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Topical Review

Renin: origin, secretion and synthesis

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Renin is a central hormone in the control of blood pressure and various other physiological functions. In spite of the very early discovery of renin over 100 years ago, we have only recently gained a deeper understanding of the origin of renin-producing cells and of the mechanisms responsible for renin synthesis and secretion. The main source of renin is the juxtaglomerular cells (JGCs), which release renin from storage granules. Besides the renin–angiotensin system (RAS) in the JGCs, there exist local RASs in various tissues. JGCs originate *in situ* within the metanephric kidney from mesenchymal cells that are not related to smooth muscle lineages, as hitherto assumed. The previous notion that JGCs stem from vascular smooth muscle cells may be explained by JGC differentiation: they acquire smooth muscle markers that are maintained throughout adulthood. It has become clear that increasing intracellular free [Ca^{2+}] inhibits renin secretion in JGCs. In contrast, cAMP stimulates renin release. Over the last decade, numerous studies on isolated JGCs and intact animals have provided contradictory results as to whether cGMP has a stimulatory or inhibitory action on renin release. More recent results strongly suggest that the effects of cGMP on renin release from JGCs involve the degradation of cAMP, which is modulated by cGMP. Finally, it has been found that not only is the production of renin modulated by enhancing or attenuating renin transcription, but renin mRNA stability is controlled by various proteins present in renin-producing cells.

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Recent studies shed new light on renin production and release, thereby considerably modifying our view of this important hormone. Renin was one of the first hormones to be discovered: during an IUPS meeting in St Petersburg in 1898, the first data were presented indirectly suggesting the existence of a renally derived factor that increases blood pressure. This hormone enzyme initiates the enzymatic cascade generating the angiotensin peptides that regulate blood pressure, cell growth, apoptosis and electrolyte balance, to mention only some of the foremost-recognized functions. Renin is rate limiting in the production of angiotensin II (Ang II), a hormone that ultimately integrates cardiovascular and renal function in the control of blood pressure as well as salt and volume homeostasis. For instance, renin seems to be of vast importance for maintaining arterial blood pressure in the face of variations in salt intake: in mice, constant blood pressure is found during alterations in sodium intake, and this relies on controlling the activity of the renin–angiotensin system (RAS). Once the RAS is experimentally kept constant, salt sensitivity of blood pressure regulation becomes apparent (Cholewa & Mattson, 2001).

Recently we have gained a different view of the origin of renin-producing cells, renin secretion and the control of renin production at the level of transcription and translation. These issues will be discussed in the following sections.

The origin of renin-producing cells

The juxtaglomerular cells (JGCs) constitute the most important source of circulating renin.

Generally it is assumed that JGCs are metaplastically modified smooth muscle cells, because adult mammal JGCs contain myofilaments (Taugner & Hackenthal, 1989). However, only recently has a study has been performed to determine the lineage of these cells. In an extensive study, Sequeira Lopez *et al.* (2001) used single cell PCR and double immunostaining combined with lineage markers to define the lineage of JGCs. The authors transplanted embryonic kidneys between genetically marked and wild-type mice and labelled them for renin, smooth muscle and endothelial cells at different developmental stages. Normal cell culture systems cannot be applied, since no vessels form (under conventional conditions) and therefore renin cells do not assemble into

arterioles. Thus, they remain dispersed in the interstitium. Sequeria Lopez *et al.* suggest that there are at least two distinct populations of cells, expressing either renin or smooth muscle markers, but never both. From E12 to E15, renin cells do not yet express smooth muscle or endothelial markers. Subsequently, the subpopulation of renin-expressing cells remarkably exhibit the capacity to express smooth muscle markers. Thus renin cells can give rise to smooth muscle cells rather than originating from them (Fig. 1) (Sequeira Lopez *et al.* 2001). In line with this interpretation, renin precursor cells are capable of assembling into the appropriate vessel types and segments. Moreover, smooth muscle cells derived from renin precursors seem to be those capable of undergoing metaplasia to renin cells when required.

The functioning of a fetal and early postnatal RAS is a prerequisite for normal nephrogenesis in the rat. In the fetal kidney, the renin–angiotensin system is markedly activated. The metanephros at embryonic day 14 (E14), contains renin and Ang II and both the Ang II receptors (AT1 and AT2). At this stage, renin is found in cells scattered within the mesenchyme (Norwood *et al.* 2000). Insulin-like growth factor (IGF)-I seems to be pivotal in this process, as underlined by the observation that angiotensin-converting enzyme inhibition suppresses renal IGF-I expression. Interestingly, treatment with IGF-I normalizes renal function and histology after early ACE inhibition (Nilsson *et al.* 2000). Moreover, IGF-1 stimulates renin production in the late gestation fetal sheep (Marsh *et al.* 2001).

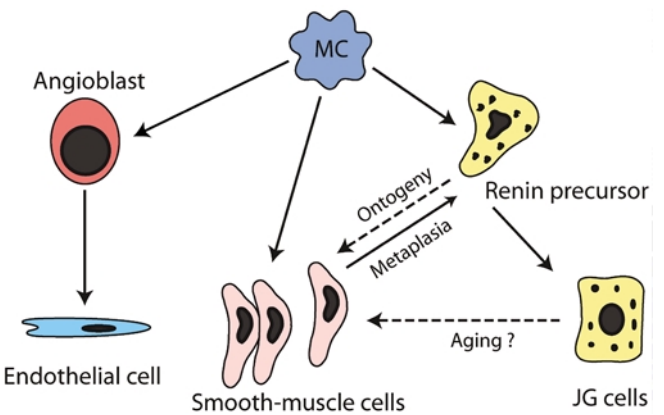

Figure 1. The lineage of the JGC

Metanephric mesenchymal cells (MC) are the origin of angioblasts, from which the endothelial cells stem. MCs are also the origin of vascular smooth muscle cells and of renin precursor cells. During ontogeny, these renin precursor cells can give rise to JGCs and to a subset of arteriolar smooth muscle cells. The smooth muscle cells originating from the renin precursors seem to be the cells capable of undergoing metaplasia to renin cells. Reproduced with permission from Sequeira Lopez *et al.* (2001).

Besides the circulating RAS, there exist several local ones within various tissues including the heart, brain and adrenal glands. Even though all components of the RAS are expressed in these tissues, the mode of action can be quite different (Peters & Clausmeyer, 2002), as underscored by the remarkable discovery of renin in inclusion bodies of the mitochondria. Although the exact function of mitochondrial renin remains to be fully elucidated, the fact that aldosterone production takes place in adrenal mitochondria indicates a potential role of mitochodrial renin in its control. This functional link between mitochondrial renin and aldosterone has been shown experimentally after bilateral nephrectomy (Peters *et al.* 1999), where the amount of mitochodria containing renin increases with the augmented aldosterone production.

It seems that only a truncated form of prorenin can be imported into the mitochondria. This truncated prorenin is synthesized by an alternative transcript characterized by an alternative exon 1A. Interestingly, this is the only renin transcript expressed in the heart. The classical mRNA coding for preprorenin does not respond to stimuli of the cardiac RAS such as hypertrophy or myocardial ischaemia. Conversely, exon 1A renin trancript increases promptly (Peters *et al.* 2002). In addition to renin located in the mitochondria, the cardiac cells can also internalize circulating renin. There are two proposed mechanisms by which this can occur, one by mannose-6-phosphate receptor-mediated endocytosis (Saris *et al.* 2002), the other by the internalization of nonglycosylated prorenin (Peters & Clausmeyer, 2002). The mannose-6-phosphate receptor-mediated uptake may simply constitute a clearance mechanism, i.e. it inactivates the circulating RAS. The second form of internalization may be crucial for the intracardiac RAS and its various functions (Peters & Clausmeyer, 2002).

Renin secretion

The regulation of renin release from the kidney is complex (Skott, 2002; Todorov *et al.* 2002; Cheng *et al.* 2002; Kammerl *et al.* 2002). Among the manifold controllers is the macula densa mechanism, which couples the tubular chloride concentration inversely to the plasma renin concentration in the rat. This occurs in the thick ascending limb of the tubule of Henle. Changes in regional renin release in the kidney help to determine the sensitivity of the tubuloglomerular feedback mechanism, consequently modifying the set point for autoregulation of renal blood flow.

A second control element is sympathetic nervous discharge to the kidney, which stimulates renin secretion through β-adrenergic receptors on the JGCs (DiBona, 2000). Increased renin secretion in response to

β-adreneroceptor stimulation is mediated via the formation of cAMP. Cyclic nucleotides are critical second messengers that determine renin secretory rate and there seems to exist a common pathway of stimulating renin secretion via cAMP. Hormones, neurotransmitter, and autacoids that raise the intracellular production of cAMP enhance renin secretion and augment renin mRNA levels. Thus, it is unequivocally held that cAMP stimulates renin secretion. In contrast, the effects of cGMP on renin secretion have been discussed controversially. Some find a stimulatory action; others have reported an inhibitory effect of cGMP on renin release. Chiu and Reid demonstrated an important interaction between cAMP and cGMP for the secretion of renin by hydrolysis of cAMP that occurs in response to various phosphodiesterase (PDE) isoforms (Chiu & Reid, 1996; Chiu *et al.* 1996). Among these, PDE 3 may play a particular role since it is inhibited by cGMP. As shown by Chiu and Reid, cGMP can stimulate renin release by decreasing cAMP breakdown via attenuated PDE 3-activity. Accordingly, inhibition of PDE 3 also augments the renin secretory response to β-adrenoceptor stimulation (Chiu & Reid, 1996). Friis and colleagues very recently employed an elegant technique to further test the concept that cGMP enhances renin release through the blunting of cAMP degradation (Fig. 2; Friis *et al.* 2002). Two PDE 3 subtypes (A and B) have been suggested to mediate cross-talk between cAMP and cGMP pathways in JGCs. The PDE 3 enzymes are specific for cAMP and, as mentioned above, they are inhibited by cGMP. In the study by Friis *et al.*, patch clamp experiments were made on isolated JGCs. Increases in membrane capacitance indicate cell surface area increase, and this is an accepted measure of exocytosis at the level of the single cell. cGMP had similar effects on membrane capacitance to cAMP, although 10-fold higher concentrations were required. This effect of cGMP on membrane capacitance was inhibited by blocking PKA, thus underscoring that the cAMP–PKA pathway takes part in cGMP-mediated responses of JGCs. Therefore renin release from JGCs is significantly regulated by degradation of cAMP, which is modulated by cGMP inhibition of PDEs.

There is also a potent pressure-sensitive mechanism for renin release; its stimulation is associated with activation of the sympathetic nervous system and release of hormones, such as oxytocin, which stimulate renin release in rats via a β-adrenergic receptor-dependent mechanism (Huang *et al.* 2001).

As mentioned before, the release of active renin from JGCs is considered to be the main rate-limiting step in providing circulating renin. Remarkably, the stimuli that inhibit renin secretion, e.g. increased arterial pressure or Ang II, increase intracellular free calcium. This is in contrast to the other secretory cells of the organism, in which augmented free calcium levels lead to enhanced depletion of secretory granules. Thus, this unique feature of renin secretion is commonly referred to as the calcium paradox. The reason for the opposite effect of calcium on renin secretion can be

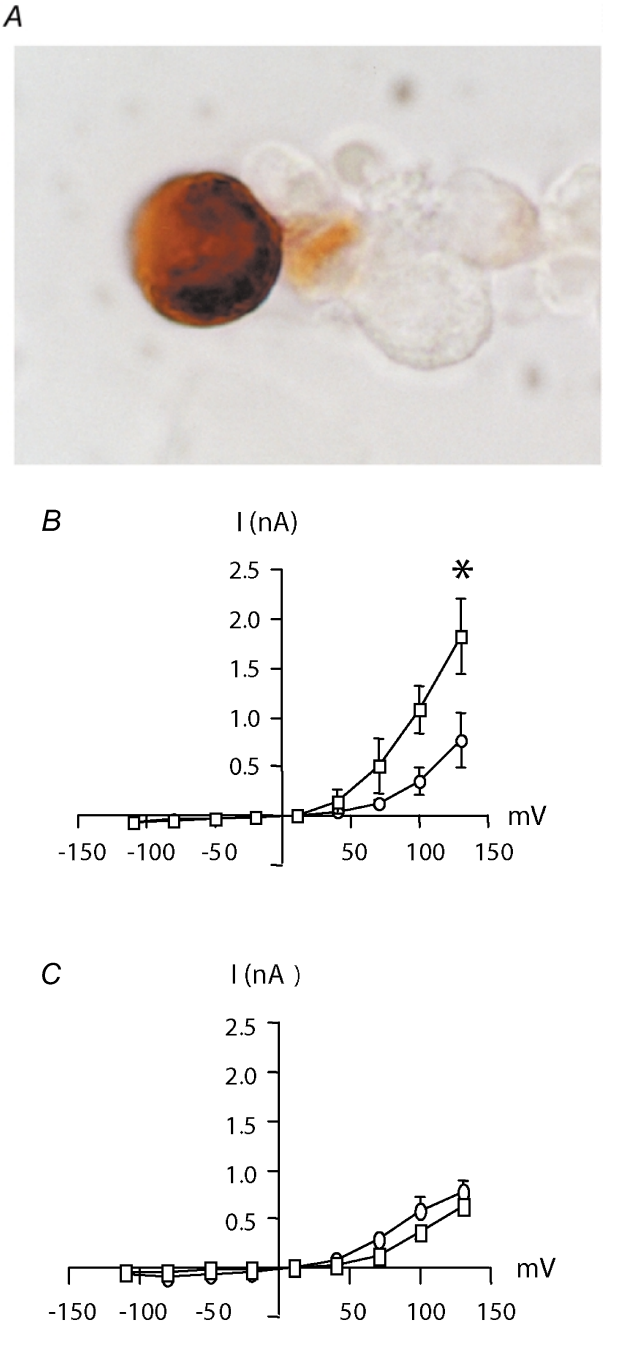

Figure 2

A, immunolabelling of renin protein in a JGC using rabbit anti-mouse renin antibody, *B*, effects of cGMP on whole-cell currents and C_m of isolated JGCs. *I–V* relationships before (●) and after (■) JGCs were dialysed with 10 μmol l^{-1} cGMP. *C*, as before, but JGC were dialysed with cGMP (10 μmol l^{-1}) together with the PKA blocker Rp-cAMP (25 μmol l^{-1}). Adapted from Friis *et al.* (2002).

found in the origin of the renin storage granules that appear to be modified lysosomes (for review see Peters & Clausmeyer, 2002).

The membrane potential of JGCs acts in concert with the various mechanisms controlling renin secretion. Again, calcium seems to play an important role in this context. For instance, voltage-dependent L-type calcium channels and calcium-sensitive voltage-gated calcium channels have been identified in JGCs (Friis *et al.* 2003). The latter channels are sensitive to cAMP as they belong to the ZERO variant, and play an important role in determining membrane potential. However, they are not directly responsible for renin secretion, as their blockade does not affect renin secretion. Conversely, activating the L-type calcium channels inhibits cAMP-mediated renin secretion, thus providing evidence for a functional link between these channels and the control of renin secretion (Friis *et al.* 2003).

Control of renin synthesis

Transcriptional control depends on the DNA located immediately upstream of the gene itself. As for other peptides, transcription of renin RNA requires the binding of RNA polymerase II to the basic promoter region of the gene. For the renin gene, there are additional regulatory elements considerably further upstream of the cap site which activate or repress transcription (Petrovic *et al.* 1996). These regulatory elements are found in areas where remarkable interspecies homology can occur (Mrowka *et al.* 2003; Persson *et al.* 2003). One of the homologous sequences has been a matter of extensive investigation (Shi *et al.* 2001*a*,*b*). It was termed the 'renin enhancer' before it was recognized that this sequence is a compound regulatory element with several stimulatory and inhibitory activities. In the renin enhancer, cAMP response elements have been identified. For murine renin gene transcription, the steroid hormone receptors LXR and retinoic acid receptor–RXR complex, transcriptional factors CREB/CREM and USF1/USF2, and finally HOX gene family members are known to be important (Pan *et al.* 2001*a*,*b*). In order to exert a specific action, the transcription factors binding at the enhancer region must interact with other transcription factors acting in the closer vicinity of the site of transcription, e.g. the proximal promoter element. Interestingly, vitamin D_3 and its receptor seem to play an important role in the complex regulation of renin transcription (Li *et al.* 2002). The vitamin D receptor belongs to the thyroid hormone (T_3) subfamily of nuclear hormone receptor transcription factors. These also include the retinoic acid receptor. The latter shares a binding site with the vitamin D receptor. As an example of the clinical importance of this binding site, it has been shown by observations in patients that there is an inverse relationship between plasma vitamin D_3 levels and plasma renin activity as well as with blood pressure (Burgess *et al.* 1990). Moreover, it has been known for some time that vitamin D_3 supplementation lowers blood pressure in hypertensive subjects (Lind *et al.* 1989). Thus, the vitamin D_3 receptor may constitute an important negative regulator of renin expression.

These are examples of the significance of the hitherto identified renin enhancer region, yet there may exist further regions of importance. Indeed, the interspecies homology that has been found in two more upstream areas of this enhancer sequence underscores the potental existence of more regulatory sites (Mrowka *et al.* 2003). Their functional importance, however, remains to be quantified.

Finally, in addition to the control of renin via transcriptional regulation, strong evidence points to the paramount role of post-transcriptional modulation. In particular, renin mRNA stability can be modified by cAMP (Chen *et al.* 1993; Sinn & Sigmund, 1999). Currently, light is being shed on possible mechanisms behind this effect: beyond the coding region of about 200 nucleotides, renin mRNA contains an untranslated region (UTR). The homology of this region is striking between sheep, rats, mice and humans (Skalweit *et al.* 2003). Several RNA-binding regulatory proteins, which were identified by MALDI-TOF, bind to this UTR. Remarkably, as shown in *in vitro* experiments, adding these proteins to cell lysates markedly protects renin mRNA from degradation, thereby increasing the amounts of renin being synthesized (Skalweit *et al.* 2003).

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