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3D structural analysis of renin-producing kidney cells

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The protease renin is the key enzyme regulating the activity of the renin–angiotensin–aldosterone system [1]. It is mainly produced and secreted in the kidney by specialized juxtaglomerular cells, which are located in the terminal parts of afferent arterioles. After synthesis and glycosylation, prorenin is transferred and stored into storage vesicles [2], and then further proteolytically processed to renin. The release of renin is controlled by two oppositely acting signaling pathways. The cyclic adenosine monophosphate (cAMP) signaling pathway stimulates renin release, whereas a Ca^{2+}-related signaling pathway inhibits the release of renin. The mode of renin release from the cells into the circulating blood is still unsolved in several aspects: morphological signs of exocytosis, such as omega-shaped figures, have very rarely been reported in renin-producing cells [3].

For a better understanding of the renin release process, we studied the morphology of renin-storage vesicles inside the renin-producing cells, and the changes during controlled modulation of renin release [4]. For this goal, we combined the model of the isolated perfused mouse kidney, which allows us to modulate renin release under nearly physiological conditions, with an analysis of the 3D ultrastructure of those cells, using serial section electron microscopy.

The mouse kidney was chemically fixed by perfusion with PBS buffer containing 2% glutardialdehyde (GA) at constant pressure (90 mm Hg). Kidneys were cut in half, stored at 4°C overnight in PBS/2% GA, trimmed to 1 mm^3 cubes and further processed in a microwave processor (Leica AMW), including fixation and staining with 1% OsO_4, dehydration, and embedding in resin. The dehydration / embedding protocol lasted about 3 hrs 30 min, and the resin hardening about 2 hrs 10 min. For some kidney samples, OsO_4 was omitted completely; for other samples, perfusion fixation was followed by high-pressure freezing, freeze-substitution fixation in acetone / 2% GA / 0.5% UAc / 5% water and embedding in Epon. Micrographs of up to 110 sections were recorded digitally in form of montages, using a 1k CCD camera (TVIPS, Gauting, Germany) on a CM12 TEM (FEI Co., Eindhoven, NL). Image stacks, generated using ImageJ, were segmented and visualized using AMIRA. Surfaces were computed from the material data, resulting in a 3D model of the juxtaglomerular cell. In most experiments, two cells were reconstructed per kidney.

Ultrathin sections of selected samples were tested for intracellular distribution of renin by post-embedding on-section immuno-labeling using an antibody specifically raised in chicken against mouse renin, and a secondary goat anti-chicken antibody coupled with ultrasmall Gold (Aurion). The quality of the kidney sample was good enough to specifically detect the renin in the storage vesicles [Fig 1], even after post-fixation with OsO_4, although with weaker detection efficiency.

Our 3D structural analysis revealed that renin is not stored in simple round vesicles; the vesicles containing renin have variable shapes, and they form a network of single granules and huge interconnected cavern-like structures (Fig 2). The vesicles cover about 36% of the extranuclear space in the renin-producing cells. Acute stimulation of renin release led to increased exocytosis in combination with intracellular fusion of vesicles to larger caverns and their subsequent emptying [4]. The results obtained so far suggest that renin is released by mechanisms similar to compound exocytosis. We intend to continue our studies by improving the 3D resolution using FIB-SEM analysis [5], and in addition to analyse selected semithin sections by TEM tomography, aiming eventually for a detailed visualization of rare exocytosis events.

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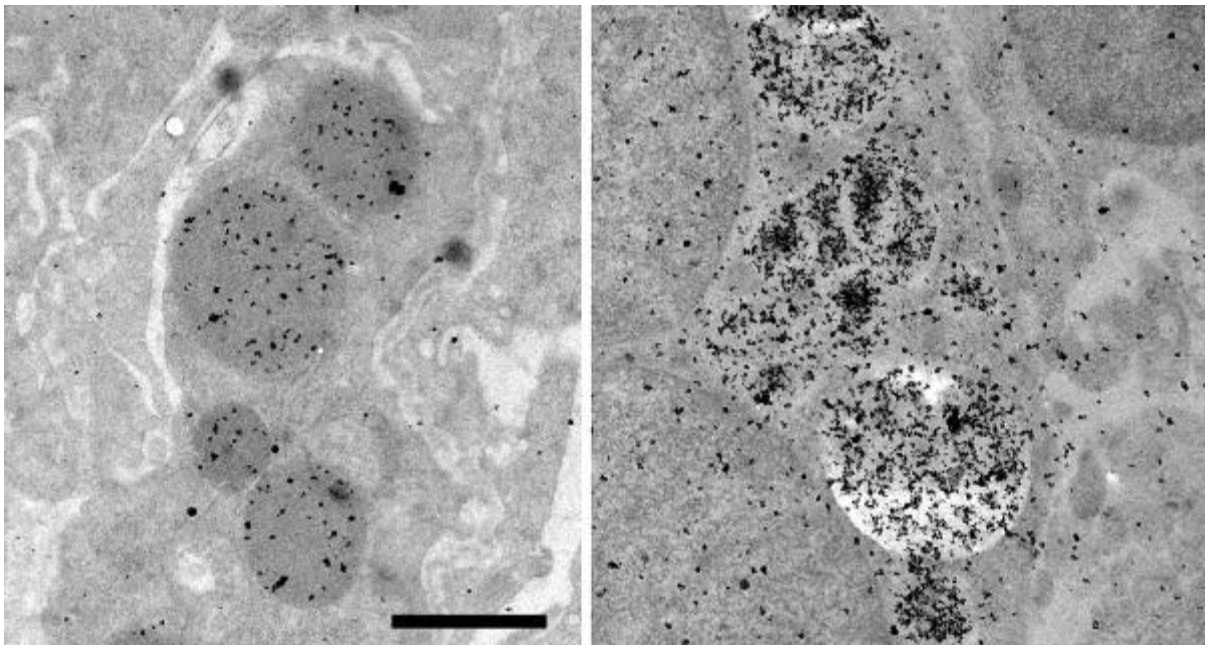

Figure 1. Subcellular distribution of renin in juxtaglomerular renin-producing cells, studied by postembedding immuno-labeling. Kidneys were fixed by perfusion with PBS / 2% GA. Left, postfixation with 1% OsO_4 and AMW embedding in Epon; right, HPF, FSF with GA and UAc, but without OsO_4, and embedding in Epon. Postembedding immuno-labeling with primary and secondary antibodies using standard protocols. Bar, 1 µm for both images.

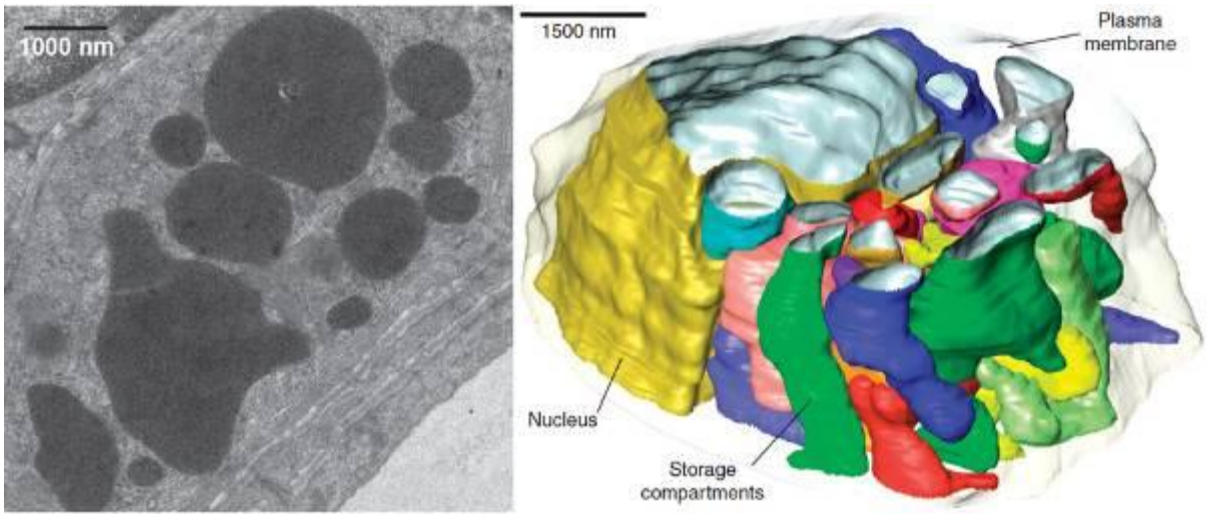

Figure 2. Analysis of juxtaglomerular renin-producing cells by serial-section TEM. Left, single TEM section, original TEM micrograph. Right, 3D reconstruction of the cell with individual renin storage compartments (or vesicles) in different colors.