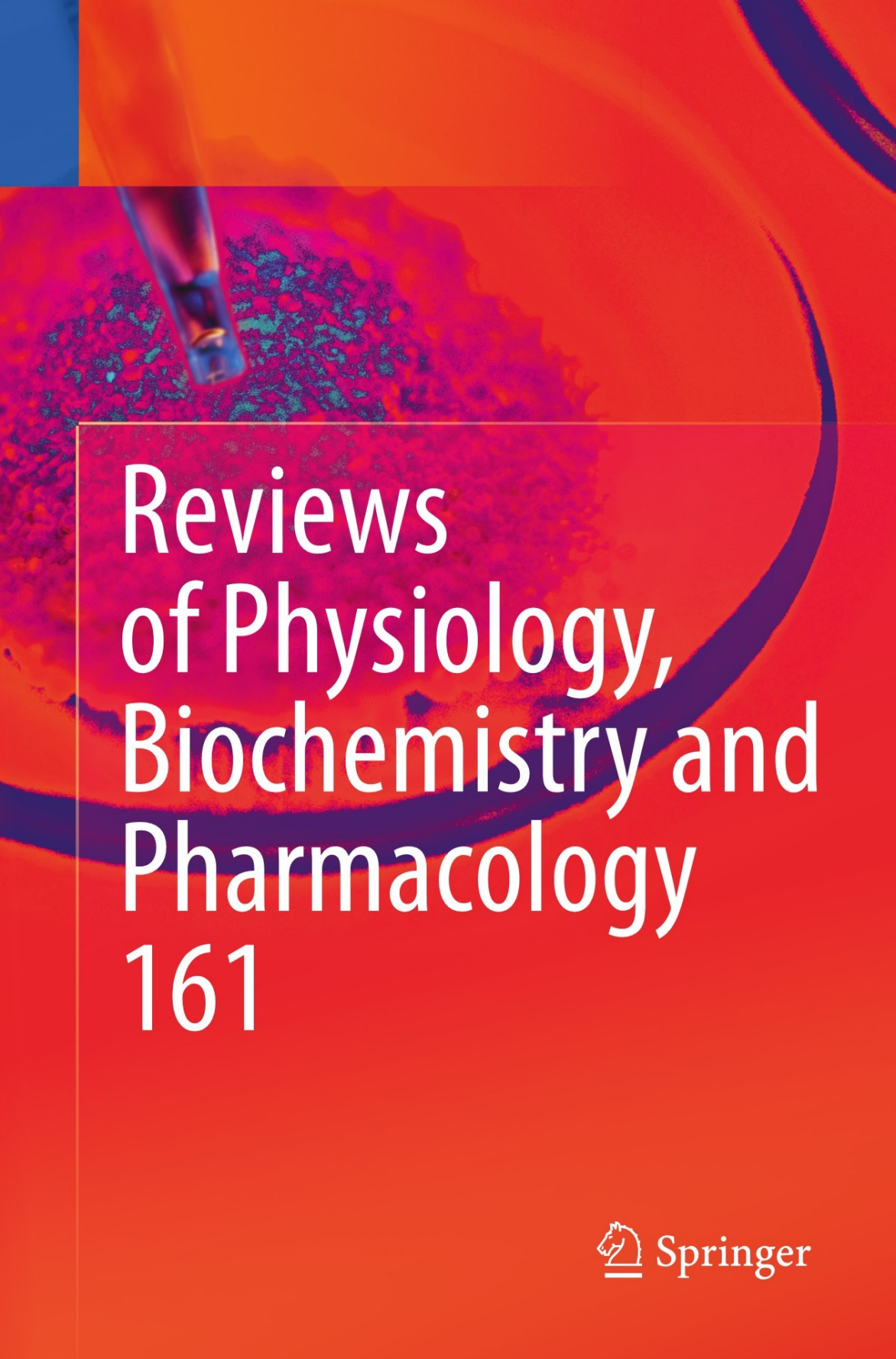
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# Reviews of Physiology, Biochemistry and Pharmacology 161

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# Contents

# Regulation of Renin Release by Local and Systemic Factors

**F. Schweda and A. Kurtz**

**Abstract** The renin-angiotensin system (RAS) is critically involved in the regulation of the salt and volume status of the body and blood pressure. The activity of the RAS is controlled by the protease renin, which is released from the renal juxtaglomerular epithelioid cells into the circulation. Renin release is regulated in negative feedback-loops by blood pressure, salt intake, and angiotensin II. Moreover, sympathetic nerves and renal autacoids such as prostaglandins and nitric oxide stimulate renin secretion. Despite numerous studies there remained substantial gaps in the understanding of the control of renin release at the organ or cellular level. Some of these gaps have been closed in the last years by means of gene-targeted mice and advanced imaging and electrophysiological methods. In our review, we discuss these recent advances together with the relevant previous literature on the regulation of renin release.

## Introduction

The renin-angiotensin system (RAS) regulates internal salt and fluid balance and blood pressure. Although the discovery of renin is credited to the work of Tigerstedt and Bergmann over a hundred years ago (Tigerstedt and Bergmann 1898), and although the RAS has been a focus of numerous studies ever since, substantial gaps remain in our understanding of how renin release is controlled.

According to the classical view of the RAS, the aspartyl protease renin is produced, stored, and released by the juxtaglomerular (JG) cells, which are located within the media layer of the afferent arterioles of the kidney (for reviews on renin release, see Friis et al. 2000, 2005, 2004; Hackenthal et al. 1990; Kurtz 1989; Kurtz and Wagner 1999; Schweda et al. 2007; Schweda and Kurtz 2004; Wagner and Kurtz 1998). Once secreted into circulation, renin cleaves the liver-derived angiotensinogen

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at its N-terminus to form the decapeptide angiotensin I (Ang I). Ang I is rapidly converted into angiotensin II (Ang II) by the cleavage of two amino acids, a reaction catalyzed by the angiotensin-converting enzyme (ACE). Ang II is considered the main biological effector of the RAS as it mediates its classical functions, such as vasoconstriction, stimulation of renal salt reabsorption, enhancement of catecholamine and aldosterone release, and stimulation of thirst and salt appetite. Since all of the aforementioned effects of Ang II increase blood pressure, the effect that led to the discovery of renin by Tigerstedt and Bergman (Tigerstedt and Bergmann 1898), blood pressure stabilization is the main physiological function of the RAS. This straight forward picture of the RAS has since become more complicated with the discovery of renin expression in extrarenal tissues such as the heart, blood vessels, adrenal glands, reproductive organs, pancreas, brain, and mast cells (Dzau et al. 1987; Ekker et al. 1989; Ganten et al. 1971; Itskovitz et al. 1992; Pandey et al. 1984; Paul et al. 1988, 1993; Mackins et al. 2006; Veerappan et al. 2008). Even renin in the kidney is found not only in its classical position at the vascular pole of the glomerulus but also in the proximal tubules, the connecting tubule, and the collecting ducts (Chen et al. 1994; Moe et al. 1993; Prieto-Carrasquero et al. 2004, 2005; Rohrwasser et al. 2003, 1999; Taugner et al. 1982). In addition to renin, the other components of the renin-angiotensin cascade are more or less completely present in these aforementioned tissues, where they form locally active tissue renin-angiotensin systems that are involved in the regulation of organ function in physiological and pathophysiological states. (For reviews of local RAS, please see Bader et al. 2001; Baltatu and Bader 2003; Danser 2003; Dzau 1993; Ichihara et al. 2004; Kobori et al. 2007; Leung 2007; Leung and Sernia 2003; Paul et al. 2006; Re 2004).

The classical view of the systemic RAS, in which Ang II is the only biological effector, has been complicated by the discovery of additional bioactive components of the RAS. For instance, Ang II can be further metabolized by aminopeptidases to angiotensin III (Ang III; 7 amino acids) and angiotensin IV (Ang IV; 6 amino acids) (Reudelhuber 2005). Furthermore, a biologically active angiotensin peptide consisting of seven amino acids, Ang(1-7), can be formed from Ang I or from Ang II by the angiotensin-converting enzyme ACE2 (Crackower et al. 2002; Tipnis et al. 2000). Finally, renin and its precursor prorenin can bind to the renin/prorenin receptor, thereby activating signaling cascades independent of their enzymatic activity (Danser et al. 2007; Nguyen 2006). These discoveries about the local RAS and the biochemistry of angiotensin peptides have been the focus of a number of recent review articles (Kobori et al. 2007; Paul et al. 2006; Reudelhuber 2005) and are not within the scope of the present review. Instead, we focus on recent advances in the understanding of the control of renin release from the renin-producing JG cells.

## The Process of Renin secretion

Renin is encoded by a single gene in humans and in most animal species, but not in mice. Laboratory mice, widely used in renin research, fall into two categories: strains (e.g., C57BL/6 and BALB/c) that carry one renin gene, designated Ren-1c,

and other strains (e.g., 129Sv and Swiss) that have two renin genes, Ren-1d and Ren-2. The latter two genes arose through gene duplication and are therefore located in close proximity to each other on chromosome 1 (Abel and Gross 1990; Dickinson et al. 1984). All three renin genes encode highly homologous proteins (97% homology at the amino acid level), which nevertheless possess different glycosylation potentials (Sigmund and Gross 1991). While the Ren-1 proteins can be glycosylated at three asparagine residues, the Ren-2 enzyme lacks these putative glycosylation sites.

The renin gene in rats and mice consists of nine exons and eight introns, while the human renin gene contains an additional mini-exon (exon Va) encoding only three amino acids (Hardman et al. 1984). The gene is transcribed, and the mRNA is processed and finally translated into the protein preprorenin (401 amino acids). Preprorenin is translocated into the endoplasmic reticulum (ER), where a 20-residue ER-targeting signal sequence is cleaved from preprorenin, thereby generating prorenin (Fig. 1). The enzymatically inactive prorenin can either be released continuously

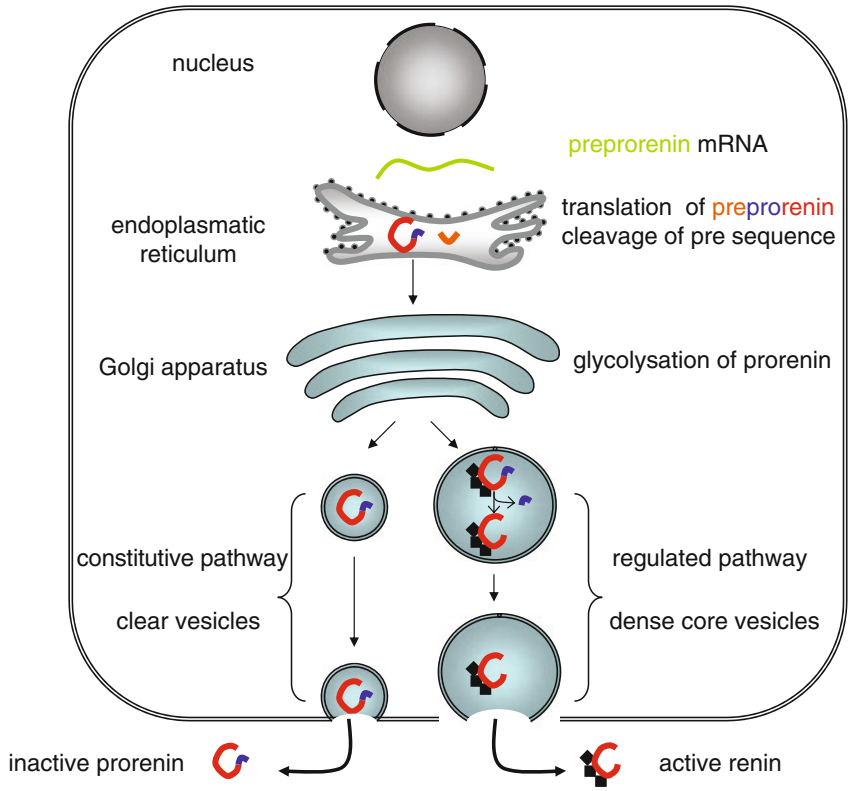


**Fig. 1** Intracellular synthesis of renin in juxtaglomerular cells. After transcription, preprorenin is transferred into the endoplasmic reticulum, where the presequence is cleaved. Prorenin is transported to the Golgi apparatus, where it can be glycosylated and tagged for the regulated exocytosis pathway. Prorenin is activated by cleavage of its prosequence in the dense core vesicles destined for regulated exocytosis. Untagged prorenin that is sorted for constitutive exocytosis remains inactive, since its prosequence is not cleaved

into circulation or be packed into secretory vesicles by the Golgi apparatus, leading to the formation of rhomboid or fusiform protogranules. During the maturation of protogranules to mature granules, prorenin is further processed into active renin via cleavage of the 46-residue prosequence from its N-terminus (Fig. 1).While the constitutive pathway of prorenin release is determined primarily by the renin synthesis rate, reflected in the abundance of renin mRNA, the release of active renin from the secretory granules is rapidly regulated. In this way, stimulation or suppression of the renin system in the long term (over days or weeks) regulates plasma levels of both active renin and prorenin, while in the short term (up to 2 h) only active renin levels are changed (Toffelmire et al. 1989).

Similar to other aspartyl proteases, renin has a bilobular form and its catalytic site is buried in the cleft between the two lobes (Blundell et al. 1983). Since the prosegment hinders the access of angiotensinogen to the active site, prorenin was originally thought to be biologically inactive. However, as mentioned above, this view is questioned since the discovery of the renin/prorenin receptor.

A critical factor determining whether prorenin is sorted into the pathway of constitutive or regulated release appears to be the glycosylation of prorenin with mannose-6-phosphate residues. The importance of the glycosylation of prorenin for storage and for generation of secretory granules has recently been suggested in studies using renin knockout (KO) mice. As mentioned above, the Ren-2 protein lacks the potential glycosylation sites that are present in the Ren-1 proteins. Genetic deletion of the Ren-1d gene in a mouse strain with two renin genes resulted in a complete loss of renin-containing granules in the JG cells (Clark et al. 1997), whereas the granules are intact in Ren-2 KO mice (Sharp et al. 1996).

The proteolytic mechanism by which prorenin is cleaved from its prosequence inside the protogranules has not been identified. Since cathepsin B coexists with renin in the secretory granules (Matsuba et al. 1989; Taugner and Hackenthal 1988) and has the potential to convert prorenin to renin (Jutras and Reudelhuber 1999; Neves et al. 1996; Wang et al. 1991), this protease is a putative candidate for processing prorenin. Kallikreins have also been suggested as possible activators of prorenin (Kikkawa et al. 1998; Yokosawa et al. 1979). In addition, the proprotein convertase PC5 was found to be capable of activating prorenin in cell culture experiments (Laframboise et al. 1997; Mercure et al. 1996). However, despite low expression of PC5 mRNA in the kidney, attempts to demonstrate the presence of PC5 protein in the human kidney by immunohistochemistry have failed (Mercure et al. 1996). Since the activation of prorenin is obviously an important step in the biological activity of the RAS, further studies should focus on identifying the protease responsible. Moreover, the central position of this activation step in the renin-angiotensin cascade leads to the speculation that this proteolytic reaction may itself be regulated such that the activity of the RAS is controlled not only by the rate of renin release but also by the transformation rate of inactive prorenin into active renin within the secretory granules.

The mode of renin release from JG cells has been a matter of debate for a long time. However, despite reports arguing for different mechanisms of renin extrusion (King et al. 1993), convincing morphological evidence has accumulated in favor of an exocytotic

release of renin by JG cells (Ogawa et al. 1995; Taugner et al. 1984a). In a recent study using multiphoton fluorescence imaging, renin vesicles that were located deep in the renin-producing cells disappeared without showing any movement (Peti-Peterdi et al. 2004), suggesting that the granules do not necessarily have to move toward the cell surface to contact the plasma membrane. This somewhat unexpected finding may be explained by channel-like invaginations in the plasma membrane of JG cells that had been observed earlier (Peter 1976; Ryan et al. 1982) and that likely result from the preceding fusion of vesicles with the plasma membrane.

Functional evidence for renin exocytosis was derived from the in vitro observation that renin is released from afferent arterioles not continuously, but instead in an episodic,"quanta-like" fashion (Skott 1986). In line with these data, acute stimulation of renin secretion in vivo markedly reduces the number of renin storage granules, while the average size of the remaining granules remains constant (Rasch et al. 1998). It can be inferred from these findings that upon stimulation, a renin storage granule releases its contents in an "all or none" fashion typical of exocytosis. This interpretation has been corroborated by direct visualization of renin release (Peti-Peterdi et al. 2004). Finally, electrophysiological evidence for the exocytosis of renin also exists,which is based on the rationale that in the process of exocytosis the membrane of the renin storage granule fuses with the plasma membrane, thereby enlarging the cell surface of the JG cell. Since whole-cell electrical capacitance changes in parallel with the cell surface, exocytotic events are accompanied by increases in whole-cell capacitance (Neher and Marty 1982). Applying this concept to isolated mouse JG cells, patch-clamp studies have demonstrated that cell membrane capacitance is increased by classical stimulators of renin release such as cAMP, which argues for membrane insertion via exocytosis (Friis et al. 1999).

How the fusion of the renin storage granule with the plasma membrane occurs and how it is regulated in JG cells is not understood. In other secretory cells the exocytotic process involves a sequence of vesicle docking, tethering, and finally fusion of the vesicle membrane with the plasma membrane. Three SNARE proteins are involved in these processes (Weber et al. 1998). Syntaxin and SNAP25 are localized at the plasma membrane (target or t-SNAREs), while synaptobrevin resides at the vesicle membrane (vesicle or v-SNARE) (Weber et al. 1998). In addition to the SNARE proteins, at least two other protein families, the Rab proteins and the SM proteins (Sec1/Munc18-like proteins), are required for vesicle fusion (Jahn et al. 2003). Since a broad range of cell types and organisms appear to use the above-mentioned processes to achieve vesiclefusion, it might be speculated that the same exocytosis machinery exists in JG cells. Thus far, however, no data have been reported confirming this hypothesis.

## Intracellular signaling pathways controlling renin release

Similar to hormone release from other secretory cells, renin secretion is controlled by the classical intracellular second messengers cyclic AMP, cyclic GMP, and calcium ($Ca^{2+}$) (Fig. 2).