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Structural analysis suggests that renin is released by compound exocytosis

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The mode of renin release from renal juxtaglomerular cells into circulation is still unsolved in several aspects. Here we studied the intracellular organization of renin-storage vesicles and their changes during controlled stimulation of renin release. This was accomplished using isolated perfused mouse kidneys with 3-dimensional electron microscopic analyses of renin-producing cells. Renin was found to be stored in a network of single granules and cavern-like structures, and dependent on the synthesis of glycosylated prorenin. Acute stimulation of renin release led to increased exocytosis in combination with intracellular fusion of vesicles to larger caverns and their subsequent emptying. Renin release from the kidneys of SCID-beige mice, which contain few but gigantic renin-storage vesicles, was no different from that of kidneys from wild-type mice. Thus, our findings suggest that renin is released by mechanisms similar to compound exocytosis.

Kidney International (2013) **83,** 233–241; doi:10.1038/ki.2012.392; published online 12 December 2012

KEYWORDS: afferent arteriole; angiotensin; renin–angiotensin system

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Received 8 August 2012; revised 10 September 2012; accepted 13 September 2012; published online 12 December 2012

The protease renin is the key enzyme regulating the activity of the renin–angiotensin–aldosterone system.[1] It is mainly produced and secreted by specialized kidney cells located in the terminal parts of afferent arterioles.[2,3] In these cells, renin is synthesized as a precursor protein prorenin, which is glycosylated in the Golgi apparatus and then transferred into storage vesicles.[1,4–6] Within these storage vesicles, prorenin is proteolytically processed to renin.[5] The release of renin from the cells is mainly controlled by two oppositely acting signaling pathways. The cyclic adenosine monophosphate (cAMP) signaling pathway stimulates renin release, whereas a calcium-related signaling pathway inhibits the release of renin.[7–10] Inhibition of secretion by calcium is unusual, as calcium is considered to be essential for the induction or maintainance of secretory events, in particular of exocytosis.[11] In fact, morphological signs of exocytosis, such as omega-shaped figures, have very rarely been reported in renin-producing cells,[12,13] in spite of the numerous and prominent renin-containing vesicles, which often show an irregular shape at the ultrastructural level.[14,15] Therefore, it has been hypothesized that the release of renin from storage vesicles may occur through intracellular solubilization of renin, which then is transported through the plasma membrane.[16,17] Functional data such as a quant-like release of renin,[18] increase of membrane capacitance[19,20], and the disappearance of renin-storage vesicles upon stimulation of renin release,[21] however, would support the idea of a controlled exocytosis of renin. Others have described membrane invaginations in the course of renin release.[22–24] Signs of exocytosis of renin-containing vesicles such as omega-shaped membrane structures have been occasionally described for kidneys of animals, in which renin synthesis and secretion had been stimulated *in vivo* by adrenalectomy in combination with treatment with the loop diurectics.[12] Notably, no signs of exocytosis have been reported for animals chronically treated with angiotensin-converting-enzyme (ACE) inhibitors,[22] although the number of renin-storage vesicles clearly decreases during acute ACE-I treatment.[22,25] In addition, periods of acute renal hypoperfusion lead to a decrease of the renin-storage vesicle number without morphological signs of exocytosis.[22] Finally, beige mice lacking the Lyst protein produce only very few but huge renin-storage vesicles, without signs of

exocytosis.[26] The plasma renin concentration reflecting secreted renin, is lower in these mice, however, in proportion with a decreased number of renin-expressing cells in the kidneys. Therefore, renin release from individual renin-producing cells may not be very different between wild-type and beige mice. Experiments with a more defined stimulation of renin release have been performed with incubated kidney slices, which were incubated with isoprenaline, calcium chelators, furosemide, cytochalasin B, or combinations of these compounds.[12,13] Again, morphological signs of exocytosis were very rarely seen. More evident was the appearance of electron-lucent and/or empty vesicles, which were considered as indirect signs for exocytosis.[12,13] Notably, isoproterenol, which is a powerful and immediately acting stimulator of renin release, did not induce morphological changes of renin-secreting cells.[13] It was the combination of the calcium chelator ethylene glycol tetraacetic acid (EGTA) with cytochalasin B that produced a partial emptying of renin-storage vesicles within 20 min of incubation.[13] However, it is not clear as to whether the combination of these nonphysiological compounds might have induced effects that do not reflect the physiological release mode of renin. In summary, the mode of renin release is still rather mysterious in several aspects. For a better understanding of this process, we were therefore interested to learn more about the morphology of renin-storage vesicles and their possible changes during controlled modulation of renin release. For this goal, we combined the model of the isolated perfused mouse kidney, which allows us to modulate renin release under quasi physiological conditions with three-dimensional (3D) electron microscopy of renin-producing cells in the kidney.

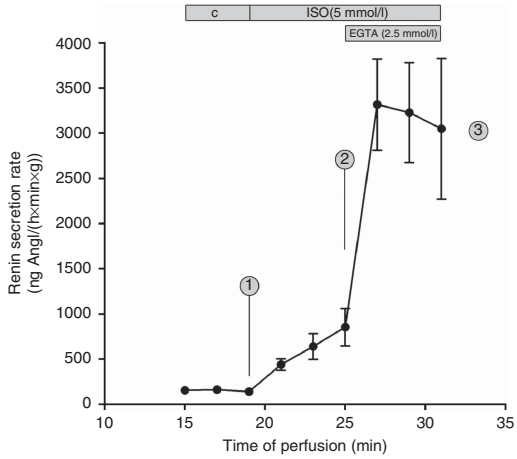

Figure 1 | Perfusion protocol and renin secretion rates of isolated wild-type mouse kidneys. After a stabilization period of 15 min, the samples were taken for the determination of basal renin release (control period C). For determination of the normal juxtaglomerular cell structure, kidneys were fixed at the end of the control period (time point 1). After the control period, isoproterenol (ISO, 5 nmol/l) was added to the perfusate. Five minutes later, kidneys were fixed for electron microscopical (ELMI) analysis (time point 2). Further, during isoproterenol infusion, ethylene glycol tetraacetic acid (EGTA) (2.5 m) was added to the perfusate to lower the extracellular concentration of calcium. At 5 min after the start of EGTA infusion, kidneys were fixed for ELMI analysis (time point 3). Renin secretion data are means ± s.e.m. of nine kidneys for the control period, six kidneys for the period during ISO infusion only, and three kidneys after the start of EGTA infusion. AngI, angiotensin I.

RESULTS

Renin secretion rates of normal mouse kidneys perfused under standard conditions reached stable renin release rates after 15 min (Figure 1). After a control perfusion period of 5 min, kidneys were perfusion-fixed for electron microscopic analysis. Renin-producing cells in the juxtaglomerular areas contained numerous electron-dense vesicles that frequently showed an irregular circumference on 2D sections (Figure 2a). Up to 100 serial sections per cell were prepared to generate a 3D picture of renin-storing vesicles. These 3D reconstructions revealed that different forms of vesicles existed, ranging from single granules to huge interconnected caverns (Figure 2b and c). We analyzed a total of six cells from three kidneys, which all displayed a rather similar appearance. The distribution of single granules and caverns showed some variability even among renin-producing cells of the same kidneys under control conditions, suggesting that granules and cisternae are states of a dynamic equilibrium (Figure 2d). Common to all of these cells was that the vesicles covered around 36% of the extranuclear space of the cells (Table 1). The average volume of a vesicle was around 0.6 fl (Table 1). In six individual cells under control conditions, we reconstructed up to 50% of the cell surfaces. However, we did not observe membrane figures indicative for exocytosis, such as omega-shaped structures. We also did not observe electron-lucent or empty vesicles in these cells.

In parallel, we also perfused and analyzed kidneys that expressed the *ren-2*, but not the *ren-1d*, gene. In the perfusate of these kidneys, very low enzymatic renin activity (5 ng AngI/h×ml×g) could be measured, which, however, was not increased by isoproterenol, nor by lowering of calcium. In juxtaglomerular cells of these kidneys, electron-dense interconnected vesicular structures could be seen as well (Figure 3a–c). The average volume of these vesicles in ren-2 kidneys was clearly smaller than those found in ren-1d/ren-2 kidneys (Table 1). Moreover, the total vesicular volume in ren-2 kidneys was less than 20% of those measured in ren-1d/ren-2 kidneys (Table 1).

We next analyzed cells from ren-1d/ren-2 kidneys in which renin secretion was prestimulated by isoproterenol (5 nmol/l) for 5 min, which led to a fivefold increase of renin secretion from the isolated kidneys (Figure 1). In none of the six cells analyzed did we find signs of exocytosis or structural alterations of the vesicular renin-storage system (not shown).

During isoproterenol infusion (5 nmol/l), we then lowered the extracellular concentration of calcium (by the addition of EGTA 2.5 mmol/l) for another 5 min. This maneuver led to a 20-fold stimulation of renin release over basal values (Figure 1). Now, rearrangment of the vesicles became apparent on 2D sections (Figure 4a). Larger caverns due to

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