The role of calcium in the regulation of renin secretion

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Beierwaltes WH. The role of calcium in the regulation of renin secretion. Am J Physiol Renal Physiol 298: F1–F11, 2010. First published July 29, 2009; doi:10.1152/ajprenal.00143.2009.—Renin is the enzyme which is the rate-limiting step in the formation of the hormone angiotensin II. Therefore, the regulation of renin secretion is critical in understanding the control of the renin-angiotensin-aldosterone system and its many biological and pathological actions. Renin is synthesized, stored in, and released from the juxtaglomerular (JG) cells of the kidney. While renin secretion is positively regulated by the “second messenger” cAMP, unlike most secretory cells, renin secretion from the JG cell is inversely related to the extracellular and intracellular calcium concentrations. This novel relationship is referred to as the “calcium paradox.” This review will address observations made over the past 30 years regarding calcium and the regulation of renin secretion, and focus on recent observations which address this scientific conundrum. These include 1) receptor-mediated pathways for changing intracellular calcium; 2) the discovery of a calcium-inhibitable isoform of adenylyl cyclase associated with renin in the JG cells; 3) calcium-sensing receptors in the JG cells; 4) calcium-calmodulin-mediated signals; 5) the role of phosphodiesterases; and 6) connexins, gap junctions, calcium waves, and the cortical extracellular calcium environment. While cAMP is the dominant second messenger for renin secretion, calcium appears to modulate the integrated activities of the enzymes, which balance cAMP synthesis and degradation. Thus this review concludes that calcium modifies the amplitude of cAMP-mediated renin-signaling pathways. While calcium does not directly control renin secretion, increased calcium inhibits and decreased calcium amplifies cAMP-stimulated renin secretion.

angiotensin; cAMP; juxtaglomerular; adenylyl cyclase; phosphodiesterase

RENIN IS AN ASPARTYL-PROTEASE enzyme produced and activated within the juxtaglomerular (JG) cells of the afferent arteriole in the kidney. It is a monogamous enzyme, acting only on the leucyl-leucine (in mouse and rat) or leucyl-valine (in humans) bond of its single substrate, angiotensinogen, to release the amino-terminal decapeptide angiotensin I (51). Angiotensin I is the precursor of the octapeptide angiotensin II, which is the primary biologically active hormone of the renin-angiotensin system. The vast array of actions of angiotensin II include the regulation of blood pressure, vasoconstriction, increasing aldosterone secretion, amplifying sympathetic activity, increasing sodium retention, promoting cell growth and angiogenesis, as well as numerous other actions. It is considered a factor in virtually every form of hypertension, and it is a therapeutic target in numerous diseases including hypertension and heart failure. Since the half-life of angiotensin is less than a minute and the circulating level of angiotensinogen from the liver normally remains constant and in excess (except in mice) (66), renin secretion is the critical rate-limiting step in the activity of the entire system. Because of this, the factors regulating renin secretion from the JG cells are of particular interest and importance in understanding the physiology of the renin-angiotensin-aldosterone system as well as understanding therapeutic targets for a myriad of cardiovascular diseases.

Calcium is a factor in virtually all modes of biological secretion, and its role is particularly novel in the JG cells. This review will limit its scope, focusing on the role(s) of calcium in regulating renin secretion. Also, this review will focus only on regulation of active renin from the JG cells. There are other secondary intrarenal (afferent and interlobular vessels, connecting segment-collecting duct) and extrarenal (submandibular gland, blood vessels, brain, adipose tissue, gonads, etc.) sites of renin production, but there is little information on how renin synthesis, release, or secretion may be influenced by calcium at these sites. Additionally, the JG cell constitutively releases inactive renin by a nonregulated pathway into the circulation as a function of prorenin synthesis and storage. The reader can find more extensive reviews on renin, including the older yet still definitive work of Keaton and Campbell (51), and more recently the excellent review of Schweda et al. (99).

The JG cells lie in the wall of the distal terminus of the afferent arteriole abutting the entrance to the glomerulus (Fig. 1). On one side they have an endothelial interface with the vascular lumen into which the active renin is released into the circulation from the JG cells. Juxtaposed to this, on the other side, is the interstitial space separating the JG cells from the macroa densa at the terminal end of the thick ascending limb (TAL) of the loop of Henle. Renin is also released into this renal cortical interstitium. However, since the renal circu-
lation constantly sweeps away secreted renin, it may create a diffusion sink favoring movement of renin toward the vascular lumen. At the distal end of the juxtaglomerular apparatus (JGA), the JG cells contact the extraglomerular mesangial cells at the hylus of the glomerulus, while at the proximal end they interface with the vascular smooth muscle cells of the afferent arteriole with which they share derivation from common progenitor cells (104).

The primary stimulatory second messenger for renin secretion is the cyclic nucleotide cAMP (15). It is thought that renin secretion is not initiated through classic calcium-mediated vesicle-cell membrane fusion but rather by granule-to-granule fusion (34). However, the actual mechanism has yet to be resolved. Intracellular calcium is proposed to be an important “second messenger” in the control of renin secretion (89). Renin secretion is stimulated by decreasing extracellular calcium in vitro (2, 15, 34, 88), and also decreasing intracellular calcium (15, 34, 79, 124) in the JG cell. These two phenomena are presumed to be linked (34). The calcium concentration gradient into JG cells is one million to one, producing a large electrochemical gradient for calcium influx. Stability of the JG cell requires low calcium permeability, an efficient calcium efflux mechanism, and an intracellular system to sequester excess calcium. While extracellular concentrations of ionized free calcium are generally high, \( \sim 1–1.5 \text{ mM} \) (69), calcium concentration inside the cell is tightly controlled and generally kept at or below 100 nM. Calcium enters the cytoplasm from either the extracellular fluid or by release from intracellular stores. Calcium is buffered by mitochondrial uptake and calcium sequestration in the endoplasmic reticulum (100, 101), but there must also be pathways for calcium extrusion to maintain this gradient. While factors that increase intracellular calcium can retard renin stimulation by cAMP (2, 7, 120), such stimulation can proceed despite changes in intracellular calcium or calmodulin activity (2, 20, 33). Thus, while calcium may modify the magnitude of cAMP’s effect, cAMP can stimulate renin independently from changes in intracellular calcium. This is because calcium acts indirectly on renin by modifying the activity of adenylyl cyclase (40, 80, 81), as described later in this review. Angiotensin II, which increases intracellular calcium (29), also inhibits adenylyl cyclase (41). All of these observations suggest that calcium does not directly affect renin secretion but acts indirectly by decreasing adenylyl cyclase activity in the JG cells and therefore retarding the synthesis of cAMP and the release of renin.

While a number of in vivo and in vitro studies suggest an inverse relationship between plasma, perfusate, or incubate calcium concentration and renin secretion, it was Park and Malvin (87) who first suggested a direct inverse relationship between intracellular calcium concentration in the JG cell and its release of renin. Because this inverse relationship between calcium and secretion is opposite to all other secretory cells (except for the chief cells of the parathyroid), it is often referred to as the calcium paradox (40, 81).

Fuchs et al. (35) reported that increased JG intracellular calcium also suppressed renin gene expression. They found that a negative calcium response element is involved in the calcium repression of renin expression and that this is mediated by calcium-induced translocation of Ref-1 to the nucleus, where it binds and represses the renin promoter and therefore transcription of the renin gene. Thus, not only does calcium acutely suppress renin secretion, but more chronic changes in intracellular calcium may also suppress renin synthesis. However, regulation of renin synthesis by calcium is not a focus of this review.

**In Vivo Studies on Calcium and Renin**

In the 1970s, a series of studies used acute or chronic hypercalcemia in vivo to try to define the relationship between calcium and renin secretion. Kotchen et al. (58) used acute intrarenal CaCl\(_2\) or calcium gluconate (57) infusion in dogs to
increase the plasma calcium concentration by 50%. While neither renal blood flow (RBF) nor blood pressure changed, they observed increased calcium excretion and a significant 70% decrease in plasma renin activity (PRA). Similar experiments using increased renal calcium delivery in sodium-deplete dogs (120) also found renin suppressed by 70%, but this was accompanied by a significant decrease in RBF. However, Iwao et al. (50) found direct intrarenal infusion of CaCl₂ in dogs increased renal venous plasma calcium by 50% but did not affect peripheral circulating calcium levels. This increased calcium in the renal circulation produced no net change in the renin secretion rate. In rats, chronic calcium loading (56) changed neither serum calcium levels nor PRA, although calcium excretion was increased. However, in sodium-depleted rats fed a high-CaCl₂ diet (56), PRA was decreased by 36%, even though serum calcium remained unchanged. In humans, CaCl₂ infusion at a level which more than doubled serum calcium had no effect on PRA (28), but if the subjects were sodium-depleted, infusion of either calcium or NaCl decreased PRA (54). Attempts to induce hypocalcemia have not resulted in changes in PRA (64). Due to the inconsistencies under basal conditions, and the inhibition by calcium observed when renin was first stimulated, the conclusion derived from these various in vivo studies, as articulated by Kotchen (56), was that changes in intrarenal serum calcium concentration probably do not directly modify renin secretion but may affect its stimulation by modifying the activity of renin-stimulating pathways.

**Calcium Channels**

Renin secretion is coupled to the electrical potential of the JG cell. Depolarization inhibits renin release (19) while hyperpolarization increases renin release. A large number of in vitro studies have used KCl depolarization to modify renin-releasing signals (reviewed in Ref. 51), and elegant patch-clamp studies have addressed the changes in potential during granular release in the JG cell (31). While there is no doubt the polarity of the cell affects renin, the question becomes whether these changes in JG cell potential are critical in the calcium-mediated pathways regulating renin. One such pathway is the voltage-gated L-type calcium channels. L-type calcium channels appear to be the pathway for calcium entry in response to angiotensin II-induced renin inhibition, and inhibition of the L-type channels stimulates renin release (14, 19, 31). However, it has been questioned whether changes in potential necessary to open L-type channels can occur in vivo (99). In addition to L-type calcium channels, T-type calcium channels are also voltage dependent and mediate calcium influx. However, whether T-type channels are directly involved in regulating renin release from JG cells, or coupled with the changes in JG cell intracellular calcium, has not been tested.

The release of calcium from the endoplasmic reticulum (100) is an effect magnified by store-operated calcium entry. Store-operated calcium entry occurs when sequestered calcium release depletes stores in the endoplasmic reticulum, and this increase in cytosolic free calcium is coupled to opening divalent cation channel(s) in the plasma membrane, permitting entry of extracellular calcium. Store-operated calcium channels are distinct from voltage-gated L-type channels and from arachidonic acid metabolite-stimulated calcium entry. Schweda et al. (101) have suggested that calcium entry and suppression of renin release from the JG cells is at least partially mediated by these store-operated calcium channels. Induction of store-operated calcium entry by inhibitors of the endoplasmic reticulum calcium ATPase also suppresses renin release from JG cells (101). Angiotensin II stimulation of the G protein-coupled AT₁ receptor results in transient calcium release from the endoplasmic reticulum. This calcium signal can be amplified by activation of the ryanodine receptor (RyR) (30). The RyR is a gate for calcium-induced calcium release (30) such that increases in intracellular calcium trigger additional calcium release from intracellular stores. Calcium release mediated by RyR is enhanced by cyclic adenine diphosphate ribose, the physiological allosteric modulator of the RyR. Cyclic adenine diphosphate ribose and calmodulin sensitize the RyR to calcium, augmenting additional calcium-stimulated calcium release into the cytoplasm. While the RyR is considered ubiquitous, it has not been directly shown in the JG cells. However, Fellner and Arendshorst (29, 30, 107) have shown that the RyR exists in the renal afferent arteriolar vascular smooth muscle where its activation exaggerates renal vasoconstriction and increases intracellular calcium. Thus, since the afferent smooth muscle cells and the JG cells are derived from common progenitor cells (104), it is possible that calcium-mediated pathways seen in the afferent arteriole are also present in the JG cells.

**Adenylyl Cyclase and cAMP**

All factors that stimulate cAMP in the JG cells, including prostaglandins, kinins, and β-adrenergic agonists, have a stimulatory effect on renin. Protein kinases are important signal transducers of cyclic nucleotides, and cAMP acts on protein kinase A, promoting an undefined cascade that results in active renin release from storage granules in the JG cells (94). Thus the common element in all renin-stimulating pathways is the product of adenylyl cyclase activity, the second messenger cAMP (15, 100). There are at least nine isoforms of adenylyl cyclase (23). Two of these isoforms (types V and VI) are inhibited by elevated intracellular calcium (72). The fact that renin release is inhibited by increased intracellular calcium and stimulated by cAMP prompted two laboratories to simultaneously publish evidence that when they reduced intracellular calcium, renin release was stimulated by enhancing cAMP levels through activation of adenylyl cyclase V and/or VI. Ortiz-Capisano et al. (81) reported that in isolated mouse JG cells, Western blots using an antibody that recognized both adenylyl cyclase types V and VI were positive. Using the same antibody for immunofluorescence in a primary culture of the JG cells showed intense focal cytoplasmic staining in the renin-positive cells. Reducing intracellular calcium with the cytosolic calcium chelator 5’5 dimethyl BAPTA-AM increased both JG cell cAMP content and renin release, and an adenylyl cyclase inhibitor completely blocked this response. They concluded (81) that low calcium increased the activity of one or both of the calcium-inhibitable isoforms of adenylyl cyclase, types V and/or VI. These exist within the JG cell such that decreased intracellular calcium stimulates adenylyl cyclase, resulting in cAMP synthesis and consequently renin release.

At the same time, the laboratory of Schweda (40), also using isolated JG cells, reported that calcium-dependent inhibition of renin by such agents as angiotensin, endothelin, and thapsig-
Renin secretion is controlled through a complex interaction of at least four different regulatory pathways (22): the renal baroreceptor, renal nerves, the macula densa, and the actions of certain hormones and autacoids (15). The common element in all of these renin-stimulating pathways is the second messenger, cAMP (15). This review will focus primarily on proposed involvement of calcium in each of these pathways.

The renal baroreceptor. The idea of a renal baroreceptor mechanism was first introduced by Goormaghtigh (37) in 1944. However, it was Tobian (110) who showed that prolonged high pressure resulted in a 50% decrease in JG cell granulation, suggesting renin was inversely related to renal perfusion pressure. This association between theafferent arteriolar perfusion pressure and regulation of renin secretion by the JG cells independently of other renin-stimulating pathways was shown using nonfiltering kidneys (22, 122). In the unanesthetized experimental animal, basal renin secretion remained low throughout the range of RBF autoregulation, but starts increasing dramatically below this range, peaking at ~60 mmHg (22). Anesthesia tends to increase the basal renin secretion concurrent with reduced renal perfusion within the autoregulatory range, but the secretion increases at a faster rate when pressure drops below the lower limits of autoregulation (102). While the baroreceptor remains one of the most potent signaling pathways for renin, its mechanism is still unclear. Hemodynamic forces, including vascular shear stress (3, 22, 39, 116) and cyclic strain (3, 22, 39), may influence the afferent arteriolar smooth muscle cells, the JG cells, or the arteriolar endothelium.

Renin release has been shown to depend on decreased perfusion pressure but not changes in RBF (73). Renin secretion is inhibited by high renal perfusion pressure through the renal baroreceptor mechanism (32, 51). Elevated renal perfusion with myogenic vasoconstriction leads to increased shear stress, the primary stimulus of endothelial nitric oxide (NO). Its second messenger, cGMP, can activate protein kinase G-II (PKG-II) in JG cells, which has been shown to inhibit renin (46). While not resolved, this signal may contribute to the suppression of renin at high levels of renal perfusion. Additionally, Schweda et al. (102) have recently reported renin inhibition following elevated renal perfusion is mediated by adenosine A1 receptors, suggesting local adenosine formation is a key factor in the inhibitory arm of the renal baroreceptor. It is also proposed that calcium plays a role in the coupling of renin secretion to the renal baroreceptor, as increasing perfusion pressure depolarizes afferent arteriolar smooth muscle cells and presumably also JG cells, constricts the arterioles, and induces calcium influx (25). Depolarization of JG cells inhibits renin secretion (19). Decreased renal perfusion reverses these steps, leading to vasodilation and changes permissive for cAMP formation and increased renin secretion.

Yao et al. (124) have described that mechanical stimulation of a single JG cell initiated propagation of a calcium wave to surrounding cells, and this was dependent on ATP and purinergic receptors. The data suggested that ATP mediates propagation of intercellular calcium signaling. Mechanical stretching of primary cultures of JG cells increased ATP release threefold, and ATP infused into an isolated, perfused kidney model produced rapid and persistent inhibition of renin release and an increase in renal vascular resistance. The stimulus for renin produced by decreasing renal perfusion pressure was largely blocked in the presence of ATP. The authors concluded (124) the mechanical stimulation of the afferent arteriole and JG cells by altered perfusion results in ATP release, which is a paracrine mediator of renin release through modulation of intra- and intercellular calcium. Furthermore, the increase in renal perfusion pressure leads to mechanically coupled ATP release, which acts on purinergic receptors to coordinate a local increase in intracellular calcium. This leads to inhibition of renin secretion.

The β-adrenergic pathway. The sympathetic nervous system is an important regulator of renin secretion (22). The classic sympathetic pathway for regulating renin is through β-adrenergic sympathetic activation, which stimulates the JG cells to release renin. JG cells contain β1-adrenergic receptors (9, 21), which are G protein-coupled transmembrane receptors which, when activated, propagate signals activating adenylyl cyclase (67, 108), stimulating phospholipases, and altering ion channels to reduce intracellular calcium (93, 108). β-Adrenergic activation stimulates renin secretion, both in vivo (21, 47) and in vitro (25), via a cAMP-dependent mechanism. This pathway seems to be independent of baroreceptor or macula densa.

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gin was prevented by small interfering RNA-mediated knockdown of adenylyl V and adenylyl VI. They concluded that “the suppressive effect of calcium liberators on renin release is mediated by inhibition of adenylyl cyclase activity” (40). Thus these two laboratories present a novel resolution to the controversy of the calcium paradox: that increasing intracellular calcium inhibits adenylyl cyclase (V and/or VI) activity, decreasing cAMP formation, and therefore inhibiting renin.

A follow-up study by Ortiz-Capisano et al. (80) found that only the adenylyl cyclase isoform V was present in mouse JG cells. They looked for both isoforms V and VI using Western blots with antibodies specific for either of the two calcium-inhibitable isoforms. They also found the immunolabeling of JG cells was only positive for adenylyl cyclase V and not for isoform VI. They found JG cell cAMP formation and renin release were stimulated by reducing intracellular calcium with BAPTA-AM. This positive response was completely blocked with a selective adenylyl cyclase V inhibitor, while an isoform VI inhibitor had no effect. They concluded (80) that lowering JG cell intracellular calcium allows an increased activity of the calcium-inhibitable isoform adenylyl cyclase V (but not adenylyl cyclase VI), stimulating cAMP and renin release. This result, in concert with the previous two studies (40, 81), suggested the calcium-inhibitable adenylyl cyclase V isoform mediates the calcium paradox. Thus changes in intracellular calcium directly decreased the activity of the adenylyl cyclase, modifying the amplitude of its response to various cAMP-driven renin stimuli. Because the increase in renin was completely accounted for by adenylyl cyclase V activity, it suggests there is no alternative (non-cAMP-mediated) pathway by which a decrease in intracellular calcium might stimulate renin release and that basal intracellular calcium levels quench adenylyl cyclase activity as predicted by Park and Frey (84). Furthermore, reducing intracellular calcium should stimulate (or disinhibit) adenylyl cyclase V’s enzymatic activity. The subsequent pathway by which cAMP initiates renin release is controversial and remains largely unresolved.

Classic Pathways for Stimulating Renin

Renin secretion is controlled through a complex interaction of at least four different regulatory pathways (22): the renal baroreceptor, renal nerves, the macula densa, and the actions of certain hormones and autacoids (15). The common element in all of these renin-stimulating pathways is the second messenger cAMP (15). This review will focus primarily on proposed involvement of calcium in each of these pathways.
signaling (27). \( \beta_1 \)-Adrenergic stimulation of renin has been shown to be (partially) dependent on Gs-coupled phospholipase A activation and prostaglandin (PG) \( E_2 \) synthesis (55, 108). Basal tonic \( \beta \)-adrenergic activity is thought to be necessary to maintain normal PRA and maintain an adequate “pool” of renin to respond to acute stimuli (52). Low level \( \beta \)-adrenergic stimulation, at a level which does not influence RBF or blood pressure, has been shown to enhance other renin-stimulating signals (108).

While \( \beta \)-adrenergic agonists stimulate adenyl cyclase activity, they have also been suggested to stimulate calcium efflux from a variety of smooth muscle cells (15). This may result from increased Na-K-ATPase activity, which stimulates calcium efflux through the sodium-calcium exchanger (15), or through activation of a calcium ATPase (112) which would increase calcium transport, reducing intracellular calcium. Such mechanisms shown in vascular smooth muscle cells could be extrapolated into the JG cell to explain how \( \beta \)-adrenergic activation may also alter calcium, although such a pathway has not been directly shown in JG cells.

In contrast, any distinct and consistent role for \( \alpha \)-adrenergic regulation of renin is inconclusive and controversial. In large part, changes in renin secretion mediated by \( \alpha \)-adrenergic activity can be explained by coincident hemodynamic and reabsorptive changes in the kidney, as previously reviewed by Moss et al. (68). Any role for calcium, other than secondary to renal vasoconstriction, is not defined.

**The macula densa.** Renin is also regulated through the macula densa, a specialized region of the distal tubule adjacent to the glomerular hilus and the JG cells (Fig. 1). Changes in tubular NaCl delivery to the macula densa create a signal to modify renin release from the JG cells (22). An increase in glomerular filtration rate increases distal delivery of NaCl, and these changes are sensed by the cells of the macula densa. This message is then fed back to the JG cells and afferent arteriole. Increased sodium delivery to the TAL increases Na-K-2Cl (NKCC2) cotransporter activity, resulting in increased sodium in the macula densa cells and the TAL.

In vivo, reducing luminal NaCl concentration delivered to the macula densa stimulates renin secretion in a cylooxygenase (COX)-2-mediated mechanism (112). Lorenz et al. (65) first showed that when the apical macula densa is exposed to high chloride concentrations, renin secretion was suppressed. This was the first demonstration of the chloride dependence of the macula densa regulation of renin secretion. Similarly, in rabbit TAL cells in primary culture (11), COX-2 expression increased with low NaCl, or when NKCC2 was inhibited with a diuretic. Substitution of chloride increased COX-2 expression but substitution of sodium did not. Graded reductions in NaCl delivery to the macula densa resulted in PGE\(_2\) synthesis (92), which was exaggerated by a low-salt diet. The response was blocked by a COX-2 inhibitor, showing that reduced distal NaCl delivery produced an increase in COX-2-derived PGE\(_2\) synthesis from the macula densa, and the signal was enhanced by chronic salt restriction.

In a macula densa cell line, reduced NaCl exposure resulted in PGE\(_2\) synthesis due to increased COX-2 activity (123). Prolonged low-salt exposure also increased COX-2 expression in a chloride-dependent fashion. This was accompanied by phosphorylated (activated) p38 kinase and ERK1/2 kinases. Mitogen-activated protein (MAP) kinase inhibition disrupted the stimulation by low NaCl of PGE\(_2\) release and COX-2 expression. Decreased NaCl or chloride delivery alone upregulates cortical thick ascending limb COX-2 expression via a p38 kinase-dependent pathway (10, 11, 12, 123). p38 stimulates COX-2 expression in the macula densa by transcriptional regulation, predominantly via a nuclear factor (NF)-\( \kappa B \)-dependent pathway and by posttranscriptional increases in mRNA stability (10).

Cheng et al. (12) found NO increased p38 expression in the macula densa, stimulated NF-\( \kappa B \) binding activity, and induced COX-2 expression in cultured TAL cells. These data suggest upregulation of neuronal nitric oxide synthase (nNOS) in the macula densa amplifies the COX-2 mediated response to distal chloride delivery and further enhances renin secretion. Peti-Peterdi and Bell (91) found increases in the luminal chloride signal to the macula densa were directly proportional to increases in macula densa cytosolic calcium. These changes were blocked by luminal furosemide, basolateral nifedipine, or inhibition of chloride channels. They suggested that cells in the macula densa detect changes in distal chloride delivery through a pathway including the NKCC2 cotransporter, basolateral membrane depolarization through chloride channels, and calcium entry through voltage-gated calcium channels (91). The role of increased macula densa calcium is not clear, but could be involved as a cofactor in nNOS activation.

Increased activity of the NKCC2 cotransporter in the TAL and the macula densa results in salt-induced changes in p38 and ERK1/2 MAPK activity. This results in COX-2 upregulation and activation, increasing PGE\(_2\) synthesis and release (46). PGE\(_2\) synthesis in the macula densa, transmitted to JG cell PGE\(_2\) (EP4) receptors, is the primary macula densa pathway for stimulating JG cell adenyl cyclase and cAMP production, leading to increased renin secretion (75). In addition, diminished NaCl reabsorption results in decreased reabsorption and reduced renal cortical interstitial calcium (57).

While nNOS in the macula densa is upregulated by low salt (46), the PGE\(_2\)-mediated stimulation of cAMP (75) is amplified by nNOS-NO-generated cGMP (98). The cGMP inhibits phosphodiesterase (PDE)-3 catabolism of cAMP, further potentiating the PGE\(_2\)-stimulated formation of cAMP and amplifying its stimulation of renin release (6, 94, 98). While reduced distal chloride delivery or chronic salt deprivation stimulates renin secretion, increased distal salt delivery or chronic sodium loading inhibits it. This may be due to a reversal of the signaling described for the response to decreased chloride delivery (see above). Thus stimulation of COX-2 and nNOS would be reversed, suppressing the PGE\(_2\)-mediated signal for renin secretion. Additionally, increased sodium reabsorption in the TAL stimulates the sodium-calcium exchanger on the basolateral membrane to move out sodium. This allows calcium to enter transiently, increasing its intracellular concentration (95). Increased sodium load to the TAL enhances sodium reabsorption and consequently the movement of calcium from the lumen to the interstitial space surrounding the JG cells. However, only small changes in intracellular calcium occur in response to variations in tubular salt delivery (91), suggesting calcium does not remain in the cells. Calcium may be rapidly extruded into the cortical interstitium, or may not enter the cells in any great amount. Calcium exits the TAL predominantly through a paracellular pathway driven by the lumen-positive potential. Thus increased NaCl reabsorption increases...
calcium reabsorption (1). Since the TAL is water-impermeable, the interstitial calcium concentration increases as calcium reabsorption occurs.

Tight junctions play a key role in mediating paracellular calcium reabsorption in the TAL. Paracellular reabsorption of calcium in the TAL occurs through paracellin-1, an epithelial protein in the claudin family of tight junction proteins (105). It is located primarily in the TAL, as well as in the distal tubule and collecting duct (121). Deactivating mutations of paracellin-1 results in hypercalciuria hypomagnesaemia syndrome, characterized by impaired calcium and magnesium reabsorption in the TAL (8). Furthermore, paracellin-1 mutations eliminate furosemide-induced increases in calcium excretion, as the paracellular transport of calcium is already impaired (8). Thus increases in TAL and distal NaCl delivery result in increased calcium reabsorption, increasing interstitial calcium in the cortical environment of the JG cell. Palmer et al. (82) have reported chronic dietary sodium loading significantly increased the concentration of cortical interstitial calcium by 25%. Renal cortical interstitial calcium was determined by the Bukoski dialysis technique (69). While it is established sodium loading suppresses renin synthesis and secretion, the study did not directly address this interaction. However, such remarkable increases in interstitial calcium, similar to what has been reported after increasing the calcium concentration in incubation media in vitro (18, 88, 100), should increase intracellular calcium, and therefore reduce calcium-inhibitable adenyl cyclase activity, cAMP formation, and ultimately reduce renin release.

Tubuloglomerular feedback (TGF) responses to increased NaCl delivery to the macula densa are transmitted via adenosine (17) or ATP (111). The TGF response operates when renin secretion is suppressed. Therefore, part of the renin-inhibiting message from the macula densa in response to increased distal delivery is transmitted to the JG cells by adenosine (17) or possibly ATP (111). Adenosine receptors have different effects on renin, in that adenosine A1 agonists decrease and A2 agonists increase renin concentration (16). Adenosine at nanomolar concentrations or adenosine A1-selective agonists constrict the renal vasculature and inhibit renin release in vitro (70,) and in vivo (71). This inhibition is blocked by calcium chelation, suggesting A1 activation increases intracellular calcium via calcium entry (17). It was also proposed at higher, micromolar concentrations, when adenosine A1 receptors are saturated, adenosine acts on A2 receptors, decreasing intracellular calcium, causing afferent vasoconstriction, and stimulating renin release in vitro (17, 70). The macula densa signal for renin should be permissive in the absence of adenosine. These studies suggest a concentration-dependent biphasic calcium-mediated effect of adenosine on renin release via adenosine created by TGF and the macula densa. However, A2 receptor stimulation of renin has not been shown in vivo.

Similar to adenosine, ATP has been shown to directly constrain renal afferent arterioles through a P2 type ATP receptor-mediated mechanism, resulting in a concentration-dependent increase in intracellular calcium (49). Activation of P2Y G-protein coupled ATP receptors has been linked to increased intracellular calcium and calcium oscillatory transients in HELA cell culture (77). Liu et al. (63) have reported P2Y receptors on the basal lateral surface of the macula densa respond to ATP (but not adenosine) to increase intracellular calcium. This response is mediated by phospholipase C. While such receptors have not been characterized in the JG cell, ATP P2 receptors may exist, such that ATP receptor activation would increase intracellular calcium (74), inhibiting renin.

Receptor-Mediated Calcium Mobilization

Arthur Vander first proposed direct negative feedback inhibition of renin secretion by angiotensin in 1965 (114). This observation has been confirmed by numerous studies in vivo and in vitro, both on basal renin and stimulated renin secretion (51). Vandongen and Pert (115) used changes in perfusate calcium in an isolated, perfused kidney to show that the angiotensin II inhibition of renin release is dependent on the presence of extracellular calcium. Angiotensin II is a powerful vasoconstrictor and is well known to induce vasoconstriction through AT1 receptor-mediated increases in intracellular calcium (97). This response is similar to the response to other G protein-coupled, receptor-mediated, calcium-mobilizing hormones like vasopressin (61) and endothelin (96), which also inhibit renin release from JG cells (Fig. 2). Angiotensin AT1 receptors have been described in the renal afferent arteriole, JGA, and mesangial cells (125). AT1 receptor activation by angiotensin II to increase intracellular calcium in the JG cell may constitute an important component of the negative feedback suppression of renin under conditions of high circulating angiotensin levels.

The calcium-sensing receptor and the JG cell. Because the parathyroid gland exhibits the calcium paradox similar to the JG cell, and the intracellular calcium and calcium signaling in this gland are mediated by calcium-sensing receptors (CaSR), it seemed possible JG cells might also contain CaSR (Fig. 2). This would provide a signaling pathway between the changes in extracellular calcium and intracellular calcium-mediated events regulating cAMP formation and stimulating or inhibiting renin release. The CaSR is a G protein-coupled receptor whose activation increases intracellular calcium by activating phospholipase C (PLC) via Gq (117). CaSR sense and translate micromolar changes in extracellular calcium into changes in intracellular calcium (97). It has been shown in several systems (48, 76, 118) that CaSR coupling to a Gq11 subtype protein activates PLC. This results in increased membrane phosphatidylinositol biphosphate (PIP2)-turnover, and the resultant production of inositol-1,4,5-triphosphate (IP3) and diacylglycerol. IP3 activation interacts with IP3 receptors on the endoplasmic reticulum to release calcium from intracellular stores (100). Diacylglycerol production leads to activation of calcium-dependent protein kinase C (PKC) and subsequent increases in intracellular calcium (116). It has also been shown that IP3-sensitive calcium store depletion activates plasma membrane and store-operated calcium channels (109), while PKC may act on either intracellular calcium stores or plasma membrane channels to increase intracellular calcium. The half-maximal effective calcium concentration (EC50) for the CaSR is 1.2 mM, which is the normal concentration of interstitial free ionized calcium in the renal cortical interstitium (82). These data provide a possible signaling pathway between CaSR activation and increased intracellular calcium in the JG cells.

Ortiz-Capisano et al. (79) found CaSR in freshly isolated or primary cultures of mouse JG cells. They used RT-PCR, Western blotting, and immunofluorescence. To test whether
CaSR actually regulate renin release, JG cells were incubated with the calcimimetic cinacalcet HCl, a CaSR agonist. Activation of the CaSR significantly decreased both JG cell cAMP formation and renin release by ~45%. They concluded that JG cells express CaSR, and CaSR respond to changes in extracellular calcium to modify or activate calcium-mediated intracellular signaling (79). This is consistent with their hypothesis that adenyl cyclase V is the calcium-inhibitable source for cAMP regulation of renin in the JG cell (80), and so CaSR activation leads to inhibition of cAMP formation and suppression of renin release (Fig. 2). They also found CaSR activation by a calcimimetic inhibits renin release from JG cells only when extracellular calcium is present (unpublished observations), such that when extracellular calcium was reduced to zero, CaSR activation has no effect on renin. This suggests extracellular calcium is necessary for CaSR activity. However, the mechanisms by which activation of CaSR changes intracellular free calcium concentration, or how calcium-regulated signals downstream are activated, is unclear. There is likely more than one such pathway involving calcium entry or release (Fig. 2).

**Calcium-Calmodulin**

Calmodulin is a calcium-binding protein which, when complexed with calcium, may mediate many calcium-dependent cellular activities (113). It has been reported since 1983 that calmodulin is involved in some way with the calcium paradox and calcium-mediated inhibition of renin secretion (24, 42, 85). A variety of calmodulin inhibitors, including calmidazolium, W-7, W-13, and trifluoperazine, all similarly increased renin release (85) from renal cortical slices in various species. W-7 reversed the inhibition of renin induced by a high extracellular calcium medium in rat renal cortical slices (7). Kurtz et al. (24) found calmodulin inhibition stimulates exocytosis of renin in isolated mouse JG cells. Calmodulin inhibition has also been found to stimulate renin secretion in isolated, perfused kidneys (42). Several studies have shown (24, 42, 85) that W-7 increased basal renin release, as well as cAMP formation.

The laboratory of Park (53) proposed that myosin light chain in the JG cell may be a target protein involved in calcium-calmodulin regulation of renin secretion. They suggested formation of the calcium-calmodulin complex results in reversible phosphorylation of the myosin light chain by calcium-calmodulin-dependent myosin light chain kinase, inhibiting renin secretion. They had previously shown calmodulin inhibition with calmidazolium (83) reversed inhibition of renin secretion by calcium. In their model of rat renal cortical slices, the myosin light chain kinase inhibitor ML-7 increased renin release under basal conditions and reversed calcium-mediated renin inhibition (53). They proposed renin release stimulated by osmotic swelling of the renin-containing granules in the JG cell could be inhibited by addition of calcium, and calcium inhibition could be reversed by the myosin light chain kinase inhibitor ML-9 (86). They concluded renin secretion may involve chemiosmotic swelling of the JG cell renin-secretory granules, which is regulated by calcium-calmodulin-dependent myosin light chain kinase regulation of an actomyosin interaction (86). While Park presents a novel and provocative explanation of calcium-calmodulin regulation of renin, classic characterization of the JG cell found it devoid of myosin (106), even though they do share a common progenitor origin with the arteriolar smooth muscle cells of the afferent arteriole (103) which do contain myosin. Furthermore, Park’s in vitro model of renal cortical slices contains all the non-JG cell types of the renal cortex including the adjacent mesangium and vascular smooth muscle cells, which, under the experimental conditions, could possibly influence renin release in this preparation.

**Phosphodiesterases**

Phosphodiesterases (PDEs) are enzymes that hydrolyze the 3’-phosphodiester bond of cAMP and cGMP and degrade them into their respective inactive non-cyclic nucleotide metabolites, thereby regulating both their steady-state levels and function (4, 27). While there are as many as 11 families of PDE’s (and most consist of more than one gene), only a few have been localized to the kidney and may play an important role in renal function (4, 27). Many in vitro studies of renin using various preparations have used nonselective PDE inhibition to enhance renin release, suggesting that PDE degradation of cAMP within the JG cell is a significant factor in regulating renin release.
release. PDE-3 hydrolyzes both cAMP and cGMP but has a much higher \( V_{\text{max}} \) for cAMP than for cGMP, so it behaves more as a selective cAMP-degrading PDE (27). Since PDE-3 binds cGMP, which is poorly hydrolyzed, cGMP acts as an inhibitor of PDE-3 and is often referred to as the cGMP-inhibitable, cAMP-degrading PDE (36). Chiu and Reid (13) noted cAMP levels in the kidney were regulated by both its production and its metabolism. Inhibition of PDE-3, which should decrease cAMP degradation, increased basal and isoproterenol-stimulated renin. Kurtz et al. (60) also presented evidence that cAMP-stimulated regulation of renin is mediated by PDE-3. It has also been shown in vivo (6, 98) that endogenous production of cGMP by NO might directly inhibit PDE-3, thus indirectly promoting increased cAMP as a stimulus for renin.

Unlike other families, the PDE-1 family is inhibited by calcium, but only the PDE-1C isoform efficiently degrades cAMP. In a preliminary report, Ortiz-Capisano et al. (78) found that PDE-1C was present in JG cells and could be activated by increases in intracellular calcium to degrade cAMP and inhibit renin release. Enzymatic activity of the PDE1 family is calcium-calmodulin dependent (4, 5), and its binding of the calcium-calmodulin complex stimulates cyclic nucleotide hydrolysis (38), which could provide a novel coupling of the role of calmodulin with calcium-inhibition of renin. Thus, intracellular calcium would balance both cAMP synthesis and degradation.

**Connexins, Gap Junctions, and Calcium Waves**

Connexins (Cx) are transmembrane proteins that combine to form hemichannels in the plasma membrane. Two Cx in adjacent cells can combine into a large intercellular channel, forming a gap junction linking the cytoplasm of the two cells. The lumen sizes of these channels vary but are certainly large enough to permit the diffusional movement of such ions as sodium, potassium, and calcium as well as larger molecules such as cyclic nucleotides. An excellent review of connexins in the kidney has been recently published by Wagner (118). The gap junctions associated with JG cells and adjacent endothelial (but not macula densa) cells have been suggested as possible conduit pathways for propagating a calcium-mediated, renin-inhibiting signal throughout the plaque of JG cells. In a cultured monolayer of renal endothelial cells, mechanical distortion of a single cell led to propagation of a calcium wave across the monolayer increasing in intracellular calcium, and also ATP production (111, 124). An siRNA against Cx40 virtually eliminated the calcium wave, as did degradation of ATP or P2 receptor blockade, suggesting Cx40 hemichannels and ATP may be important in the juxtaglomerular calcium signaling (111). However, Yao et al. (124) were unable to detect gap junctions using a similar preparation. They concluded “ATP functions as a paracellular mediator to regulate (inhibit) renin secretion, possibly through modulating intra-and intercellular calcium signaling” (124).

Renin-producing JG cells contain Cx37, Cx40 (119), and Cx45 (45), which form gap junctions between the JG cell and the adjacent endothelium (118). In Cx40 knockout mice renin-producing JG cells are absent from the afferent arteriole, but appear dispersed throughout the extra glomerular mesangium, glomerular tuft and periglomerular interstitium (62). These knockouts cannot stimulate renin through the macula densa pathway (62), suggesting gap junctions are critical in the proper anatomical positioning of renin-containing JG cells. Wagner et al. (119) reported that deletion of Cx40 results in the elimination of the calcium-mediated negative feedback response of renin to both angiotensin and increased renal perfusion of the renal baroreceptor, with no effect on \( \beta \)-adrenergic or macula densa/salt regulation of renin. The Cx40 knockout mice also had elevated blood pressure and high plasma renin concentration. Krattinger et al. (59) found Cx40-based channels couple the JG cells to each other and to the endothelial cells of the afferent arteriole, which facilitates a coordinated signaling pathway within the JGA. This was coupled with their observation that Cx40 knockouts had increased numbers of renin-secreting cells, increased renin biosynthesis, and were hypertensive (59). These data suggest Cx40-dependent gap junctions of the JG cells may mediate calcium-dependent inhibition of renin by angiotensin and renal perfusion, and also the macula densa, operating via a unique endothelial pathway (59, 119).

Haefliger et al. (43) found Cx43 in the afferent arteriole and in its endothelium. In mice in which the coding region of Cx43 was replaced by Cx32, there was reduced renin expression and renal renin content, but no differences in blood pressure, and a refractory response to renal artery clipping (43). Both Cx40 and Cx43 were studied in rats with two-kidney, one-clip (2K,1C) renovascular hypertension, and Cx40 was expressed by endothelial cells and renin-secreting cells, while Cx43 was in tubular elements and sparsely in endothelium. In their 2K,1C model, Cx40 expression increased in both kidneys, including in renin-secreting cells. Conversely, Cx43 increased only in the contralateral, unclipped kidney. These authors suggest that cell-to-cell communication mediated by Cx40, but not Cx43, may be involved in renin-secreting cells in the kidney (44).

While it is clear that JG cells contain Cx-dependent gap junctions associated with propagation of calcium waves that lead to increases in intracellular calcium, it is not completely clear what signals in vivo initiate these responses. There are at least two different pathways through which a calcium wave might traverse the JG cells: direct Cx45-mediated gap junctional coupling of a calcium signal or cell-to-cell signaling mediated by ATP production (45). Both of these would facilitate a calcium wave-instigated increase in intracellular calcium in the JG cell, leading to the inhibition of renin release. Using a microperfused afferent arteriole-JGA-glomerulus preparation, Peti-Peterdi (90) observed that changes in tubular flow rate which activate TGF lead to propagation of a broad calcium wave. This wave increased intracellular calcium across the JGA to the afferent arteriole and glomerulus, as well as in the JG cells. The wave was abolished by gap junction uncoupling, ATP scavenging, and P2 purinergic blockade (90), supporting a TGF-induced inhibition of renin initiated by sodium delivery to the macula densa. In other reports, gap junctions exist between adjacent endothelial cells (111) or connect the JG cells with their bordering endothelial cells (119, 124). The anatomic location of such connections suggest gap junction-facilitated calcium signaling might be initiated from the luminal/endothelial interface, perhaps in response to hemodynamic changes, or increased angiotensin, as alternative pathways initiating calcium-mediated inhibition of renin (119). The diversity of the locations and types of the different connexins
in the JGA raises a number of interesting questions about how they actually work. Our understanding of what activates them, and where and in what direction(s) the calcium waves they facilitate may travel, is still to be resolved. However, it is clear they are important in the calcium-mediated regulation of renin and seem to be initiated by classic renin signaling via the macula densa (90), renal baroreceptor (111, 119, 124), and possibly also β1-adrenergic signals.

Conclusion

It has been proposed there are several important second messengers which regulate renin secretion, including cAMP, calcium (15), and cGMP (99). However, it has become increasingly clear that cAMP is the dominant second messenger for renin secretion. cAMP synthesis by adenylyl cyclase V activity is the target for the classic renin-stimulating pathways: the renal baroreceptor, renal nerves, and the macula densa. While calcium is involved in many aspects of renin signaling, the role of calcium is to modulate the enzymatic activities which integrate to balance cAMP synthesis and degradation. Thus calcium itself does not directly control renin secretion but modifies the amplitude of the response to classic signaling pathways. This conclusion is reflected by what now seems a remarkably insightful observation by Kotchen (56) in 1974, where he suggested changes in calcium probably do not directly modify renin secretion but may affect its stimulation via the JG cells through modifying the stimulating signals of other pathways. It has taken 35 years to understand how calcium acts as the target for the classic renin-stimulating pathways: the macula densa (90), renal baroreceptor (111, 119, 124), and possibly also β1-adrenergic signals.

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