was crossed onto a new genetic background, the animals were found to exhibit symptoms of malignant hypertension (sudden rapid increase in blood pressure followed by death). Subsequent breeding onto Lewis and Fischer backgrounds led to the mapping of modifier loci on chromosomes 10 and 17, at or near the Ace and the AT<sub>1</sub> (Agtr1) loci, respectively, which affect susceptibility to malignant hypertension (154). The inducible MH model is proving valuable for studying the transition to hypertension and for discerning the pattern and reversibility of end-organ damage in this model of hypertension (153).

Transgenic rats expressing rat prorenin exclusively to the liver developed heart and renal pathology independent of hypertension, suggesting that prorenin may play a role in development of vascular damage (362). Transgenic rats harboring either the human renin gene or the human angiotensinogen gene do not develop hypertension, because there is strict species specificity in the reaction between renin and angiotensinogen (115). The double transgenic strain reconstitutes an enzymatically functional RAS (86) and has proven a useful experimental model for studying human-specific enzyme kinetics and drug development.

Knockout of the Ren2 gene in a two-renin gene mouse strain (129/Ola) has no discernible effect on blood pressure (309). On the other hand, knockout of Ren1 in a two renin-gene strain causes reduction of blood pressure in females (56), together with characteristic changes in
morphology of the macula densa, and loss of granulation in the juxtaglomerular cells of the kidney. All aspects of the phenotype can be rescued by the introduction of a wild-type BAC carrying Ren2 and Ren1, but not by a BAC in which Ren1 has been insertionally inactivated with a reporter construct before microinjection (234). Knockout of Ren1 in a single renin-gene strain reduces viability, with 80% of the pups dying within a few days of birth, from dehydration. Survivors show significant lowering of blood pressure (20–30 mmHg); undetectable levels of plasma renin, ANG I, and ANG II; increased urine and drinking volume; reduced urinary aldosterone; and altered renal morphology (337, 386). Heterozygous Ren1c+/− mice are indistinguishable from wild type. In the dual renin knockout, vascular hypertrophy is evident (213).

Placement of the GFP reporter downstream of a 4-kb Ren1c promoter visualizes expression patterns of renin in mouse embryonic, extraembryonic, and adult tissues (144, 179). Such reporter constructs can also help to identify short-range control elements (259, 260, 266). The use of much larger transgenes, such as the 160-kb PAC containing the human renin gene (321), have allowed the identification of numerous potential control element binding sites upstream of the renin gene. Detailed analysis of the expression patterns of all genes encoded within the PAC has hinted at the location of longer range locus control elements (241). Three-way sequence comparisons between mouse, rat, and human (90) should confirm those enhancer sequences and transcription factor-binding sites that are conserved between species and are likely to be of major physiological significance.

Transgenesis has been used to address the relative contributions of endocrine and paracrine RAS (181). Recently, animals expressing both human renin and angiotensinogen, specifically in the proximal tubule under an androgen-regulated promoter, were reported to have increased blood pressure following testosterone treatment (67, 180). This is clear evidence that tissue-specific RAS can contribute to the regulation of systemic blood pressure (115). The subject of brain RAS activity remains contentious and is reviewed elsewhere (17, 230).

2. Angiotensinogen transgenics

Genetic linkage between certain variants of the human angiotensinogen gene and essential hypertension (T235 vs. M235; A-6 vs. G-6) is well established (132, 136). The physiological significance of these variants was investigated in transgenic mice by targeting two haplotypes [−6G/235Met (GM) and −6A/235Thr (AT)], as a single copy transgene, to the mouse hypoxanthine phosphoribosyltransferase locus. This allowed direct comparison of the two haplotypes in vivo. Both transgenes exhibit similar transcriptional activity and produce similar levels of hAGT protein in the plasma. When each line was bred to mice expressing human renin, however, only mice carrying the GM haplotype showed a small but significant increase in blood pressure and compensatory downregulation of renin expression, suggesting the haplotypes may affect blood pressure regulation differently (64).

Transgenic mice carrying an additional rat angiotensinogen (Agt) gene have been shown to exhibit hypertension (because angiotensinogen is rate limiting in the mouse, Ref. 162), whereas knockout of angiotensinogen results in hypotension (340). A more elegant demonstration of the importance of angiotensinogen in blood pressure control is the titration of gene copy number through the breeding of targeted gene disruption and gene duplication mouse mutants (see Fig. 9B) (160, 322). Gene duplication was achieved by a modification of gap repair, such that the gene, together with 5′- and 3′-flanking sequences, was tandemly repeated. All animals were genetically identical except for having zero, one, two, three, or four copies of the angiotensinogen gene. Plasma angiotensinogen levels increase progressively from zero, in the zero-copy animals, to 145% of normal in the four-copy animals. [Steady-state Agt levels increase progressively, but not linearly, with Agt gene copy number because the endogenous and duplicated genes are not functionally equivalent (160).] Mice of all genotypes are normal at birth, but most null animals die before weaning. Surviving homozygous null animals exhibit pathological changes in the kidney. Blood pressures of one- to four-copy animals show increases of ~8 mmHg per gene copy, establishing a direct causal relationship between Agt genotype and blood pressure in this species.

Interestingly, Agt(−/−) animals on a C57Bl/6 background can be rescued by saline injection for 7 days after birth. Hydronephrosis develops by day 14, and polyuria by day 30, and the mice are chronically hypotensive. Agt(−/−) pups born to Agt(−/−) mothers develop fetal hydrenephrosis and die of respiratory failure at birth (339), suggesting that maternal RAS may affect structural maturation of fetal kidney and lung.

Major sites of angiotensinogen expression include the liver, kidney, and adipose tissue (64). A transgenic model, with exon 2 of the human angiotensinogen gene flanked by loxP sites, achieved tissue-specific ablation of angiotensinogen using the Cre-loxP recombinase system (delivered by intravenous administration of adenovirus, which mainly infects the liver). A significant acute decrease in circulating angiotensinogen was achieved, within 5 days, paralleled by a fall in blood pressure in animals also carrying the human renin gene (327).

A transgenic model with overexpression of Hsd11b1 (which activates deoxycorticosterone to corticosterone), specifically targeted to adipose tissue (211), exhibited hypertension, in addition to classic symptoms of metabolic syndrome (visceral obesity, hypolipidemia, insulin resistance, and glucose intolerance). The increased local
glucocorticoid levels cause an increase in angiotensinogen and ANG II, which ultimately raises blood pressure. Interestingly, a related transgenic mouse, with Hsd11b1 targeted to the liver, exhibited metabolic syndrome and hypertension without obesity (262).

The effect of angiotensinogen gene titration on homeostatic compensation in other components involved in blood pressure control has been investigated (161). High-throughput molecular phenotyping, using RT-PCR to assess the tissue-RNA levels of 10 genes, revealed both positive and negative responses. A twofold increase in Agt leads to a threefold increase in aldosterone synthase expression in the adrenal gland and a twofold decrease in renal renin expression. Feedback inhibition of renin by ANG II is to be expected and represents a large compensatory adjustment. This is not sufficient to return blood pressure to normal because of the ANG II-induced inositol trisphosphate (InsP3) response. ANG II is generated in the proximal tubule, had a marked reduction in blood pressure, had deficiencies in the RAS and tissue-associated ACE (76). These mice were incapable of forming ANG II in the proximal tubule, had a marked reduction in blood pressure, had deficiencies in the RAS system, and lacked TGF. Blood pressure could be restored by ectopically expressing ACE in the liver, but TGF response was still markedly reduced compared with wild type (111). This again exemplifies the intimate connection between TGF and tissue RAS.

4. ANG II receptor

The physiological actions of ANG II are elicited by specific cell surface receptors, belonging to the large family of G protein-coupled receptors. The receptors can be divided into two pharmacologically distinct types: AT1, which mediates the major actions of ANG II and is inhibited by compounds such as losartan, and AT2, which is inhibited by compounds such as PD123177. In mouse, the AT1 receptors are divided into subtypes AT1A and AT1B, which show differential tissue distribution and regulation, further modulating the effects of ANG II. Knockout of the AT1A receptor (Agtr1a) gene in mice causes a drop in blood pressure in both heterozygotes and homozygotes (134). Blood pressure can be further reduced in the null animals, by administration of losartan, suggesting that the AT1B receptor can contribute to blood pressure regulation in the absence of AT1A receptor (250). Animals null for the AT1A receptor exhibited no TGF response (300) and showed hypertrophy of the JGA (254). Animals doubly homozygous for Agtr1a and Agtr1b knockout have a phenotype similar to Agt knockout and the Ace knockout, with reduced growth, vascular thickening within the kidney, atrophy of the inner renal medulla, reduced blood pressure, and, additionally, no systemic pressor response to ANG II infusion (253, 350). Paradoxically, administration of ACE inhibitor to these animals causes an increase in blood pressure, suggesting that the AT2 receptor opposes the actions of AT1 receptors on blood pressure.

In Agtr1a knockout animals, the already low blood pressure falls even further on a low-salt diet, and their sodium balance becomes negative despite a sixfold increase in urinary aldosterone, indicating that the AT1A
receptor is critical for modulating sodium handling (251) and water excretion (252). The severity of kidney histopathology was found to be variable on a mixed genetic background (129 and C57Bl/6), suggesting the presence of genes that can substantially modify the phenotype of AT1A receptor deficiency. Suitable backcrossing has led to the identification of a modifier locus on chromosome 3, with significant linkage to the development of renal vascular lesions (182).

Gene titration of the Agtr1a gene locus, to yield animals with three and four copies of the gene, has shown that AT1A mRNA and ANG II binding capacity increase in proportion to copy number (182). In females (but not in males), there is a strong positive correlation between Agtr1a gene copy number, blood pressure, and AT1A receptor expression, a significant increase in aldosterone synthase, a positive correlation between kallikrein and AT1A receptor mRNA levels, and an inverse correlation with renin mRNA.

The AT1 receptors are expressed in many tissues, making it difficult to discern their role in particular sites such as the kidney. An elegant strategy to overcome this problem has utilized microsurgery for kidney transplantation between Agtr1a-knockout and wild-type mice (62). Four groups of mice consisted of wild-type donors and recipients (D+R+), wild-type donors and knockout recipients (D+R−), knockout donors and wild-type recipients (D−R+), and knockout donors and recipients (D−R−). Animals in the D+R+ group had normal blood pressure, whereas the D−R− group showed a 35-mmHg reduction in blood pressure. Animals in groups D+R+ and, more surprisingly, in the D+R− group showed a 20-mmHg reduction in blood pressure, clearly demonstrating that regulation of blood pressure by the RAS is mediated, to approximately equivalent extents, by both kidney and systemic specific AT1 receptors. As aldosterone levels are unaffected in the D−R+ group, reduction in BP in this case is probably due to the lack of AT1A receptor action on renal vasculature and/or epithelia (which would normally reduce urinary sodium excretion). Urinary aldosterone levels in the D+R− group were ∼50% of wild type, but aldosterone replacement failed to restore blood pressure, suggesting that a lack of AT1A receptors in the vasculature may account for the reduction in blood pressure in this group. Only groups D−R− and D−R+ were found to be sensitive to a high-salt diet, indicating that salt sensitivity was associated with a lack of renal AT1A receptors. The contribution of AT1A receptors in the central nervous system could not be assessed in this study, because kidney transplantation reduced sympathetic innervation in all groups. However, the marked (10-fold) increase in renin mRNA levels in the D−R− group suggests that below a certain blood pressure, a baroreceptor mechanism stimulates renin production (62). In summary, a clear distinction between systemic and renal AT receptor contribution to blood pressure is apparent.

B. Natriuretic Peptides and Receptors

Natriuretic peptides (NP) are physiological regulators of cardiovascular and renal function. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are released from the atria and the ventricles, respectively, upon stretching of the cardiac myocytes, while C-type natriuretic peptide (CNP) is found mainly in the brain and is thought to act as a neurotransmitter (37). All three peptides are venous dilators, but ANP and BNP also evoke natriuresis.

There are three NP receptor subtypes: NPA, at which ANP is the most potent agonist; NPB, which preferentially binds CNP; and NPC, which binds all NPs with equal affinity. NPA and NPB, both coupled to guanylate cyclase activity, have an overlapping distribution in the kidney, vasculature, and brain. NPC, the most abundant NP receptor (>90%), lacks guanylate cyclase activity and functions as a clearance receptor (212). The high density of NPC in the brush-border membrane of the proximal tubule ensures that filtered NPs are removed from the tubule fluid, and thereby protects the paracrine function of renal NPs. Short-term administration of ANP/BNP reduces MABP by lowering cardiac output and total peripheral resistance. Cardiac output falls due to the combined effect of hemoconcentration (i.e., ANP promotes a redistribution of plasma water into the interstitium, an intrinsically hypotensive event) and suppression of autonomic reflexes that would normally promote a reflex tachycardia to oppose a reduction in MABP (303). The reduction in total peripheral resistance reflects both direct, cGMP-dependent actions on smooth muscle (247) and indirect relaxation due to inhibition of central sympathetic drive (303). ANP/BNP also increase sodium and water excretion by altering renal hemodynamics and tubule sodium reabsorption (25). NPs cause relaxation of afferent arteriole and contraction of the efferent arteriole, thereby increasing SNGFR and filtered sodium load. Additionally, tubular sodium/water reabsorption is inhibited either directly (an inhibitory action on ENaC) or indirectly [antagonism of the effects of antidiuretic hormone (ADH) and ANG II]. ANP also influences volume status through inhibition of both aldosterone and ADH secretion.

I. Natriuretic peptides

Targeted overexpression of ANP to the liver causes a significant reduction in blood pressure (328), indicating that diuresis and natriuresis are not induced. Anp(Nppa)−/− mice have pronounced left ventricular hypertrophy, suggesting that ANP is an important modulator of cardiac function (229). Additionally, homozygous null animals fed
Salt sensitivity in the reduced ANP can lead to salt-sensitive hypertension. Gotes become hypertensive, indicating that genetically activated in these mice (222). On a high-salt diet, heterozygous mice have increased blood pressure, and male mice suggest subtle interactions between NPA and hypertension, which is insensitive to ANP infusion and is salt resistant (194). These mice also have a blunted pressure natriuresis response (163). Gene titration of NPA levels, generating animals with one to four copies of the gene, demonstrate that below normal Npr1 expression leads to a salt-sensitive increase in blood pressure, whereas above normal Npr1 expression lowers blood pressure and protects against high dietary salt (249).

Assessment of the RAS in zero-, one-, and two-copy male mice suggests subtle interactions between NPA and renin (311). Following blood volume expansion, the zero-copy mice show blunted increases in urinary flow and Na+ excretion responses, despite increased arterial pressure, compared with nontransgenic controls, while four-copy animals augment these responses and also have an increased GFR and renal plasma flow (312). The only association between natriuretic receptor peptide clearance genes and hypertension in humans has been shown for a novel promoter variant of NPC. The variant was associated with lower ANP levels and higher blood pressure in obese hypertensives (292).

2. Natriuretic peptide receptors

Knockout of NPC increases the half-life of ANP, and both heterozygotes and homozygotes have a reduced ability to concentrate urine, mild diuresis, and depleted blood volume. The homozygous animals are mildly hypotensive (and unexpectedly have skeletal deformities).

Knockout of the NPA gene (Npr1) results in chronic hypertension, which is insensitive to ANP infusion and is salt resistant (194). These mice also have a blunted pressure natriuresis response (163). Gene titration of NPA levels, generating animals with one to four copies of the gene, demonstrate that below normal Npr1 expression leads to a salt-sensitive increase in blood pressure, whereas above normal Npr1 expression lowers blood pressure and protects against high dietary salt (249).

Assessment of the RAS in zero-, one-, and two-copy male mice suggests subtle interactions between NPA and renin (311). Following blood volume expansion, the zero-copy mice show blunted increases in urinary flow and Na+ excretion responses, despite increased arterial pressure, compared with nontransgenic controls, while four-copy animals augment these responses and also have an increased GFR and renal plasma flow (312). The only association between natriuretic receptor peptide clearance genes and hypertension in humans has been shown for a novel promoter variant of NPC. The variant was associated with lower ANP levels and higher blood pressure in obese hypertensives (292).

3. Guanylin and uroguanylin

Guanylin and uroguanylin are synthesized in the intestine and kidney and appear to function as intestinal natriuretic hormones (stimulating cGMP production via guanylate cyclase C). Ablation of uroguanylin gene expression resulted in impaired ability to excrete an enteral load of NaCl, due to an inappropriate increase in renal Na absorption. The knockout animals were also found to have increased blood pressure (196). Uroguanylin may complement the renal effects of the cardiac natriuretic peptides, through suppression of the TGF, although its effects on glomerular microcirculation are far less than those of ANP (373).

It would be interesting to see the effect of targeted knockout of NPs or their receptors in the kidney, to directly assess their systemic versus renal paracrine functions.

C. Endothelin System

The endothelin (ET) system involves the actions of three peptides, ET-1, ET-2, and ET-3, on two G protein-coupled receptors, ET_A and ET_B (188). Endothelins are formed from large peptide precursors, by endothelin converting enzyme (ECE), in a variety of cell types. ET-1 and ET-3 are detectable in plasma whereas ET-2 appears to be an intrarenal peptide. Both receptors have an extensive distribution, but ET_A predominates in the heart and kidney.

A role for the endothelins in blood pressure homeostasis is advocated by studies in healthy humans in which nonselective receptor antagonism reduces MABP. Receptor antagonism also normalizes blood pressure in various forms of human and experimental hypertension. A polymorphism in ET-1 has been associated with blood pressure levels in overweight people (346).

The receptors are generally coupled to the activation of phospholipase C (PLC), leading to an increase in intracellular calcium. Activation of ET_A receptors leads to vasoconstriction, cell proliferation, and matrix deposition. Activation of ET_B receptors on endothelial cells leads to release of PGL_2 and NO, which inhibit ET-1 synthesis in the vascular wall, by a cGMP-dependent mechanism. ET_B receptor activation thus offsets the vasoconstriction of ET-1, but ET_B does elicit vasoconstrictive actions on the renal circulation.

Administration of ET-1 into the renal artery causes profound, long-lasting vasoconstriction of the renal circulation, and depression of renal blood flow and GFR (through constriction of the afferent arteriole), leading to reduced renal sodium excretion. The renal circulation is particularly sensitive to vasoconstrictive actions of ET. This has important ramifications for both physiology and pathophysiology since subthreshold levels of ET can sensitize the renal vasculature to the actions of other vasoconstrictors (167). ET-1 is secreted by proximal tubular cells, and microinjection of ET-1 into the proximal tubule lumen, and/or exposure of the macula densa to ET-1, caused a significant increase in SNGFR (286). This may reflect a physiological mechanism for activating the TGF. Intrarenal, paracrine endothelins, synthesized in the collecting duct, promote renal salt loss through activation of renal epithelial ET_B receptors and may play a substantial role in blood pressure homeostasis (1). Although systemic effects on glomerular microcirculation are far less than those of ANP (373).
endothelin concentration does not correlate well with either the development or degree of hypertension, the renal excretion of ET-1 (reflecting renal synthesis) does, at least in DOCA-treated rats.

Transgenic mice overexpressing ET-1 are normotensive (suggesting counterregulatory compensation) (121), but chronic overproduction of ET-1 leads to pathological changes in the kidney (consistent with matrix deposition), increased urinary protein excretion, and salt sensitive hypertension (316). When the NO system is antagonized with NO synthase inhibitor (L-NAME) vascular contraction is reduced, indicating its involvement in the ET-1 transgenic phenotype (275).

ET-1, ECE, and ET\textsubscript{A} knockout mice have craniofacial abnormalities and die at birth. Animals heterozygous for the ET-1 (Edn1) null allele are mildly hypertensive (175), reflecting long-term hypoxia, a disturbance in central cardiorespiratory regulation, or an ET\textsubscript{A}/ET\textsubscript{B} imbalance. [ET\textsubscript{B} (Ednrb) +/- animals have raised blood pressure, which is restored to normal by ET\textsubscript{A} inhibitors (28).] A high-salt diet significantly reduces renal ET-1 levels but does not induce salt sensitivity in the ET-1 heterozygous knockout (231). Renal sympathetic nerve activity in anesthetized mice is higher in Edn1 (+/-) mice than in wild-type mice, suggesting that ET-1 normally participates in this reflex and that the baroreceptor is reset at a higher level in ET-1-deficient animals (193).

Cre-lox technology was used to overcome ET\textsubscript{A} knockout lethality, targeting gene deletion to the collecting duct (5). Plasma ET-1 levels were normal in these mice, but urinary excretion of ET-1 was significantly reduced, consistent with a separation of the systemic and renal endothelin systems. On a normal-salt diet, -/- mice were hypertensive, but renal sodium handling and plasma renin activity were normal. Salt loading exacerbated the hypertension through renal sodium retention, which was ameliorated by amiloride. Since control mice doubled their renal excretion of ET-1 during salt loading, and the -/- animals did not, this strongly implicates collecting duct ET-1 synthesis in the normal response to sodium.

Cre-lox technology has also been used to target ET\textsubscript{A} knockout to the heart. Animals develop normally and show normal stress-induced responses to ANG II (158). Likewise, ET\textsubscript{B} receptor knockout mice can be rescued from neonatal lethality by expression of a dopamine \(\beta\)-hydroxylase promoter/ET\textsubscript{B} receptor transgene. Studies on acid balance and NHE3 levels suggest that metabolic acidosis increases ET-1 expression, which increases NHE3 activity via the ET\textsubscript{B} receptor (176). Rats carrying a natural deletion of ET\textsubscript{B} have similarly been rescued and exhibit severe hypertension on a high-salt diet (87). In summary, transgenic models have provided a clear distinction between systemic and renal endothelin functions.

**D. Nitric Oxide Signaling Pathways**

The potent vasodilator NO, produced from the oxidation of \(\ell\)-arginine by NO synthase (NOS) enzymes, regulates a wide variety of cardiovascular and renal functions and is important in both the short- and long-term control of blood pressure (376). Three different forms of the enzyme have been identified: endothelial (eNOS or type 3), neuronal (nNOS or type 1), and inducible (iNOS or type 2) (226), which are differentially expressed in the cardiovascular system and the kidney. Mice with targeted disruptions in each of the Nos genes have been used to provide insights into the role of NO in blood pressure homeostasis (255).

Overexpression of eNOS leads to hypotension and a reduced response to the endothelium-derived relaxing factor NO (246). Mice lacking eNOS are hypertensive (174, 326), partly due to the lack of constitutively active NO, which favors vasoconstriction and leads to an apparent increase in sympathetic tone. Pharmacological inhibition of NOS activity also augments sympathetic-induced vasoconstriction (165, 365), and eNOS (Nos3) null mice have an increased basal vasoreactivity in the pulmonary circulation and markedly elevated, hypoxia-mediated vasoconstriction (77). Inhibition of nNOS or iNOS fails to exacerbate hypertension in Nos3 null mice (164), indicating that there is little compensatory NO production. In fact, selective inhibition of nNOS, either chronically or acutely, evokes a marked reduction in MABP in eNOS knockouts, suggesting that nNOS has a basal pressor action (174). This apparent paradox can be explained by the fact that NO derived from central nervous system nNOS facilitates sympathetic nerve activity (215, 220) and exerts a permissive effect on the baroreceptor reflex (70). An attenuated baroreceptor reflex in eNOS knockout mice means they do not elevate heart rate after \(\ell\)-NAME-induced hypotension (174). Aortic rings, taken from eNOS null mice (126), and to a lesser extent heterozygous mice (79), fail to relax in response to acetylcholine (ACH). The response is restored by transfection with a viral vector carrying the eNOS gene (307), indicating that in large vessels, eNOS mediates ACh-induced vasodilation.

In the kidney, eNOS is expressed in the vasculature and in several segments of the tubular epithelium. Although NO profoundly influences renal circulation, Nos3 null mice maintain renal blood flow (23), due to compensatory vasodilators. In the proximal tubule, eNOS-derived NO exerts a basal inhibition of Na\textsuperscript{+} reabsorption (3), although this is not a consistent finding (372). Plasma renin activity is either normal (23) or inappropriately elevated (310) in Nos3 -/- mice, given the high blood pressure. Renin mRNA in the kidney, however, is consistently decreased (310, 366). The substrate for NO, \(\ell\)-arginine, decreases NaCl absorption by the TAL. Gene transfer of Nos3 (by recombinant adenovirus vector) to
the TAL of Nos3 −/− restores l-arginine-induced inhibition of NaCl transport (256), confirming the importance of eNOS in this process.

Animals lacking eNOS also exhibit insulin resistance and hyperlipidemia, indicating that eNOS may represent a link between metabolic and cardiovascular disease (73). Under normal conditions, Nos3 heterozygotes are normotensive, but they have an accelerated hypertensive response to a high-fat diet (59). The link between eNOS and the metabolic syndrome is further supported by a significant linkage between two polymorphisms in the NOS3 gene and the metabolic syndrome (82). In addition, male Nos3 −/− mice have a markedly reduced life span and develop pronounced cardiac dysfunction with age (190). The reason for this sexual dimorphism is unclear, but recent data suggest that endothelium-derived hyperpolarizing factor (EDHF), rather than NO, is the predominant relaxing factor in female mice (306).

Blood pressure in nNOS knockout mice is normal (238, 357), suggesting that NO derived from this source plays only a minor role in BP management or can be fully compensated for by other vasodilators. In the kidney, nNOS is expressed in the collecting duct and TAL but the highest expression is in the cells of the macula densa, consistent with the known modulatory role of NO on renin release, afferent arteriolar tone and TGF. Despite this, TGF is normal in Nos1 null mice (357), and renal renin levels are comparable to wild type (366).

Although the Nos2 gene is linked with blood pressure in Dahl salt-sensitive rats, its role is unclear. In knockout mice, early hypertension disappears as the mice reach adulthood, and salt loading has no adverse effect (130). Constitutive expression of iNOS is observed in the kidney, especially in the proximal tubules. It remains unclear whether iNOS-derived NO has an inhibitory (106) or stimulatory (372) action on sodium reabsorption. Clearly, more studies are required to reveal the complex relationships between NOS isoforms, NO, and blood pressure control.

E. Kallikrein-Kinin System

Evidence for the importance of the kallikrein-kinin system (KKS) in long-term blood pressure regulation comes from epidemiological studies in which genetically reduced kallikrein activity was associated with the development of high blood pressure even before the onset of clinical hypertension. This suggests an intact KKS may be required to avoid hypertension when additional risk factors are present.

The KKS is a multienzyme system consisting of the substrate kininogen, the activating enzyme kallikrein, and active metabolites, of which bradykinin is predominant (see Fig. 10). The substrate kininogen is encoded by a single gene (in humans), which produces two kininogen products by alternative splicing. Plasma kininogen is synthesized primarily in the liver but also by platelets, neutrophils, and endothelial cells (31). Tissue kininogen, the lower molecular weight product, is synthesized in the peripheral tissues, notably in the kidney, where it is localized to the collecting duct.

There are two bradykinin receptors, B1 and B2, but most known physiological effects are mediated through the B2 receptor: B1 receptor expression is largely induced by tissue injury or inflammation. Kinins are rapidly inactivated by ACE (kininase II) and neutral endopeptidase. The KKS mediates both inflammatory and constitutive responses. During inflammation, plasma KKS increases vascular permeability, leading to edema, and contributes to the fall in blood pressure associated with sepsis. Tissue KKS operates constitutively, controlling...
organ perfusion by evoking vasodilation in the myocardium, kidneys, and other microvascular beds, via increases in endothelial mediators such as NO, cGMP, and prostacyclin (see Fig. 10). Although kinins cause vasodilation of both the afferent and efferent arterioles, the effect on the afferent is more pronounced, and GFR is thus increased. Bradykinin also increases blood flow in the renal medulla through complex interactions with vasoconstrictors such as ANG II (351).

Systemic administration of bradykinin evokes, through activation of B2 receptors, natriuresis and diuresis. This relates both to inhibition of sodium reabsorption in the segments of the medullary collecting duct and reduced medullary hyperosmolarity as a consequence of increased vasa recta blood flow. Bradykinin exerts actions at the JGA, reducing the sensitivity of the TGF response and directly stimulating renin release.

1. Kallikreins

Kallikrein levels are lower in nonmodulating hypertensives than modulating hypertensives or controls (291). Additionally, patients with a loss-of-function polymorphic variant of the human tissue kallikrein gene exhibit arterial dysfunction (15). To assess the role of the kallikrein-kinin system in blood pressure control, human tissue kallikrein was introduced into mice, under the metallothionein promoter. Transgene expression was identified in the pancreas, salivary gland, kidney, liver, and spleen, and animals had significantly reduced blood pressure (370), which was restored to normal by administration of the kallikrein inhibitor, aprotinin. Human tissue kallikrein has been used for gene therapy in several hypertensive rat models, including the Goldblatt hypertensive rat (389), the SHR (392), and the fructose-induced hypertensive rat (393). In all cases, hypertension was significantly reduced. Tissue kallikrein-deficient mice are unable to produce kinins and have marked cardiovascular abnormalities (224). Despite this, blood pressure is normal. The flow-dependent vasodilation of resistance vessels involves TK production of bradykinin, which is attenuated in TK (Klk1b1) −/− mice (26). Kinins have been implicated in the beneficial effects of ACE inhibitors (318).

In a rat model bred for low kallikrein levels (LKR), kallikrein was reduced by 60% in the kidney (although it was increased in the heart and unaltered in pancreas, liver, and salivary glands). LKR exhibited an increased mean arterial blood pressure, polyuria, glomerular hyperfiltration, and reduced sodium excretion, and renal vaso- dilation in response to volume expansion was impaired. The phenotype was attenuated by AT1 blockade, suggesting that a balance between the KKS and the RAS is essential for normal renal function (203). One should remember that animal models bred for a specific phenotype may carry contributing variants at a number of loci.

2. Kinin receptors

The effect of bradykinin B2 receptor (Bdkrb2) knock-out is contentious. Some groups found a mild increase in blood pressure in knock-out mice (202), while others found that knockout animals on a normal salt diet had normal blood pressure (10). Both null animals and those heterozygous for the knock-out allele show exaggerated vasopressor responses to ANG II (202). Null animals show increased sensitivity to deoxycorticosterone, with a more rapid and pronounced increase in blood pressure than controls (75), and increased sensitivity to high-salt diet hypertension, with renal blood flow reduced by 20%, and renal vascular resistance doubled compared with a normal-salt diet (9), indicating a tonic vasodilatory effect of bradykinin in this vascular bed. Salt-sensitive hypertension can be reversed by selective ET A and ET B antagonists, and also by inhibitors of ACE and AT 1 receptors, suggesting that ET-1 and ANG II are involved in the development of hypertension (39).

The antidiuretic effect of arginine vasopressin in B2 receptor null mice is also enhanced (8), suggesting that endogenous kinins act through the B2 receptor to oppose the effects of arginine vasopressin in vivo. ACE inhibition leads to increases in bradykinin, COX-2, and renin, suggesting that renin may be regulated through a kinin-COX-2 pathway. In B2 receptor knockout mice, renal COX-2 levels are reduced by 40–50% and renal renin levels by 60%, but other components of the RAS remain unchanged (131). Blockade of the AT 1 receptor in B2 receptor knockouts prevents the cardiac remodeling seen in this model, suggesting that interaction between ANG II and bradykinin is important for normal heart development (201). A deletion polymorphism of human B2R gene has been associated with exaggerated cardiac growth response to physical exercise (42). In a transgenic model overexpressing the B2 receptor, the animals develop chronic hypertension. Urine volume, K+, and pH are all increased, but Na+ excretion is unaltered. The animals exhibit enhanced renal function, with increased renal blood flow, GFR, and urine flow. Significant increases in urinary nitrate, cGMP, and cAMP suggest that the enhanced renal function is achieved via activation of the NO signal transduction pathway (369).

In summary, the noted hypotensive actions of the KKS relate to effects on both renal hemodynamics and tubular sodium reabsorption. Transgenic approaches have demonstrated that the KKS interacts with a variety of separate vasoactive systems. Among these, an interaction with the RAS is of particular clinical significance. ACE inhibition will increase bradykinin levels, the effects of which are uncertain. On the one hand, the natriuretic effect of bradykinin would be beneficial in the treatment of hypertension; on the other hand, bradykinin can induce
a marked fall in GFR, thereby attenuating the salt-shedding impact of ACE inhibition (81).

F. The Dopaminergic System

Dopamine, better known as a neurotransmitter, can act as a hormonal modulator of blood pressure and is implicated in hypertension on the basis of studies in humans and rodent models (391). Dopamine produced by the kidney in response to increased Na+/H+ loading is an intrarenal regulator of sodium transport. It acts through G protein-coupled receptor(s) in renal proximal tubules to effect natriuresis through the inhibition of apical NHE3 and basolateral Na+/K+-ATPase (see Fig. 11). Kidney cell experiments suggest that signaling cascades downstream of the dopamine D1 receptor include adenyl cyclase protein kinase A-dependent phosphorylation followed by PLC- and protein kinase C-ε-dependent phosphorylation (93, 94), which ultimately leads to the inactivation of the major Na+ transporters. Dopamine-induced reduction of Na+/H+ exchange occurs through endocytosis, while recruitment to the plasma membrane, induced by serotonin or the phorbol ester phorbol 12-myristate 13-acetate (via protein kinase C-ε-dependent phosphorylation), leads to an increase in Na+/K+-ATPase activity (43, 74). The relative balance between activation or inactivation of Na+/K+-ATPase appears to be controlled by intracellular Na+ concentration (74).

In mice lacking one or both copies of the D1 receptor, both systolic and diastolic blood pressures are increased (7). In SHR rats, in spite of normal dopamine production and receptor density in the kidney, defective transduction of the D1 receptor signal results in decreased inhibition of sodium transport. The defective coupling between D1 receptor and its G protein/effecter enzyme complex is due, at least in part, to excessive serine phosphorylation of the receptor by a mutant G protein-coupled receptor kinase (GRK). The GRK4 locus has been linked with human essential hypertension (145), and several SNPs have been identified (271). Of these, the GRK4γA142V SNP has the greatest effect on D1 function in vitro. Transgenic mice expressing this variant were hypertensive and showed impaired diuretic and natriuretic, but not hypotensive, effects of D1 agonist stimulation (80).

The D3 receptor is expressed in the proximal tubules and the juxtaglomerular cells and also appears to affect natriuresis and diuresis. Knockout of D3 receptor increases systolic and diastolic blood pressures and renal renin activity and causes sodium retention (12). The increased urine flow rate and Na+ excretion associated with acute salt loading are attenuated in the homozygous null animal.

The dopamine receptor D5 is also involved in blood pressure control, since its knockout results in significant hypertension by 3 mo of age (122). The hypertension appears to be caused by increased sympathetic tone, mediated through a central nervous system defect.

G. Cyclooxygenase Pathway of Arachidonic Acid Metabolism

Arachidonic acid is metabolized into its active products, the eicosanoids, by three distinct enzyme pathways (see Fig. 12). COXs produce prostaglandins and thromboxanes; lipoxygenase gives rise to hydroxyeicosatetraenoic acids (HETEs), the leukotrienes, and lipoxins; and cytochrome P-450 (CYP450) produces 20-HETE and the epoxyeicosatrienoic acids (EETs). All of these active products can modulate hemodynamics and renal salt homeostasis and therefore have the potential to regulate blood pressure. Although genetic modification has been used to interfere with parts of this pathway, most of these models have been directed toward studies of inflammation, rather than blood pressure regulation, and will not be considered in this review.

The importance of COXs in the control of blood pressure is illustrated by the use of nonsteroidal anti-inflammatory drugs in humans, which inhibit both COX isoforms (COX-1 and COX-2), cause Na+ retention and hypertension (51), and interfere with the efficacy of ACE inhibitors (13). Additionally, COX-2 expression is stimulated by ACE inhibitors and low salt (47, 149). COX-1 is constitutively expressed in a variety of tissues, including the kidney. The exact function of renal COX-1 remains uncertain: the gene does not appear to be dynamically
regulated by alterations in salt and water intake, and COX-1 (Ptgs1) knockout mice display neither renal abnormalities nor misregulation of blood pressure (177).

COX-2 has a more restricted distribution, being limited in the kidney to the thick limb of Henle and cells of the macula densa. Expression of COX-2 is dramatically upregulated, at both the mRNA and protein level, by low-salt diet intake, consistent with its key role in the stimulation of renin release. Knockout of COX-2 (Ptgs2) is associated with decreased renin synthesis and granularity in juxtaglomerular cells, which does not increase on a low-salt diet (387) or in response to ACE inhibition (52). Ptgs2 knockout mice also display a marked but variable renal pathology, featuring glomerular hypoplasia and tubular atrophy (71, 228), yet blood pressure regulation in the Ptgs2 mice is normal. In both of these studies, the gene deletion was on a mixed C57/Bl6 and 129/Sv background. A backcross breeding strategy was used to introduce the Ptgs2 deletion onto 129/Sv, Balb/C, or C57/B6 congenic backgrounds (388). All strains displayed the renal pathology, but the most severe phenotype was observed on the 129/Sv background in which the males also developed a malignant hypertension. This demonstrates that genetic background is an important factor in the development of hypertension and can modify the severity of a single gene disorder. The development of hypertension may be related to the RAS. Both C57BL/6 and BalbC mice have the single Ren1C and the RAS would be expected to be suppressed following deletion of Ptgs2. Mice from the 129/Sv strain have, in addition, the constitutively expressed Ren2 gene, which may not be affected by Ptgs2 deletion.

Although PGE2 is a major product of COX, PGE2 receptor null mice (Ptger2 null) have no gross abnormalities, and blood pressure is normal. They do lose the hypotensive response to intravenous PGE2 infusion (although ability to vasodilate is inherently normal) and develop a marked hypertension on a high-salt diet (159).

H. Other Potential Contributing Factors

The general usefulness of α- and β-blockers in reducing blood pressure points to the involvement of adrenergic receptors in blood pressure control and their inclusion as candidate genes. There are nine adrenergic receptor subtypes (three α1, three α2, and three β), and the lack of selective pharmacological agents means that transgenic and knockout strategies have been very useful in distinguishing the various roles of the receptors (see Table 1). The α1-adrenoceptors (ARs) are postsynaptic receptors, which mediate some of the main actions of the catecholamines, epinephrine and norepinephrine, and play a crucial role in blood pressure regulation. The α2-AR are largely presynaptic and distributed in the central nervous system, although they are the numerically predominant AR type in the kidney. The β-AR appear to cross-compensate for each other, since mice with a single knockout of each have normal resting blood pressure and response to
exercise. From analysis of these single knockouts, plus double knockouts, it appears that all three $\beta$-ARs control vascular relaxation (283). Interestingly, positional genomic analysis has identified the $\beta$-AR gene as a susceptibility locus for human hypertension (36). Caution is required in translating the wealth of information from animal studies to the human situation, because of different expression patterns of the various receptor subtypes, especially in the heart (341).

Numerous other genes have been shown to affect blood pressure in transgenic or gene targeting models, hinting at their possible involvement in essential hypertension. Several of these genes have been summarized in Table 2 for the interested reader. The relationships between obesity, insulin resistance, and hypertension are complex and beyond the scope of this review. Some animal models are beginning to shed light on the interrelationships, although it is not always clear whether the variation in blood pressure seen in any of these models is directly or indirectly linked to the underlying genetic alteration (41).

### VIII. PERSPECTIVE

We have noted, throughout our review, a number of genes that exert their action both systemically and in the kidney. It has long been held as the central tenet of pressure natriuresis that the kidney is the seat of pressure control (103); an increase or decrease in arterial pressure will be matched by the appropriate excretion or retention of sodium and water, to achieve long-term balance. A clear demonstration of distinct kidney and systemic control mechanisms has been dramatically highlighted by the kidney transplantation experiment of Crowley et al. (62), which suggests that nonrenal tissues, such as the vasculature, may be potentially important contributors to blood pressure control. Equally, factors such as ANG II may contribute to vascular disease irrespective of their role in blood pressure (66).

The above description of transgenic and knockout strains is comprehensive but by no means exhaustive and broadly exemplifies the transgenic strategies currently available and the kind of questions that can be asked. The importance of genetic background cannot be overstated;

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**TABLE 1. Adrenergic receptor transgenics and their effects on blood pressure**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Transgenic Alteration</th>
<th>Effect on Blood Pressure</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_{1A}$</td>
<td>Knockout</td>
<td>Hypotension</td>
<td>285</td>
</tr>
<tr>
<td>$\alpha_{1B}$</td>
<td>Overexpression</td>
<td>Hypotension</td>
<td>397</td>
</tr>
<tr>
<td>$\alpha_{1D}$</td>
<td>Knockout</td>
<td>Hypotension</td>
<td>125, 342, 343</td>
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<tr>
<td>$\alpha_{2A}$</td>
<td>Knockout</td>
<td>Increased resting BP</td>
<td>117</td>
</tr>
<tr>
<td>$\alpha_{2B}$</td>
<td>Knockout</td>
<td>No response to salt load</td>
<td>205</td>
</tr>
<tr>
<td>$\alpha_{2C}$</td>
<td>Knockout</td>
<td>Normal response to salt</td>
<td>205</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>Knockout</td>
<td>Perinatal lethal; BP normal; no response to isoproterenol</td>
<td>284</td>
</tr>
<tr>
<td>$\beta_2$</td>
<td>Knockout</td>
<td>Blunted response to isoproterenol</td>
<td>54</td>
</tr>
<tr>
<td>$\beta_3$</td>
<td>Knockout</td>
<td>Blocks NO-dependent suppression of inotropy</td>
<td>359</td>
</tr>
</tbody>
</table>

BP, blood pressure; NO, nitric oxide.

**TABLE 2. Other factors reported to affect blood pressure in transgenic or gene targeting models**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tg/KO</th>
<th>Blood Pressure Change</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenomedullin</td>
<td>Adenoviral gene delivery</td>
<td>Attenuates hypertension</td>
<td>72, 368</td>
</tr>
<tr>
<td>Adenosine receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$A_2A$AR</td>
<td>KO</td>
<td>Hypertension</td>
<td>183</td>
</tr>
<tr>
<td>$A_2A$AR</td>
<td>KO</td>
<td>Renin expression enhanced on low salt</td>
<td>305</td>
</tr>
<tr>
<td>$\beta$-Adducin</td>
<td>KO</td>
<td>Hypertension</td>
<td>210</td>
</tr>
<tr>
<td>Parathyroid hormone-related protein (PTHrP) receptor</td>
<td>Overexpression</td>
<td>Hypotension</td>
<td>204</td>
</tr>
<tr>
<td>PTHrP receptor</td>
<td>Overexpression</td>
<td>Hypotension</td>
<td>274</td>
</tr>
<tr>
<td>Orexins</td>
<td>KO</td>
<td>Decreased basal arterial pressure</td>
<td>157</td>
</tr>
<tr>
<td>E-prostanoid receptors (EP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EP1</td>
<td>KO</td>
<td>Decreased male vasopressor response to PGE2</td>
<td>14</td>
</tr>
<tr>
<td>EP2 and EP4</td>
<td>KO</td>
<td>Decreased female vasopressor response</td>
<td></td>
</tr>
<tr>
<td>Leptin</td>
<td>Overexpression</td>
<td>Hypertension*</td>
<td>245</td>
</tr>
<tr>
<td>LDL-R</td>
<td>KO</td>
<td>Hypertension*</td>
<td>349</td>
</tr>
<tr>
<td>GLUT-4</td>
<td>Heterozygote</td>
<td>Hypertension*</td>
<td>329</td>
</tr>
<tr>
<td>Insulin receptor substrate-1</td>
<td>KO</td>
<td>Hypertension*</td>
<td>2</td>
</tr>
<tr>
<td>Thromboxane (TxA2)</td>
<td>KO</td>
<td>Hypertension caused by ANG II infusion is attenuated</td>
<td>84</td>
</tr>
</tbody>
</table>

* Hypertension may be indirectly linked to underlying genetic alteration. KO, knockout; LDL, low-density lipoprotein.
meaningful comparisons should only be made between transgenics and appropriate controls. Equally, comparisons between transgenic lines should always be made on the same genetic background, a requirement that is readily attainable with the development of speed congenics (where parent choice for each generation is determined following detailed microsatellite analysis, to assess the relative contribution of “parental” genomes). That said, crossing onto an alternative genetic background may reveal different subtleties of phenotype reflecting the presence of modifier loci. These may prove important in identifying or defining differences in susceptibility to hypertension in human populations. A note of caution: it is not implicit that any given gene identified as having a role in hypertension in the animal model will automatically be involved in human essential hypertension. However, genes identified as important in animals are potential contenders for QTL or association analysis in patients and may highlight relevance to essential hypertension in humans.

A lot can be learned from the generation of animals carrying multiple mutations (compound heterozygotes), either within a system or from different control pathways, to determine how they interact in health and disease. By introducing multiple components of human RAS into the mouse or rat, one can study elements of the human pathway in physiological context, perhaps pharmacologically, or investigate potential therapeutic interventions in the animal models generated. This may be especially helpful where species specificity means that the candidate gene is not normally functional in a heterologous species.

The vast majority of gene knockout experiments have been achieved in the mouse. Since a wealth of knowledge has accumulated from the many rat models of hypertension, it would be ideal to apply knockout technology to the rat.

One strategy for the production of knockout rats is the use of ethyl-nitroso-urea (ENU) mutagenesis (390), which randomly introduces point mutations into the genome through intercalation of ENU into the DNA. An appropriate screening protocol is necessary to look for mutations in genes of interest (390).

To date, the production of germline-competent rat ES cells, for gene-targeting, has proven elusive. Two groups have reported the culture of rat ES-like cells for many generations (44, 360), making them available for transfection with targeting constructs. Recently, nuclear cloning has been achieved using two-cell-stage embryos as donor and recipient cells (282). It may be possible, in the future, to use targeted ES-like cells as a source of nucleus for nuclear transfer, thus circumventing the need for germline competence (235).

The identification of exogenous growth factors and serum-free culture conditions, which allow continuous culture of mouse spermatogonial stem cells (SSCs) for periods in excess of 6 mo, opens up exciting possibilities for germline modification (173) by this route. The stem cells, generated from neonate, pup, and adult testes, can be transplanted into recipient seminiferous tubules, after extensive culture, and are able to reconstitute long-term spermatogenesis, or even restore fertility to infertile recipients. In vitro proliferation of SSCs should make genetic manipulation, such as targeted modification, possible. Such strategies could prove invaluable for species, such as the rat.

With the rapid advances in transgenic technology, one can anticipate the introduction of increasingly sophisticated and subtle genomic alterations to understand the genetic basis of hypertension. Equally exciting is the potential for applying knockout technology to alter genes in classical animal models, such as SHR, in an attempt to rescue the hypertensive phenotype.

Given the multigenic complexity of essential hypertension, computer simulations have an important role to play in assessing the contribution of genes to overall blood pressure. Making simple assumptions, one can begin to build more complex models, adding one enzyme at a time, building in pathways, and systems to account for perturbations in the data seen in gene titration experiments (336). Such modeling may identify groups of allelic variants or mutations, which given certain prenatal programming, or dietary or environmental stimulation, will lead to the development of hypertension. Identifying patients with similar predisposition will be helpful in tailoring therapy or even prevention.

Rapid advances in functional genomics and proteomics mean that the majority of genes identified and mapped in the whole genome sequencing programs will be classified and have functions assigned to them. Ultimately, it is the interactions between gene products that mediate physiological responses (361). The use of siRNAs to mediate gene-specific posttranscriptional silencing or knockdown is likely to prove an efficient method for studying gene function in mammalian cells. Plans to conduct large-scale screens on a genome scale should help to assign both broad and specific functions to genes, in a high-throughput manner (109, 258, 319). There is a suggestion that RNA interference could ultimately be used therapeutically (45), provided that techniques to allow efficient, stable production and, more importantly, delivery to target tissues are refined (367).

Functional genomics and proteomics will greatly advance the ability to identify genes that are differentially expressed in hypertensive and normal animals and/or patients. The challenge will be to identify the functionally relevant interactions that directly impact on essential hypertension.
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All mouse gene nomenclature is according to the Mouse Genome Informatics website (http://www.informatics.jax.org).

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