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Editorial Comments

Electrophysiology of the renin-producing juxtaglomerular cells

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Introduction

Renin is an aspartyl-proteinase hormone that is produced, stored and released by juxtaglomerular (JG) granular cells in the distal part of the renal afferent arterioles. The rate of secretion of renin from JG cells is one of the main determinants of the overall activity of the renin-angiotensin system, and is the most well regulated parameter of all constituents of that system.

At least three cellular messengers are involved in the intracellular control of renin secretion. Intracellular calcium is an inhibitory second messenger and is increased after exposure of the JG cells to vasoconstrictors such as angiotensin II, a_1-adrenergic agonists or endothelin [1]. Cyclic AMP stimulates the secretory process and is involved in the stimulation of renin secretion seen after exposure to vasodilator hormones coupled to activation of adenylyl cyclase (dopamine [2], b-adrenergic agonists [3], prostaglandin E_2, prostaglandin I_2 and adrenomedullin [4]). Cyclic GMP may inhibit or stimulate renin secretion depending on which of several pathways dominate [5]. Many of the cellular and subcellular processes involved in the renin secretory control are not well understood.

Ion channels in single JG cells

Early electrophysiological studies using sharp electrodes on JG cells are reviewed in Friis *et al.* [6]. The first patch-clamp study [7] using mouse isolated glomeruli with attached afferent arterioles showed that JG cells displayed outward rectification at positive membrane potentials, and that this current was carried by K^+. We applied the patch-clamp technique [8,9] to single rat JG cells to identify the contribution of the different ion channels to this membrane conductance [10]. The current-voltage (*I*-V) relationship was obtained by applying the pulse protocol shown in Figure 1A. An original recording of the whole-cell currents following this pulse protocol is shown in Figure 1B, and the steady-state *I*-V relationship is shown in Figure 1C. The cell displayed outward rectification and limited net currents between −30 mV and +10 mV [7]. Tetraethylammonium (TEA), which is an unselective blocker of potassium channels, inhibited most of the outward current suggesting that under resting conditions the outward current is carried by K^+. This is in accordance with results obtained from mouse JG cells [7]. Inhibition of voltage-gated K_V channels with 4-aminopyridine only slightly affected the current, indicating that only a minor part of the current is carried through K_V channels. Chelation of calcium with EGTA almost completely abolished the outward current, indicating that calcium-sensitive potassium channels were involved. Consistent with this, charybdotoxin and iberiotoxin, blockers of calcium-sensitive voltage-gated (BK_{Ca}) channels, inhibited almost all of the outward current. The current was enhanced by cAMP, suggesting that the BK_{Ca} was of the cAMP-stimulated ZERO isoform. This prediction was confirmed by RT–PCR [10].

The JG cells exhibited voltage-dependent calcium currents that were sensitive to the L-type calcium channel blocker calciseptine and they expressed mRNA and protein of the L-type channel Ca_v 1.2 [10]. A functional role of these channels was indicated by the finding that strong depolarization inhibited cAMP-mediated increases in cell membrane capacitance, but when membrane potential is not artificially clamped the action of the BK_{Ca} channels is likely to prevent depolarization in this range and, thereby, be permissive for renin release.

The cAMP pathway

Hormones, neurotransmitters, and autocoids that raise the intracellular production of cAMP stimulate renin

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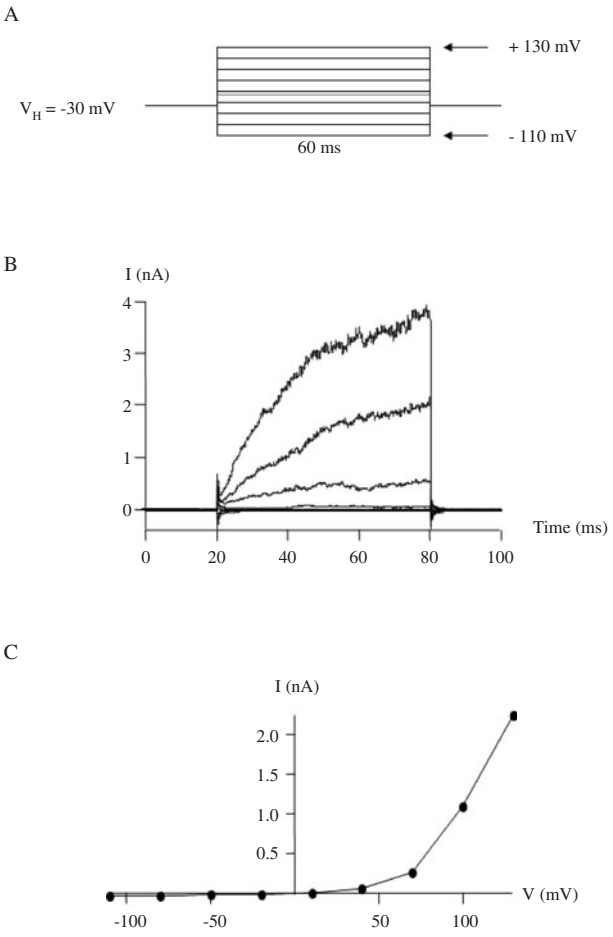

Fig. 1. Whole-cell current in a single JG cell. (**A**) Pulse protocol. (**B**) Recording of whole-cell current from a JG cell dialyzed with control solution. (**C**) Steady-state *I*-V relationship.

secretion and renin mRNA levels [11], but exactly how cAMP affects the secretory pathways is not well understood. Exocytosis involves insertion of the renin granule membrane into the cell membrane. This leads to an increase in cell surface area and therefore to an increase in cell capacitance, which can be measured by the whole-cell patch-clamp technique [12]. We have used this technique to measure renin secretion from single JG cells [13,14]. Usually C_m was followed for at least 10 min. With control solutions the capacitance was constant over time. The average capacitance of JG cells was 3.13 pF ($\pm$0.13 pF, $n = 106$, mouse) and 2.82 pF ($\pm$0.06 pF, $n = 192$, rat).

The patch-clamp technique makes it possible to introduce molecules into the cell through the pipette and simultaneously measure changes in cell capacitance. With 1 mM cAMP in the pipette, a significant increase in C_m was observed, suggesting net addition of membrane material to the cell membrane. This observation is in accordance with the hypothesis that renin secretion occurs by exocytosis. The stable cAMP analogue, Sp-cAMPs, also increased C_m. The responses to cAMP or Sp-cAMPs were completely abolished by the protein kinase A (PKA)-blocker Rp-cAMPs, indicating that the action of cAMP is mediated via PKA. In comparison to observations in other secretory cells, the rate of increase in C_m after stimulation of JG cells was quite slow. There was a gradual increase in capacitance during the first 5 min after the whole-cell configuration was established. A similar increase in JG cell capacitance was observed after addition of the b-adrenergic agonist isoproterenol to the voltage-clamped JG cells. This is probably because of the time required to generate intracellular cAMP. We conclude from these results that receptor-mediated activation of adenylyl cyclase and subsequent formation of cAMP leads to exocytotic secretion of renin.

The PDE-3 pathway

Agonists coupled to cGMP formation have been reported both to stimulate and inhibit renin release [15–19]. The concentrations of cyclic nucleotides in cells are determined by the rate of synthesis by cyclases and by the rate of degradation by cyclic nucleotide phosphodiesterases (PDEs) [20]. With regard to the control of renin release, the PDE3 and PDE4 subtypes have attracted special attention. Thus, PDE3- and PDE4-selective inhibitors increase renin release in conscious rabbits and humans [21–24]. Similar findings have been obtained with the isolated perfused rat kidney [25]. PDE3 and PDE4 use primarily cAMP as a substrate, and PDE3 is endogenously inhibited by cGMP [20]. This raises the intriguing possibility of an interaction between hormones acting through cGMP production and the cAMP pathway in the control of renin release. Recent data from whole animal studies [21] and from the isolated kidney [25] have indeed supported the concept that cGMP-dependent agonists might enhance renin release through inhibition of PDE3. We tested this hypothesis at the cellular level, where effects on renal haemodynamics, renal nerves and signals from the macula densa are excluded [14].

First, we needed to confirm the presence of PDEs in JG cells. The expression profile for PDE3 mRNA was analysed by RT–PCR, and it could be shown that PDE3A was expressed in single JG cells sampled with the patch pipette. Then the functionality of this enzyme was investigated. When the cAMP-specific PDE3 was inhibited with the PDE3-inhibitor trequinsin (10^{-5} mol/l), cAMP levels in isolated JG cells were significantly increased, confirming that PDE3 was present in JG cells and, furthermore, that it was constituently active, since its inhibition resulted in an increase in the cAMP level. Next, the effect of trequinsin on renin secretory activity was studied. Isolated JG cells were superfused and the effluent was collected with a time resolution of 2 min. In this experimental set-up, addition of trequinsin resulted in

a rapid and transient stimulation of renin release to levels significantly above time controls.

Finally, we applied the patch-clamp technique to single isolated rat JG cells for measurements of cell capacitance in response to manipulations of the cAMP and cGMP pathways. With cGMP (10^{-5}/l) in the patch pipette, a consistent and significant increase in C_m was observed, suggesting net addition of membrane material to the cell membrane. A similar increase in JG cell capacitance was observed after addition of the PDE3-blocker trequinsin to the voltage-clamped JG cells. The increase in C_m in response to cGMP or trequinsin was completely abolished by the PKA-blocker Rp-cAMPs, suggesting that PKA mediates the effect of cGMP (and trequinsin) on C_m in rat JG cells. The similar effects of cGMP and trequinsin on membrane capacitance suggest that both compounds act through inhibition of PDE3 leading to a subsequent increase in the cAMP formation and exocytosis.

Conclusions

JG cells display a characteristic *I*-V-relationship with marked outward rectification, which is mainly carried by potassium through calcium- and cAMP-sensitive voltage-gated BK_{Ca} channels (K_{Ca} 1.1, ZERO variant) and, to a lesser degree, through voltage-gated K_V channels. The BK_{Ca} channels significantly influence the resting membrane potential. JG cells express functional L-type voltage-dependent calcium channels (Ca_v 1.2) whose activation can inhibit cAMP-induced renin release. Renin release induced by the cAMP pathway is exocytotic and this action of cAMP is mediated via PKA. Stimulation of renin release by cGMP involves inhibition of phosphodiesterase type 3 resulting in enhanced cAMP formation and activation of PKA.

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Conflict of interest statement. None declared.

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Tamm–Horsfall protein or uromodulin: new ideas about an old molecule

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The discovery and re-discovery of Tamm–Horsfall protein/uromodulin

More than 50 years ago, Tamm and Horsfall isolated a mucoprotein from the human urine, and showed that the protein was able to interact and inhibit viral haemagglutination [1,2]. Of interest, the protein was found to be heavily glycosylated, containing up to 30% of its mass in carbohydrates [3]. It was then discovered that the Tamm–Horsfall protein (THP), as it was readily named, was the most abundant protein in normal human urine, with a migration pattern at ~90 kDa in SDS–PAGE [4]. In 1985, Muchmore and Decker [5] identified a 85 kDa glycoprotein in the urine of pregnant women. The protein was named uromodulin, due to its potent immunosuppressive activity reflecting its ability to inhibit antigen-induced T-cell proliferation and monocyte cytotoxicity *in vitro* [5]. Besides the molecular mass and the abundance in urine, the characterization of uromodulin revealed a number of resemblances with THP, including a ~30% carbohydrate content, a tendency to form aggregates and a significant number of intrachain disulfide bridges [5]. Based on sequence analysis, Pennica *et al*. [6] later confirmed that uromodulin was indeed THP. For the sake of clarity, we will use the term uromodulin to discuss the THP/uromodulin protein in this review.

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Uromodulin: biochemical properties and distribution

Uromodulin is a glycosylphosphatidylinositol (GPI) anchor-linked protein characterized by a remarkable structure and unique properties [reviewed in 7]. The mature protein contains 616 amino acids, including 48 cysteine residues potentially involved in 24 disulfide bridges, which are important for its conformation. Eight potential sites of *N*-glycosylation are also present, explaining the high carbohydrate content of uromodulin [3,5]. The predicted structure of uromodulin contains three epidermal growth factor (EGF) domains that contain a calcium-binding consensus sequence and mediate protein–protein interactions, as well as a zona pellucida-like domain. The C-terminus of the protein includes a stretch of hydrophobic amino acids that acts as a signal for the attachment of a preformed GPI anchor within the endoplasmic reticulum (ER). Following this addition, the membrane-bound protein is transported to the Golgi complex, where glycans are fully processed, then delivered to the luminal cell surface, and finally released into the urine by proteolytic cleavage.

Initial hybridization studies showed that uromodulin mRNA is only expressed in the kidney [6]. In this organ, uromodulin is distributed to the cells lining the thick ascending limb (TAL) of Henle's loop, with an extension to the early distal convoluted tubule (DCT) in some species [7]. Evidence obtained at the light and electron microscope levels showed that uromodulin is sorted to the apical cell membrane of the TAL epithelial cells (Figure 1), probably resulting from the addition of the GPI anchor and/or *N*-linked glycans