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Synthesis and Secretion of Renin in Mice with Induced Genetic Mutations

Jurgen Schnermann[1] and **Josephine P. Briggs**[2]

[1]National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda MD, USA

[2]National Center for Complementary and Alternative Medicine, NIH, Bethesda MD, USA

Abstract

The juxtaglomerular (JG) cell product renin is rate-limiting in the generation of the bioactive octapeptide angiotensin II. Rates of synthesis and secretion of the aspartyl protease renin by JG cells are controlled by multiple afferent and efferent pathways originating in the CNS, cardiovascular system and kidneys and making critical contributions to the maintainance of extracellular fluid volume and arterial blood pressure. Since both excesses and deficits of angiotensin II have deleterious effects it is not surprising that control of renin is secured by a complex system of feedforward and feedback relationships. Mice with genetic alterations have contributed to a better understanding of the networks controlling renin synthesis and secretion. Essential input for the setting of basal renin generation rates is provided by β-adrenergic receptors acting through cAMP, the primary intracellular activation mechanism for renin mRNA generation. Other major control mechanisms include COX-2 and nNOS affecting renin through PGE2, PGI2, and nitric oxide. Angiotensin II provides strong negative feedback inhibition of renin synthesis, largely an indirect effect mediated by baroreceptor and macula densa inputs. Adenosine appears to be a dominant factor in the inhibitory arms of the baroreceptor and macula densa mechanisms. Targeted gene mutations have also shed light on a number of novel aspects related to renin processing and the regulation of renin synthesis and secretion.

Introduction

The antihypertensive, antifibrotic, and antiproliferative effects of systemic administration of inhibitors of renin, angiotensin II receptors, or angiotensin II converting enzyme underscore the important contribution of the renin-angiotensin system (RAS) to the progression of cardiovascular and renal diseases. The formation of angiotensin II in the circulation is primarily regulated by changes in the activity of the aspartyl protease renin, which is largely a product of the juxtaglomerular granular (JG) cells at the glomerular vascular pole of the kidney. Thus, the rate of renin synthesis in JG cells and the rate of renin secretion are the main determinants of circulating angiotensin II. Under steady-state conditions synthesis and secretion of renin tend to change in parallel since transcriptional and posttranscriptional regulation of renin mRNA and secretion of renin from storage granules are partly under the control of the same intracellular mechanisms.

Commensurate with the central role of angiotensin II in volume homeostasis, synthesis and secretion of renin by JG cells is regulated by multiple inputs that in general reflect the status of extracellular fluid volume. The most important among the macroscopic control mechanisms of renin release in vivo are blood pressure (“the renal baroreceptor

Send correspondence to: Jurgen Schnermann, MD., National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 10 Center Drive-MSC 1370 Bethesda. MD 20892, USA, Tel 1-301-435-6580, Fax 1-301-435-6587, jurgens@intra.niddk.nih.gov.

mechanism"), renal sympathetic tone through β-adrenergic receptors, and tubular NaCl concentration in the macula densa segment of the nephron ("the macula densa mechanism"). A number of pharmacological, ablative, and cellular strategies have been employed to establish the distinct nature and contribution of individual regulatory pathways in controlling renin secretion. However, the cellular mechanisms underlying control of renin secretion by physiological regulatory systems in the intact animal have been difficult to identify because of complex interactions between feedforward and feedback regulation. Mice with defined gene manipulations offer a new venue to further explore the mechanisms responsible for control of renin synthesis and secretion in the intact organism. The approach in the majority of studies has been to draw conclusions from phenotypic changes caused by defined alterations of the genotype. In this review we will focus mostly on those studies of renin secretion in which interference with recognized signaling pathways has occurred at the level of receptors, ligands, ligand synthesis, and membrane transduction mechanisms, all interventions that could potentially shed light on the acute regulation of renin release. A graphical preview of the selected topics is given in Fig. 1.

It has become apparent that the generation of angiotensin II in the circulation is only part of the functionality of the RAS. Evidence is mounting that angiotensin II can be generated in a tissue-specific fashion and exert local effects, for example in the brain, heart, or kidney, but a discussion of these local effects will not be attempted here [1, 2]. Furthermore, in this short review we cannot address the important progress that has been made in defining the sequences in the renin DNA and their binding complexes that promote, enhance, or silence renin gene transcription, for example by the activation of nuclear hormone receptors such as the vitamin D3 receptor or PPAR [3-5].

Angiotensin II

Mice with deletions in the non-renin components of the renin-angiotensin system have confirmed that the expression and secretion of renin is under strong inhibitory control of angiotensin II. Mice with null mutations in the genes for angiotensinogen (AGT), angiotensin converting enzyme (ACE), and angiotensin II receptors show consistent and massive upregulation of renin expression and plasma renin concentrations [6-11]. The overexpression of renin in these conditions is typically associated with upstream recruitment and ectopic location of renin-expressing cells [7, 11-13]. Disruption of AT1a signaling contributes most to these abnormalities; intrarenal renin expression was found to be essentially normal in AT1b or AT2-deficient mice [13, 14]. In fact, plasma angiotensin II levels were lower in AT1a/AT1b/AT2 triple knockout mice than in mice with AT1 receptor deficiency suggesting that AT2 receptors may have effects that are directionally opposite to those elicited by AT1 receptors [15].

It has been a widely accepted notion that the effect of angiotensin II on renin expression is the reflection of a direct interaction of the peptide with JG cells. Several findings from genetically modified mice call this concept into question. Cross-transplantation of kidneys between AT1a-deficient and wild type mice results in a significant increase in renin expression only when AT1a receptors are missing in the entire organism. Selective absence of AT1a receptors in the kidneys did not cause detectable elevations of renal renin mRNA, suggesting that upregulation of renin expression is not the direct consequence of angiotensin withdrawal from AT1 receptors in JG cells [16]. That the stimulation of renin expression by withdrawal of angiotensin II effects is not primarily mediated by AT1 receptors on JG cells is supported by earlier findings in chimeric mice carrying both AT1a-/- and AT1a+/+ cells. In these mice increases in renin expression and JG cell hyperplasia were seen independent of whether individual JGAs did or did not express AT1 receptors [17]. In both studies increased renin expression correlated with the reduced arterial blood pressure suggesting that at least

part of the “feedback” effect of angiotensin II is baroreceptor-mediated. Increased arterial blood pressure rather than any direct effects of angiotensin II may also be responsible for the inhibition of renin expression in AGT-deficient mice on high salt intake [18-20].

G protein Gsα and cAMP

Substantial early experimental evidence has supported an important role for Gsα in activating adenylate cyclase (AC) and stimulating renin synthesis and secretion through its catalytic product cAMP [21]. The absolutely critical contribution of the Gsα-cAMP-PKA signaling cascade in both basal and regulated renin secretion has been affirmed in mice with selective deletion of Gsα in JG cells [22]. Cell-specific Gsα deletion was achieved by crossing mice that express Cre recombinase under control of the endogenous renin promoter with animals in which exon 1 of the GNAS gene is flanked by loxP sites [12, 23]. In mice homozygous for the loxP-flanked GNAS gene and heterozygous for the modified renin locus, native Gsα was found to be significantly reduced in the whole kidney and almost completely abolished in single JG cells at the DNA, mRNA and protein level [22]. This genetic deletion of Gsα in JG cells was associated with a marked reduction of renin expression and renin secretion under basal conditions. Renal cortical renin mRNA was reduced to 23% of control and renin content of isolated JG cells to about 10% of control indicating a greatly reduced rate of renin production. Furthermore, concentrations of renin in plasma and in the medium of isolated JG cells were reduced to 10% and 5% of control respectively indicating a critical role of Gsα signaling in renin secretion [22]. Thus, Gsα-dependent signal processing is needed in a non-redundant fashion for the maintenance of high basal levels of renin expression and renin release. Furthermore, the stimulation of renin secretion caused acutely by furosemide, hydralazine, or isoproterenol was virtually abolished in the Gsα-deficient mice. To the extent that furosemide stimulates renin secretion through the macula densa and hydralazine through the baroreceptor, these data suggest that Gsα is required for the major physiological inputs that regulate renin secretion in vivo. Suppression of renin expression in the absence of Gsα signaling is already apparent prenatally; the appearance of renin in distal arcuate arteries, the earliest site for renin expression in the metanephric kidney, was missing in mice with JG cell specific deletion of Gsα [24].

A somewhat unexpected observation has been that the strong stimulation of renin synthesis and secretion caused by either converting enzyme or angiotensin receptors blockers was completely abrogated in mice with JG cell-specific deletion of Gsα [25]. This finding sheds additional light on the phenomenon often described as the “short loop feedback” inhibition of renin expression. While there is substantial support for the concept that angiotensin II has a direct inhibitory effect on JG cells, mediated presumably by an increase in cytosolic calcium, there is no direct support for the assumption that the stimulatory pathway is simply the reverse, i.e. caused by a direct effect of angiotensin II on the JG cells to decrease cellular calcium [26]. The requirement for Gsα for the stimulation of renin release by angiotensin II withdrawal indicates a role for activation of Gsα-dependent pathways. Co-administration of propranolol, indomethacin, and L-NAME mimicked the effect of Gsα deficiency [25], indicating that that angiotensin II withdrawal produces Gsα activation through stimulation of catecholamines, prostaglandins, and nitric oxide, and that these agents are the actual ligands that effect renin stimulation.

Cyclooxygenase and Prostaglandins

Studies of the functional connection between renin and the prostanoid system are founded on the finding that arachidonic acid increases and indomethacin reduces plasma renin levels [27]. Numerous subsequent studies have identified various metabolites of arachidonic

acid as potential regulators of renin secretion [28] with prostaglandins of the E and I series being the most consistent stimulators of renin release [29].

The effects of PGE2 are elicited by the activation of four types of G protein coupled receptors (EP1 to EP4). Studies in mice with deletion of single PGE2 receptors have identified the Gsα-coupled EP4 as the most important receptor for stimulation of renin. Basal plasma renin concentration was significantly lower in EP4-deficient than WT mice while there was no difference in EP1-, EP2-, or EP3-deficient mice [30]. Renin release in response to a high PGE2 concentration was significantly reduced in isolated perfused kidneys of EP4-/- and EP2-/- mice while the renin-stimulating threshold concentration was increased in EP4-/- mice suggesting that PGE2 acts through EP4 and EP2 to increase renin release [30]. Stimulation of renin release by chronic administration of furosemide was significantly reduced in EP4-/- mice together with a reduced stimulation of renin expression [31] although the acute administration of bumetanide in isolated kidneys did not show a difference in the renin release response between EP4-/- mice and all other strains [30]. The effect of PGE2 on renin release is probably a direct effect since isolated JG cells have been shown to express EP4 and EP2 mRNA and to respond to PGE2 with increased renin secretion [29, 32]. In view of the strong evidence involving PGE2 in renin secretion, it is surprising that mice deficient in microsomal PGE2 synthase did not show a significant reduction in basal or furosemide-stimulated renin mRNA, suggesting that alternative pathways may exist for the synthesis of PGE2 [33].

Studies in prostacyclin receptor (IP)-deficient mice have confirmed that prostacyclin is another prostaglandin with renin-stimulatory properties. Besides having a reduced response to the stimulatory effect of furosemide, IP-deficient mice have a markedly reduced response of renin expression and release in response to renal arterial constriction or salt depletion whereas mice with deficiencies in the four PGE2 receptors showed normal responsiveness [31, 34]. Somewhat unexpectedly, mice deficient in the FP receptor the prostanoid receptor that mediates the effects of PGF2α, also had reduced levels of plasma renin and angiotensin II concentrations [35]. At the same time the number of renin-expressing cells appeared to be reduced, but exactly how PGF2α may stimulate renin release or increase the number of JG cells is unknown.

Mice with deletion in PGHS-2 (COX-2) have aided in solidifying the notion that the formation of PGE2 is a critical regulatory input to the formation and release of renin by JG cells. The presence of COX-2 in macula densa and TAL cells had suggested that this pathway may be of particular importance for macula densa control of renin [36]. Evidence in support of this notion was provided by studies in COX-2 deficient mice showing that renin enzyme activity, plasma renin and renal renin mRNA and protein expression were markedly reduced [37, 38]. [37, 39]. In contrast, plasma renin concentration and renal renin expression were not different from control in COX-1 deficient mice [40]. It is of note that the stimulatory effect of a chronic low salt diet on renin expression was virtually absent in COX-2-/- mice [38]. The magnitude of the renin release response to acute stimulation by furosemide, hydralazine, or isoproterenol were markedly diminished in the absence of COX-2 [37], as it is in most states of chronically suppressed renin.

It is noteworthy that chronic inhibition of COX-2 enzyme activity by inhibitors such as rofecoxib does not always produce the same inhibition of renin expression that is seen with genetic COX-2 deletion [41, 42]. A possible explanation for this dissociation may lie in the observation that pharmacological COX-2 inhibition is associated with a marked upregulation of renal COX-2 expression so that increased COX-2 enzyme levels may partly compensate for enzyme blockade [41]. Other evidence for unidentified modifying factors in this pathway is the observation that the reduction in plasma renin concentration was more

pronounced in mice with the COX-2 null mutation bred into congenic C57Bl/6, BALB/c, or 129 J backgrounds compared to that seen in a mixed genetic background [37, 39, 43].

Studies in COX-2 deficient mice have also provided insights about another important interaction in the relationships between prostaglandins and renin, the feedback inhibition of COX-2 expression by angiotensin II (Fig. 2). One critical experimental observation is that inhibition or genetic deletion of COX-2 attenuates the stimulatory effect of angiotensin II blockade on renin expression [37, 43]. No difference in the stimulatory effect of captopril was observed between wild type and COX-1 deficient mice [40]. Thus, a reduction in angiotensin II signaling, probably at the level of the MD and/or TAL cells, appears to lessen tonic feedback inhibition of COX-2 and thereby enhance renin expression. This is strongly supported by the observation that COX-2 expression is markedly upregulated in angiotensin-II-blocked or -deficient mice [37, 43-47]. A fivefold upregulation of COX-2 and of urinary PGE_2 excretion has also been observed in the low renin model of JG cell-specific deletion of Gsα [22]. Interestingly, whereas AT1a receptors on macula densa cells appear to mediate inhibition of COX-2 expression, AT2 receptors may cause stimulation of COX-2 expression. This is based in part on the observation that the AT1 receptor blocker candesartan elevated COX-2 expression to a lesser extent in AT2 knockouts than in wild type mice [48]. Furthermore, the stimulatory effect of candesartan in wild type mice was lessened by the AT2 receptor blocker PD123319. Thus, although under most physiological conditions COX-2 and renin expression change in parallel, during primary changes of renin expression angiotensin II feedback inhibition of COX-2 can dissociate the expression of COX-2 and renin.

Nitric Oxide

The notion that NO may contribute to the regulation of renin secretion has seemed plausible because of the high levels of expression of NOS in cells of the macula densa and vascular endothelium, two cell types in close anatomical association with JG cells. Nevertheless, conclusions from studies with NO synthase blocking agents have been contradictory. Perhaps the most convincing evidence for an important role of NOS in renin secretion comes from studies in isolated perfused kidneys from either nNOS or eNOS deficient mice, in which renin secretion is markedly reduced (to 10% or 30% respectively) [49]. Since perfusion pressure is servo-controlled in this setting, indirect mediation of the effect of NOS deficiency on renin by baroreceptor activation is unlikely. In intact nNOS-deficient mice plasma renin concentration is reduced, supporting tonic stimulation of renin secretion by NO [39, 49]. Furthermore, the stimulation of renin synthesis and secretion caused by reduced Na intake was absent in mice without nNOS [50]. In contrast to plasma renin, renal renin mRNA expression and renal renin content were found to be unchanged in nNOS knockout compared to wild type mice [51]. The strength of the evidence from these studies is somewhat marred by the fact that the recombination strategy used in the available nNOS knockout mice permits the translation of an enzymatically active NOS1 isoform called NOS1β [52]. This isoform has recently been detected in macula densa cells so that residual NOS activity in the vicinity of JG cells cannot be excluded in the “knockout” animals [53].

Evidence for a role of NO generated by eNOS in basal renin release is less convincing. Plasma renin concentration of eNOS-deficient mice was not different from wild types in two studies [49, 54] and paradoxically increased in another [55]. An increase in plasma renin concentration accompanied by an increase in plasma aldosterone was also observed in triple knockout mice devoid of all 3 NOS isoforms [56]. Since these mice show signs of cardiac and renal failure leading to a markedly reduced life span it is likely that a reduction of renal perfusion overcomes any inhibitory effects of NOS deficiency on renin release. The observations in the triple knockouts notwithstanding, renal renin expression and plasma

renin concentration were found to be significantly reduced in nNOS/eNOS double knockout mice generated in our laboratory (unpublished). Furthermore, whereas NOS inhibition with L-NAME reduced plasma renin in WT, eNOS-/- and nNOS-/-, it had no effect on plasma renin in the double knockouts suggesting that iNOS does not play a major role under normal conditions and that both eNOS and nNOS facilitate renin release.

Expression of nNOS in macula densa cells was observed to be significantly increased in mice with gene deletions of the AT1a receptor, of both AT1a and AT1b receptors, and of angiotensinogen, suggesting that angiotensin II exerts an inhibitory influence on nNOS expression [47, 57, 58]. Upregulation of nNOS in AT1a/AT1b knockout mice was noted to extend to the preglomerular vasculature [59]. The implication of these observations is that angiotensin II inhibits nNOS expression in macula densa and vascular smooth muscle cells just as it inhibits COX-2 expression in the macula densa (Fig. 1).

Catecholamines

In metanephric kidneys of mice with deletion of β1 and β2 adrenergic receptors the early expression of renin in large renal vessels is largely absent, but renin-positive cells later appear in the typical JG arteriolar location [60]. This observation implies that the early expression of renin in large renal vessels appears to be under sympathetic control, and that the subsequent establishment of the adult-type JG location does not require an earlier vascular expression.

Adult β1/β2 adrenergic receptor-deficient mice have greatly reduced levels of renin in plasma and renal tissue, indicating that renal sympathetic input and circulating catecholamines via β-receptors represent a dominant stimulatory input for basal renin expression throughout life [61]. Changes of renin release by furosemide, inhibition of angiotensin II receptors or formation, or dietary salt intake occurred in the absence of β1/β2 adrenergic receptors, but the magnitude of the secretory response to both acute or chronic perturbations was markedly smaller in all conditions [61]. A generalizable conclusion from these and similar observations in other studies is that the size of the releasable renin pool as determined by renin synthesis in the entire JG cell population is the main determinant of the amount of renin that is released in response to an acute regulatory need (Fig. 3).

Recent studies have identified two of the adenylyl cyclases that are activated by the interaction of adrenoceptors with Gsα. The response of renin release to an injection of isoproterenol was significantly reduced in mice deficient in either AC5 or AC6, suggesting that these Ca-inhibited adenylyl cyclases are causal in the generation of cAMP that mediates the increase in renin secretion with β-adrenergic stimulation [62]. In contrast to the β1/β2 adrenergic receptor knockout mice, basal plasma renin and renal renin synthesis were increased in AC6-/- and normal in AC5-/-, indicating redundancy and the capacity to compensate for the loss of an individual AC.

The pituitary adenylate cyclase-activating polypeptide (PACAP) stimulates renin secretion in the isolated perfused rat kidney as well as in JG cells in primary culture [63]. This effect is mediated by the PAC1 receptor for PACAP since PAC1-deficient mice have a significantly lower plasma renin concentration and since renin release in isolated kidneys from PAC1-deficient mice did not respond to PACAP.

A previously unknown role of IA-2 proteins inregulation of renin secretion, probably in the β-adrenergic pathway, has recently been discovered. IA-2 proteins are best known for their role as the major autoantigen in type I diabetes mellitus [64, 65]. The structure of IA-2 and IA-2β indicates that they are members of the tyrosine phosphatase family although they lack enzymatic activity [66]. IA-2 proteins are integral proteins of dense core vesicle membranes in

cells with secretory activity as well as in autonomic nerve varicosities and synaptic vesicles [66, 67]. The location of IA-2 proteins suggests a role in secretory processes, and this notion has been confirmed in regard to the release of insulin and luteinizing hormone LH [68-70]. Plasma renin concentration and renal renin expression were reduced by about 50% in mice with IA-2/IA-2β double deletion, with smaller changes in mice with the respective single deletions [71]. The regulation of renin secretion by furosemide or salt intake was intact, although the response magnitude was reduced commensurate with the lower renin expression levels. The effect of IA-2 and IA-2β is probably a consequence of a reduced tonic β-adrenergic input since the expression of IA-2 proteins co-localized with tyrosine hydroxylase, but not with that of renin, and since β-adrenergic blockade with propranolol did not further reduce renin secretion in the IA-deficient animals [71]. The use of IA-2 gene manipulation thus identified an unexpected effect that indicates a contribution of IA-2 and IA-2β to tonic adrenergic transmitter release.

Adenosine

The nucleoside adenosine exerts its effects throughout the body by activation of specific G protein coupled receptors. Over a concentration range between 10^{-10} and 10^{-6} M, adenosine causes dose-dependent inhibition of renin secretion in isolated JG cells and kidney slices, indicating a direct inhibitory interaction of adenosine with JG cells [72, 73]. Studies with selective receptor agonists have established that the inhibitory effect is mediated by the A1 adenosine receptor subtype (A1AR) [72, 73]. Mice with deletion of A1AR have elevated plasma renin concentrations, supporting a tonic inhibitory influence of adenosine on renin release [74-76]. Interestingly, repeat measurements of plasma renin concentration over a two week period have shown a significantly greater variability in individual A1AR-deficient compared to WT mice. The greater renin instability may reflect the fact that the tubuloglomerular feedback (TGF) mechanism is absent in A1AR-/- mice, and that GFR is therefore predicted to be less stabile, resulting in greater variability of NaCl concentration at the macula densa. Thus, the purported role of TGF to stabilize NaCl excretion may be achieved not only by controlling GFR, but also by minimizing variations in renin secretion.

The role of adenosine in pressure-mediated changes in renin secretion has been examined in A1AR deficient mice both in vivo and with the isolated perfused kidney. Stimulation of renin secretion by low renal perfusion pressure has been found to remain intact [76, 77], but the inhibitory effect of an increase in perfusion pressure on renin secretion was entirely absent in isolated perfused kidneys [76]. A similar asymmetric effect of A1AR-deficiency on renin release pertains in regard to the macula densa mechanism. Examination of the stimulatory arm by furosemide-induced blockade of NaCl transport showed maintenance of increased renin release in A1AR-deficient mice [77, 78]. On the other hand, intravenous injection of NaCl causing increased NaCl concentration and transport at the macula densa reduced plasma renin concentration in WT mice, but it did not alter it in A1AR knockout mice [77]. Thus, activation of A1AR by adenosine contributes to the inhibition of renin secretion by increased perfusion pressure or increased NaCl at the macula densa while the stimulatory arms of these regulatory pathways are largely A1AR-independent.

Connexins

The usefulness of genetically modified mice is exemplified by the identification of the important role of gap junctional coupling in the function of the juxtaglomerular apparatus and the regulation of renin secretion. Although it was known that the renal vasculature and the JG cells express a number of connexins (Cx37, Cx40, Cx43, Cx45) and that gap junctional coupling is likely to account for electrotonic spreading of vasomotor responses along the vasculature in general [79, 80], the use of mice with targeted deletions permitted

conclusions about the role of specific connexin proteins in renin secretion. Cx40 is the dominant connexin linking JG cells to each other, to neighboring mesangial cells, and to vascular endothelial cells. Its inactivation at the gene level is associated with dramatic abnormalities in the JG cell phenotype [81, 82]. In the adult animal, renal vascular renin expression is normally restricted to cells in the media of the distal-most part of the afferent arteriole; in Cx40-deficient mice an expansion and redistribution of renin-expressing cells into the extraglomerular mesangium, glomerular tuft, and interstitial space is observed [83]. Together with a change towards a mesenchymal cell appearance, this suggests cell dedifferentiation since gap junction coupling is a characteristic of fully differentiated cells. Total renal renin content and plasma renin under basal conditions were significantly elevated indicating that gap junctional coupling of JG cells by Cx40 exerts an inhibitory influence, perhaps through a tonic depolarizing effect and an upward shift of cytosolic calcium. The critical role of Cx40-coupling among JG cells is emphasized by the finding that the phenotype of global Cx40 deficiency is fully mimicked by JG cell-specific deletion of Cx40 [84] whereas selective deletion of Cx40 from endothelial cells did not alter renin secretion, the distribution of JG cells, or blood pressure [84, 85]. Interestingly, elevations of plasma renin and renin mislocation were observed in transgenic mice expressing a mutant Cx40 previously found in a patient with atrial fibrillation and hypertension [86, 87]. The cause for the recruitment of “new” renin-producing cells and the implicit expansion into neighboring domains is still unknown, but this abnormality emphasizes the restrictive effect of Cx40 coupling among JG cells.

Baroreceptor-mediated inhibition or stimulation of renin secretion was found to be largely obliterated in Cx40-deficient mice suggesting that the synchronized response of JG cells to the pressure signal is achieved by Cx-40 dependent cell-to-cell communication [82, 87]. Macula densa regulated renin release as assessed by responsiveness to furosemide has also been found to be attenuated in Cx40-deficient mice [83]. Since signaling through the MD pathway is thought to involve the release of prostaglandins and possibly of other diffusible mediators it is conceivable that an increased distance of renin-producing cells from the MD imposes geometrical restrictions to transmitter efficiency. It is important to note that the secretory machinery itself seems to be intact since the stimulatory response to isoproterenol and the inhibitory effect of L-NAME were maintained in Cx40-deficient mice [82, 88]. The increase in renin production is the likely cause of the elevated blood pressure of Cx40-deficient mice [81, 82, 87] although decreased levels of eNOS expression leading to reduced NO production may contribute to the hypertension [89].

Maintenance of JG cell connectivity in the absence of Cx40 by replacing its coding sequence with that of Cx45, another connexin isoform normally found in JG cells, resulted in partial rescue of the Cx40-deficient phenotype [90]. Plasma renin activity was similar to wild type, and it responded to angiotensin II with a decrease and to enalapril with an increase, changes not seen in the Cx40 null animals. Since the conductivity of Cx45 is significantly lower than that of Cx40, these results are somewhat surprising, and they raise the question of whether some property of the channel other than conductance could be responsible for the effect on renin expression. The observation that Cx45 can substitute for Cx40 in its role to suppress renin expression is also surprising in view of the observation that deletion of Cx37 has no effect on JG cell renin formation, release, or localization pattern even though Cx37 is normally expressed by JG cells [91]. The effect of Cx45 knockin described above does not imply that Cx45 is normally involved in the control of renin synthesis and release. JG cell-specific deletion of Cx45 had no effect on the RAS and did not alter blood pressure or the regulation of renin release [92]. The reason for this appears to be the absence of demonstrable expression of Cx45 in JG cells in contrast to arteriolar smooth muscle cells where Cx45 was readily found [92]. On the other hand, a role of Cx45 had been suggested in studies in which Cx45 was conditionally deleted using Cre

recombinase under control of the nestin promoter [93]. Nestin-driven Cre activity was shown to cause reporter expression in afferent and efferent arterioles, mesangial cells, as well as JG cells. The phenotype of these mice largely mimicked that of Cx40-deficient animals in that blood pressure was elevated and renin expression and secretion was increased. In view of the extensive spectrum of cells expressing nestin and therefore of Cx45 deletion in other than JG cells the effect on the RAS in these animals may have been exerted indirectly. Overall, participation of gap junctional connectivity in renin responsiveness is in retrospect perhaps not surprising; synchronization between ensembles of JG cells may be necessary for physiological inputs to cause changes in renin secretion sufficiently large to change plasma renin.

Emerging Areas of Interest

Inhibitors of Na,K,2Cl-cotransport activity such as furosemide or bumetanide are potent stimulators of renin secretion, an effect believed to be mainly due to inhibition of the kidney-specific NKCC2 isoform and subsequent activation of the macula densa pathway. However, a threefold increase of basal plasma renin and renal renin mRNA has also been observed in mice deficient in the ubiquitous NKCC1 isoform [94, 95]. Furthermore cells from these mice did not show renin exocytosis in response to furosemide. Thus, NKCC1-mediated NaCl uptake in JG cells appears to exert direct inhibition of basal renin production and release presumably by determining resting membrane potential. Increased expression of renin and elevated plasma renin concentrations have also been observed in NHE2-deficient mice, an effect that seems to be secondary to stimulation of the macula densa COX-2 pathway [96]. How NHE2-mediated transport interferes with COX-2 activity still remains to be determined, but changes in cell volume are speculated to play a critical role [96].

Significant increases in renal and plasma renin have been found in dopamine D3-receptor deficient mice, consistent with the expectation that the Gi-coupled D2-like receptors for dopamine (D2, D3, D4) inhibit renin release [97]. Although the direct effect of activation of the Gs-linked D1-like receptors is stimulation of renin release, the D1 agonist fenoldopam caused the opposite, a significant inhibition of renin release [98]. The mechanism of this indirect action of D1 activation appears to be inhibition of proximal tubular fluid reabsorption and subsequent suppression of cortical expression of COX-2 by an elevated NaCl at the macula densa. D1 agonists have in fact been shown to suppress COX-2, and the inhibition exerted by fenoldopam was converted to stimulation in COX-2-deficient mice [98, 99]. Mice with a cell-specific deletion of the dopamine synthesizing enzyme aromatic amino acid decarboxylase in the proximal tubule have an array of disturbances that can be interpreted as representing the unchecked effects of angiotensin II excess [100].

GPR91, until now a G protein-coupled orphan receptor, has recently been identified as a receptor for succinate. It has been found to be localized in the apical membrane of cells of the cortical TAL including the MD and in endothelial cells of the afferent arteriole [101]. Activation of GPR91 by succinate stimulates COX-2 activity, PGE2 release and presumably renin expression, an effect that is particularly pronounced in diabetic wild type mice and markedly reduced in GPR91-deficient animals [102].

Components of the olfactory system including the olfactory receptor Olfr90, the olfactory trimeric G protein G_{olf}, and the olfactory adenylate cyclase isoform 3 (AC3) have been found to be expressed in MD cells [103]. Plasma renin concentration in AC3-deficient mice was reduced by about 50% compared to wild types despite increased MD COX-2 expression and augmented nNOS activity suggesting that the change in renin expression was primary. The ligands that may activate olfactory receptors on MD cells in vivo remain to be determined.

Gene deletion studies have clarified some functional aspects of the issue that some mouse strains possess a single renin gene (Ren-1c) whereas other strains in addition carry a duplicated renin gene (Ren-1d and Ren-2). Deletion of the Ren-1d gene in two renin gene mice has shown that Ren-2 generates a fully functional renin providing sufficiently high levels of active plasma renin to essentially keep arterial blood pressure normal [104, 105]. Because Ren-2 expression is known to be androgen-sensitive this effect is somewhat less effective in female animals. Nevertheless, an important characteristic of Ren-1d deficiency is that plasma levels of prorenin are markedly elevated and that the granulation of JG cells appears to be reduced [104]. Thus, Ren-2 does not seem to carry the signals needed to be sorted to the regulated secretory pathway, but is preferentially secreted as prorenin through the constitutive pathway.

Cathepsin B is able to activate prorenin in vitro, and it has been considered a likely candidate as physiological prorenin-processing enzyme because of its localization in JG cell granules [106]. However, mice with deletions of the cathepsin B gene have normal plasma levels of active renin and do not display any of the phenotypic hallmarks of renin deficiency [107]. Thus, the issue of the prorenin activator in vivo still awaits resolution.

Isolated JG cells respond to hypotonicity-induced cell swelling with an increase in renin secretion. Recent studies using membrane capacitance changes as index of exocytosis have shown that the increase in capacitance caused by hypotonicity in JG cells from wild type mice was absent in cells from COX-2-deficient or AQP1-deficient mice [108]. These observations indicate that hypotonicity causes water influx through AQP1 water channels, activation of COX-2, release of PGE2 and stimulation of renin exocytosis through the cAMP/PKA pathway. The novel findings of presence of both COX-2 and AQP1 in JG cells and of their functional interactions have interesting implications that will stimulate further investigations.

Conclusion

Genetic modification in mice has become the ultimate tool for the in vivo evaluation of the chronic effects of complete and “certified” inactivation of defined regulatory factors. Given the central role of the RAS in body fluid and blood pressure regulation it is not surprising that mutational approaches have been extensively used to gain further insights into this system. Improved understanding has been gained of the feedback inhibition of renin expression by angiotensin II, the central role of Gsβ signaling in renin control, the inclusion of COX-2 and nNOS products in regulatory pathways, the inhibitory role of adenosine in baroreceptor- and macula densa control, and the fundamental contribution of β-receptors to renin expression. New directions that have greatly benefited from the availability of novel mouse models include exciting information on the roles of connexins, GPR91, AC3, specific transport proteins, and numerous other factors. Combined with vastly improved phenotypic methods, the insights gained have been dramatic, confirming some hypotheses and dashing others. Insights with the “first generation” methodology of global over- or under-expression of genes have been important, but one may expect that widened application of improved tools for temporal and spatial control as well as insertions of known mutations will further expand the application of this approach. The expected extension of gene manipulations from mice to rats will be highly welcome in that it will facilitate and expand the phenotyping possibilities [109].

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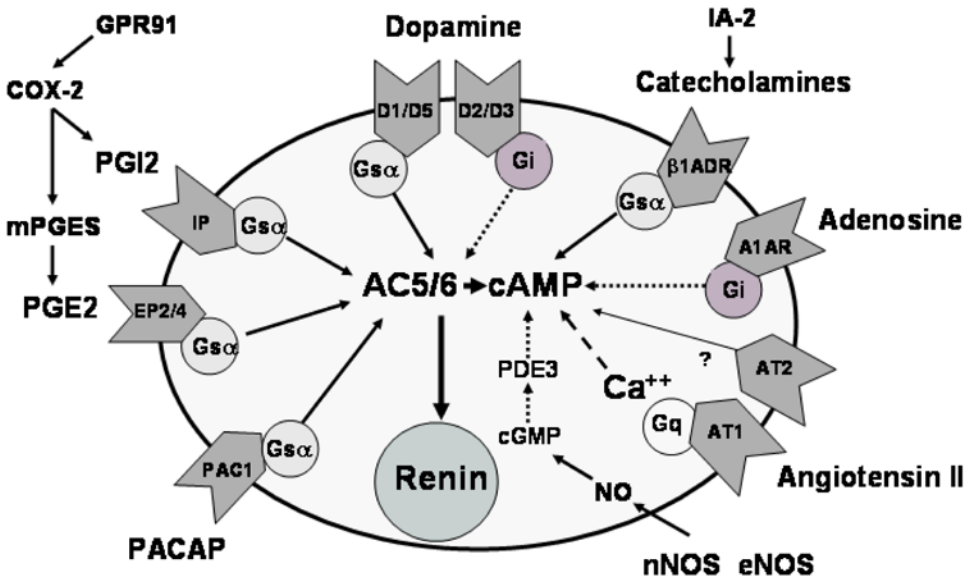

Fig 1.

Schematic overview of the majority of genetic targets discussed in this review and their effects on renin release. Oval indicates a representative JG cell and effects on renin release are indicated by arrows with solid arrows delineating direct effects (or stimulatory effects with positive deflections) and broken arrows indicating inverse effects (or inhibitory effects with positive deflections). Pathways with an even number of broken arrows (zero or two) are stimulatory and pathways with an uneven number of broken arrows are inhibitory.

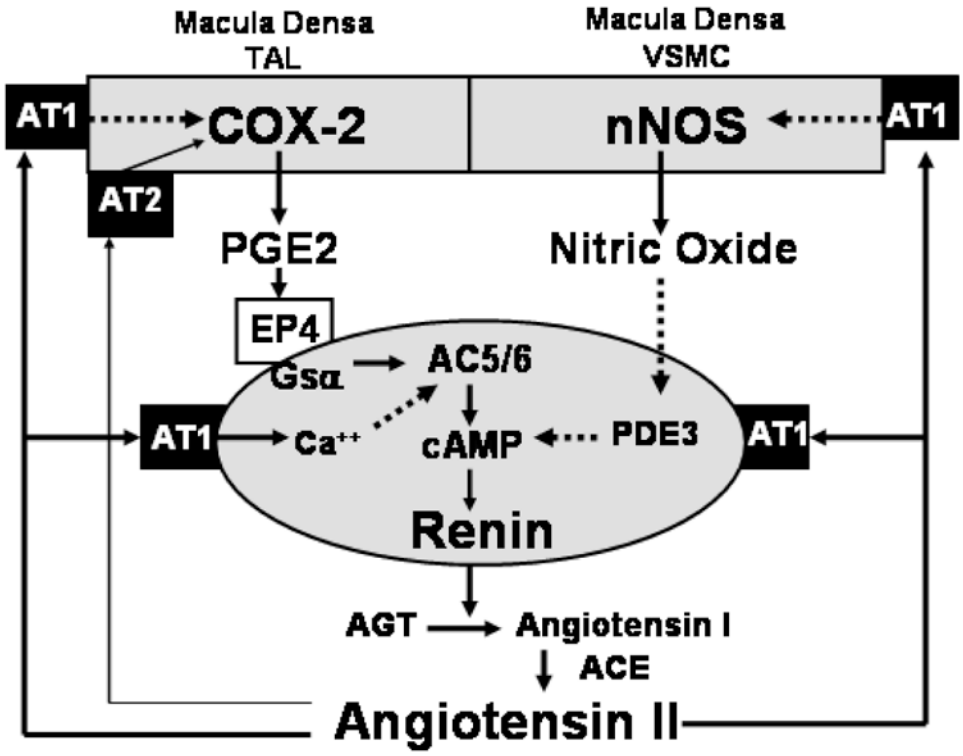

Fig. 2.

Angiotensin II feedback inhibition of renin synthesis and secretion is mediated by AT1 receptors on both JG cells and on macula densa cells. Activation of JG cell receptors directly inhibits renin whereas activation of macula densa cell receptors (perhaps also of TAL and VSMC receptors) inhibits renin indirectly through downregulation of COX-2 and nNOS. Feedback inhibition by angiotensin II is not necessarily symmetrical: reductions of angiotensin II levels rely more exclusively on the indirect pathways than increases of angiotensin II.

Solid arrows denote direct relationships or positive effects whereas broken arrows denote inverse relationships or negative effects.

TAL: thick ascending limb of Henle's loop; VSMC: vascular smooth muscle cells; PDE3: phosphodiesterase 3; AC5/6: adenylyl cyclase 5 or 6.

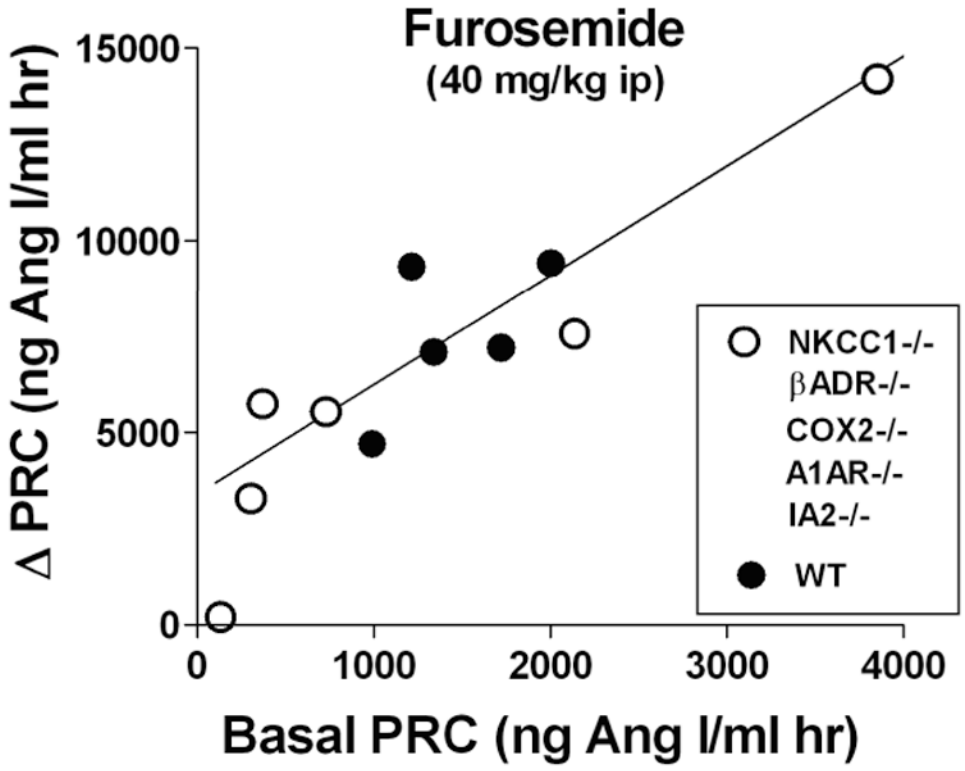

Fig. 3.

Relationship between basal plasma renin concentration (PRC) and the increase (Δ) of PRC caused by intraperitoneal injection of 40 mg/kg furosemide.

Each symbol is the mean value of 5-10 measurements of PRC in wild type (black dots) and various mutant mouse models (open circles; mutants are used to extend the limited range of basal PRC in wild type to the higher values of NKCC1-/- and the lower values of the other mutant strains). Data are taken from references [37, 61, 71, 77, 94].