Renin was discovered by Robert Tigerstedt in 1898. A half-century later, Pickering rediscovered renin and implicated it in Goldblatt’s model of renal hypertension that resembles renal artery stenosis in humans. In the 1960s, Borst argued that the primary defect responsible for essential hypertension resided in the kidney. Guyton provided support for this concept in the 1970s by means of physiological experiments and computer modeling. Dahl showed that kidneys transplanted from genetically hypertensive rats to a normotensive strain raised blood pressure (BP), and kidneys from normotensive rats lowered BP when transplanted into hypertensive rats. This suggested that the cause of hypertension was a genetic defect in the kidney. Support for this in humans was provided by Lifton, who showed that mutations in single genes involved in BP control by the kidney were responsible for rare monogenic forms of hypertension. Laragh and Sealey argued that intrarenal processes being responsible for hypertension. The existence of an intrarenal renin–angiotensin system had been suggested ever since the late 1960s, but the evidence was weak, even artifactual. Thus, my idea characterizing angiotensinogen in the kidney in the mid-1970s represented the first real evidence for the existence of a functional intrarenal renin–angiotensin system. Shortly thereafter, genuine intrarenal angiotensin II was identified. Renal angiotensinogen was present in vesicles that were distinct from those that contained renin and were located in proximal convoluted tubule cells. Angiotensinogen in segments 1 and 2 of the proximal tubule seems to be of systemic origin, whereas angiotensinogen in segment 3 is produced locally. Overexpression of tubular angiotensinogen raises BP.

Prorenin, Renin Biosynthetic Pathway, Renin Gene Isolation, and Regulation

My lifetime research interests in the field started 44 years ago with the discovery of a proteolytically activatable form of renin—prorenin. I found many proteases could activate prorenin, apparently because of a protease-sensitive sequence at the activation site. I found prorenin in the kidney and, by elucidating the biosynthetic pathway of renin, demonstrated directly the synthesis of prorenin in renin-producing cells.

After isolating mouse renin cDNAs, we used these as probes to isolate the human renin gene (REN), which was sequenced and localized to chromosome 1q42. The encoded protein consisted of a 2 kDa signal peptide, a 5 kDa pro segment, and a 38 kDa renin sequence. The 3D structure was then elucidated. Because the signal peptide was separately encoded on exon 1, it seemed to me that generation of an alternative transcript lacking exon 1 would result in a form of renin unable to enter the secretory compartment of the cell and that was therefore intracellular. This prediction has been confirmed. In the kidney, a truncated intracellular renin isoform has been identified in collecting ducts and generates local angiotensin II, leading to inflammation and systemic insulin resistance without an elevation in plasma renin.

After localizing renin mRNA in the kidney, we showed that stimuli known to affect renin secretion had similar effects on renal renin mRNA levels. Perturbations in renin mRNA were then documented in the kidney and other tissues in models of hypertension. The heart lacked renin mRNA under...
resting conditions, but expression could be switched on by low sodium diet and angiotensin-converting enzyme inhibition.31

The human REN proximal promoter was weak,57,58 although contained positive and negative regions, and there was a silencer element in intron 1.59 Most interesting was a functional cAMP response element at upstream nucleotides −218 to −2226.61 responsible for promoter activation after the attachment of a CREB-ATF-1 heterodimer to the cyclic AMP response element.62 Cyclic AMP first activated protein kinase A, leading to CREB phosphorylation, which caused it to bind to ATF-1.62 Rapid phosphorylation and dephosphorylation would facilitate tight regulation of transcription in response to physiological stimuli, such as β-adrenoceptor and possible purinergic (P2Y1 receptor)63 effects.

Transcription is regulated by a strong enhancer far upstream of the mouse renin gene.64-66 This fitted the on/off (variegation) model of gene expression. After confirming this model in vitro,66 we obtained in vivo support by creating a renin enhancer knockout mouse, which had lower BP, marked depletion of renal renin, macula densa hyperplasia,64 a blunted response in renin mRNA to physiological stimulation of renin secretion,69 including stress responses,70 but not to prepulse inhibition.71

Post-transcriptional regulation59 involved binding of multiple cellular proteins to the 3′-UTR of REN mRNA in vivo.72 The proteins HuR and CP1 stabilized REN mRNA, whereas HADHB destabilized it by binding to an AU-rich stem-loop sequence in the distal 3′UTR, leading to recruitment of the degradation machinery of the cell. HADHB colocalized with renin in juxtaglomerular cells.72

Molecular Genetics of Essential Hypertension

The first publication on the molecular genetics of hypertension involved an association study of a REN HindIII polymorphism in hypertensive and normotensive subjects.73 Although the result was negative,73-75 it led to further studies of the genetic basis of essential hypertension (reviewed in Ref. 76-79). Biological power was enhanced by restricting choice of subjects to those whose BP status was the same as both of their parents. As a result, hypertensive subjects had earlier onset, more severe hypertension when compared with subjects having only 1 hypertensive parent or sibling,80 leading to stronger associations with hypertension than found by others, for example, variants in G-protein β3 subunit gene (GNB3),81 angiotensin II type 1 receptor gene (AGTR1),82 the aldosterone synthase gene (CYP11B2),83 and other genes.84-89 It also helped exclude additional genes others found to be associated with hypertension.90-96

My work implicates a major effect of uncommon polymorphisms on hypertension, demonstrated for the functional Gly40Ser variant in the glucagon receptor gene (GCGR), which was present in 1% of normotensive subjects compared with 5%-97 to 7%-98 of hypertensives. This concept is now widely accepted99,100 and could explain in part why even large genome-wide association studies have been able to find only a small proportion of the expected number of causative genes. A current challenge is to find this missing heritability.99

The concept of survivor bias was introduced by me based on association of the I allele of the II/D angiotensin-converting enzyme gene polymorphism with essential hypertension.101 I showed that the association was actually a result of depletion of the D allele from the hypertensive group,102 which increases risk of myocardial infarction.103 A similar phenomenon was then observed for a promoter variant of NOS2A.103 Researchers should therefore check their data to see whether an association with hypertension applies to both younger and older patients. If it does not then the variant might not be associated with hypertension after all.

In one of the first genome-wide linkage scans, my Laboratory identified a locus for hypertension and hypercholesterolemia at chromosome 1p36.105 This was then confirmed by others.106,107 We discounted involvement of one gene in this region.108 Loci on other chromosomes were subsequently found after completion of the genome-wide study.109

Transcriptome-Wide Alterations in mRNA Expression in Rodent Models of Hypertension

I will review these more recent studies in more detail. This newer research began with a meta-analysis of array data for kidney, adrenal, heart, and artery of spontaneously hypertensive and Lyon hypertensive rats to find novel causative, maintenance, and responsive mechanisms and pathways.110 We then turned to a neurogenic model, the Schlagert hypertensive and normotensive mouse strains, by performing transcriptome-wide studies of hypothalamic tissue. This revealed differences in early and established hypertension in levels of transcripts encoding proteins having effects on sympathetic outflow as well as inflammation and stress responses.111

In order to identify transcripts corresponding to the exaggerated waking BP surge (that is a risk factor myocardial infarction and stroke) we obtained array data for the diurnal BP peak and trough.112 This implicated hypothalamic transcripts encoding known BP regulators, as well as novel proteins involved in inflammation, mitochondrial function, and other pathways.112

Human Kidney Transcriptome-Wide Arrays

Transcriptome-wide arrays of human kidneys showed aberrant expression of 46 mRNAs and 13 microRNAs in the renal cortex and 14 mRNAs and 11 microRNAs in the renal medulla.113 One transcript elevated in hypertension was that encoding renin. Another mRNA, whose level was reduced in hypertension, was that encoding CD36. This finding provided the first support for CD36 having a role in human hypertension just as had been shown in the spontaneously hypertensive rat kidney.114 Bioinformatic analyses identified pathways in the renal cortex and medulla that were enriched in hypertension (Figure 1).

In silico searches revealed potential targets of the microRNAs in the 3′-untranslated region of the various transcripts that were differentially expressed.115 The ability of several of these to destabilize specific mRNAs was then confirmed by cotransfection of human kidney cells with microRNA expression constructs and luciferase reporter gene constructs. We found 2 microRNAs that destabilized REN mRNA.113 These data were the first to implicate a microRNA mechanism in the regulation of renin expression. Lower miR-181a and miR-663 and elevated renin mRNA (and presumably
renin) in hypertension pointed to a mechanism whereby renin overexpression or inadequate physiological suppression might contribute to BP elevation in hypertension.

MicroRNA miR-181a, Renin, and BP in Genetically Hypertensive Mice

Schlager hypertensive mice exhibited renal hyperinnervation, and sympathetic stimulation led to an increase in renal renin mRNA. These studies suggested that besides increased sympathetic outflow from the CNS, the renin–angiotensin system was an additional contributor to hypertension in Schlager mice and did so to a greater extent in the active period. Although renal renin mRNA and BP were elevated, miR-181a was decreased, that is, was consistent with the inverse relationship we saw between miR-181a and renin in human hypertensive kidneys. (Mice do not have a miR-663.)

Preliminary data from Geoff Head’s Laboratory has shown recently that kidney-specific transfection of a miR-181a mimic caused a 42% reduction in renal renin mRNA during the active period and a 4 mm Hg reduction in diastolic BP. The miR-181a mimic abolished the reduction in BP caused by enalaprilat, and so demonstrated the importance of miR-181a in control of BP by the renin–angiotensin system. Renal sympathectomy lowered BP by 8 mm Hg and normalized both renin and miR-181a in the hypertensive, but not in the normotensive strain, and so pointed to a role for sympathetic traffic to the kidney in regulation of miR-181a, as well as of renin and miR-181a in hypertension. Figure 2 depicts a possible mechanism, although whether renal sympathetic input has this effect on miR-181a and hence renin in human hypertension remains to be shown.

Human miR-181a Co-localizes With Tubular Renin mRNA and Is Associated With BP and Immune System/Inflammatory Transcripts

Marques, Tomaszewski, Charchar, and colleagues found colocalization of miR-181a and renin in epithelial cells of the collecting duct. Such intratubular renin is not released into the bloodstream and acts locally in a paracrine or autocrine manner. Together with other components of the tubular renin–angiotensin system, collecting duct renin is known to affect mitochondrial function and contribute to hypertension. When overexpressed, collecting duct renin raises BP whereas genetic deletion of renin in the collecting duct attenuates hypertension induced by angiotensin II, as well as reducing epithelial sodium channel levels.

There was a strong correlation between BP and both kidney and serum miR-181a. The association was strongest for diastolic BP in subjects not taking antihypertensive medication, miR-181a-stratified gene set enrichment analysis, and next-generation RNA sequencing revealed that miR-181a targeted transcripts in pathways elevated in adaptive immunity and inflammation, as well as transcripts associated with reduced mitochondrial function. Others have implicated miR-181a in mitochondrial function and inflamm-aging.
The immune system and inflammation are involved in the genesis and progression of hypertension, with evidence linking the sympathetic nervous system, immune cells, production of cytokines, and, ultimately, vascular and renal dysfunction, so leading to the augmentation of hypertension. NADPH oxidase, by generating superoxide and thence other reactive oxygen species, is critical for many forms of hypertension. In hypertension, renal proteins that have been oxidatively modified by γ-ketoaldehydes (isoketals) stimulate dendritic cells to produce interleukin-6, -1, and -23 as well as costimulatory proteins CD80 and CD86 that then cause T cell proliferation, interferon-γ, and interleukin-17A production and hypertension.

In established essential hypertension, activation of immune system and inflammatory processes contribute to kidney damage, which further accentuates the hypertension. miR-181a is known to increase in response to inflammatory stimuli and, by targeting mRNAs for inflammatory cytokines, particularly interleukin-1β, interleukin-6, and tumor necrosis factor-α, helps counteract inflammation as part of a homeostatic response.

Perspectives

I deeply appreciate having been chosen as the recipient of the 2014 Irvine Page–Alva Bradley Lifetime Achievement Award and thank all of those involved in the nomination and selection process. My molecular studies of renin, genetics and hypertension have provided fundamental basic knowledge that has assisted advances in the field. My recent research perhaps represents a culmination of this line of research by suggesting that a novel microRNA-mediated mechanism may be responsible for involvement of the intrarenal renin–angiotensin system in the onset of experimental and essential hypertension. After 44 years of contributions, I expect the next generation of researchers will go on to fully solve the enduring mystery of what causes essential hypertension. Molecular studies will continue to be pivotal to such endeavors.

Acknowledgments


Disclosures

None.

References


55. Adams DJ, Beveridge DJ, van der Weyden L, Mangs H, Leedman PJ, Morris BJ. HADHB, HuR, and CPE bind to the distal 3'-untranslated region of human renin mRNA and differentially modulate renin
Morris Renin, Genes, MicroRNAs, Kidney, Hypertension


Renin, Genes, MicroRNAs, and Renal Mechanisms Involved in Hypertension
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