Renin release: role of SNAREs

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Mendez M. Renin release: role of SNAREs. Am J Physiol Regul Integr Comp Physiol 307: R484–R486, 2014. First published June 18, 2014; doi:10.1152/ajpregu.00175.2014.—Little is known about the molecular mechanism mediating renin granule exocytosis and the identity of proteins involved. We previously showed that soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNAREs), a family of proteins required for exocytosis, mediate the stimulated release of renin from juxtaglomerular cells. This minireview focuses on the current knowledge of the proteins that facilitate renin-granule exocytosis. We discuss the identity of potential candidates that mediate the signaling and final steps of exocytosis of the renin granule.

renin; SNAREs; hypertension; cAMP; exocytosis

RENIN HAS BEEN STUDIED for the past 100 years. Renin generates angiotensin I by cleaving its unique substrate angiotensinogen. This first step is indispensable for the subsequent formation of angiotensin II. Thus renin is essential for sodium homeostasis and blood pressure regulation (6). We have great knowledge on the expression of renin throughout kidney development (5), proteolytic activation of human renin by cleavage of its prosegment (3), and regulation of the renin gene and its promoter (13). Given its importance, several hormonal and intrinsic mechanisms regulate renin release (6). Most known agonists enhance intracellular cAMP (4, 6). While the stimulatory role of cAMP in renin release is clear, the molecular mechanisms by which cAMP induces release of renin from its storage granules have been less studied.

After maximal stimulation, a small fraction (3 to 5%) of the total renin content in juxtaglomerular cells (JG cells) is released. Using electron microscopy, Taugner et al. (14) first reported that after acute hemorrhage, a maximal stimulus for renin release, fusion of renin granules with the plasma membrane, was rarely observed, and the decrease in the number of granules was negligible (14). This observation was later replicated in ex vivo preparations (8), as well as in primary cultures of JG cells (11, 12). These data indicate that the release of renin from its storage granules is tightly regulated and controlled.

Renin is stored in dense core secretory granules in JG cells of the kidney. Renin is first packed in juvenile immature secretory granules carrying a higher ratio of the immature pro-renin to renin (6). Maturation of the renin secretory granule into larger dense core granules is accompanied by a predominant increase in mature/active renin (15). Despite the importance of the formation of a pool of readily releasable renin granules, the structural/scaffolding proteins involved in renin granule maturation remains unclear. Importantly, the molecular mechanism leading to renin release from its granules has remained mostly hypothetical.

In other endocrine cells, there is detailed knowledge on the mechanisms that mediate granule exocytosis (7, 17). However, renin granule exocytosis and the involvement of fusion per se in renin release have not been fully demonstrated. The small advance in this field could be attributed to the lack of a good...
model to study the biology of renin in JG cells. Immortalized tumor renal JG cells (As.4.1) have been a great resource to study renin promoter regulation (13). However, As.4.1 cells differ from native JG cells, showing fewer renin granules, a modest response to stimulation, and release of prorenin rather than active renin (10). The number of JG cells per nephron is very small (4–10 cells/nephron). JG cells are fragile, do not attach well to a matrix, and require stable culturing conditions. To understand the molecular mechanisms of renin release, our laboratory, in collaboration with others, refined a preparation of primary cultures of mouse JG cells that respond to all known physiological stimuli and conserve their characteristics.

Membrane fusion is required for exocytosis of granule content and is mediated by the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) (soluble NSF attachment protein receptor, where NSF stands for N-ethyl-maleimide-sensitive fusion protein) family of proteins (2). A minimal of three proteins form a SNARE core complex: one vesicle-associated membrane protein (VAMP), one syntaxin, and one SNAP (18). There are 8 VAMPs, 17 syntaxins, and 4 SNAP described to date (2). In most cells, selective pairing of different SNAREs confers high specificity for membrane fusion of different subcellular compartments (9). Fusion is a complex process that involves regulatory (“accessory”) proteins that differentially mediate the sequential steps leading to final fusion of the two membrane bilayers (2). Accessory proteins, such as Snapin, Munc18, and tomosyn, can be phosphorylated and serve as an additional regulated control of exocytosis by some signaling cascades.

Our laboratory showed that SNAREs and some of its accessory proteins are expressed in JG cells. We found that VAMP2 was enriched when compared with total kidney homogenates (12). In addition, silencing VAMP2 blocked cAMP-stimulated renin release. In support of a role for VAMP2 in mediating exocytosis in JG cells we found that tetanus toxin, a clostridial toxin that cleaves both VAMP2 and VAMP3, decreased cAMP-stimulated membrane exocytosis in JG cells. Synaptosome-associated proteins (SNAPs) are also required for SNARE-dependent fusion. We found that SNAP23, but not SNAP25, is expressed in JG cells (11). Inhibiting SNAP23 function by short hairpin RNA (shRNA) silencing or with a dominant negative construct blunted cAMP-stimulated renin release without affecting the total content of renin or baseline renin release (Fig. 1). These were the first evidence indicating that renin release occurs due to a fusion event that is driven by SNAREs. Despite the clear involvement of VAMP2 and SNAP23 on stimulated release, identification of the VAMP, syntaxin, and SNAP isoforms that mediate baseline renin release remains elusive.

SNARE proteins are required for exocytosis in most organs. Therefore, genetic deletion of most SNAREs is not viable. VAMP3 and VAMP8 knockout mice are viable but their renal function is not clear. In other cells, VAMP8 mediates exocytosis and is, also, critically involved in granule-to-granule fusion (1). It is unclear whether granule-to-granule fusion is required for renin processing and maturation (Fig. 1). Identification of the unique SNARE pairs involved in this process could offer a potential explanation for the differential release of prorenin over mature renin under certain conditions.

VAMP8 knockout mice show impaired water reabsorption due to impaired vasopressin-stimulated aquaporin 2 trafficking, resulting in a water-wasting phenotype (16). However, it is unclear whether VAMP8 is involved in renin release. We are currently studying the role of VAMP3 and VAMP8 in baseline renin release.

Overall, our data suggest that different SNARE proteins are likely involved in regulating baseline and stimulated renin release from juxtaglomerular cells.

Perspectives and Significance

A large percentage of hypertensive patients do not respond to the conventional antihypertensive therapies. Thus additional pharmacological targets are required to inhibit renin, control blood pressure, and improve current therapies. Identification of SNAREs, their molecular regulation, and structural protein components of renin granules in native JG cells are likely to provide new targets that may be used to inhibit renin before its release into the circulation.

REFERENCES