Distinct localization of renin and GLUT-4 in juxtaglomerular cells of mouse kidney

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Distinct localization of renin and GLUT-4 in juxtaglomerular cells of mouse kidney. Am. J. Physiol. 274 (Renal Physiol. 43): F26–F33, 1998.—The insulin-responsive glucose transporter, GLUT-4, is found primarily in adipocytes and skeletal muscle cells, where it is sequestered in a specialized recycling compartment, from which it can be recruited to the cell surface following insulin stimulation. Lower levels of GLUT-4 are also expressed in other tissues, including the kidney, where it is present particularly in cells of the afferent arteriole and juxtaglomerular apparatus (JGA). The exact nature of GLUT-4-containing compartments and their relationship to other regulated trafficking pathways in different cells are not yet well defined. The trafficking of GLUT-4 has been studied in different cells with regulated secretory pathways, and a recent study shows that, in cardiomyocytes, GLUT-4 is sorted and packaged into multiple regulated pathways (J. W. Slot, G. Garruti, S. Martin, V. Oorschot, G. Pshuma, E. W. Kraegen, R. Laybutt, G. Thibault, and D. E. James. J. Cell Biol. 137: 1243–1254, 1997). In the kidney, cells of the JGA synthesize and secrete their major product, renin, via a well-established, regulated, secretory pathway. These cells also express GLUT-4 and thus offer the potential to directly compare the localization and trafficking of GLUT-4 and renin in a unique cell type. The present study was undertaken to investigate the intracellular distribution of GLUT-4 in mouse kidney cortex and to determine whether GLUT-4 and renin are trafficked in the same or in separate regulated pathways. Ultrathin cryosections of mouse kidney were labeled by the immunogold technique and viewed by electron microscopy, demonstrating the distribution of GLUT-4 in cells of the JGA, afferent arteriole, and distal tubule. In granular cells of the JGA, renin was localized in secretory granules of the regulated secretory pathway, whereas GLUT-4 labeling in the same cells was found in a distinct tubulovesicular compartment located adjacent to the trans-Golgi network. We show that granulun cells have separate, morphologically distinct compartments for the sequestration of renin and GLUT-4, providing evidence that there may be distinct pathways for the sorting and trafficking of these two proteins.

Renin is a key enzyme in the generation of the vasoactive hormone, angiotensin II, and in this role it is essential for maintaining blood pressure and electrolyte balance. Circulating renin in the plasma is primarily derived from the juxtaglomerular apparatus (JGA) of the kidney. A group of modified smooth muscle cells in the wall of the terminal portion of the afferent arteriole, known as granular cells, are responsible for the synthesis and secretion of renin within the JGA (8). Renin is initially synthesized as an inactive precursor, prorenin, which is trafficked through the Golgi complex and packaged into immature secretory granules or proteogranules. During maturation of the granules, the prosequence is cleaved to form active renin, which is then stored in the mature secretory granules (8). Active renin is secreted via a stimulus-coupled, regulated secretory pathway in which stored renin is released into the surrounding interstitium from individual granules or by compound exocytosis from multiple granules (4, 16). In some circumstances, prorenin may also be secreted from immature granules (4, 25) or in smaller vesicles via a constitutive pathway (7).

Renin-containing granules are somewhat atypical secretory granules, having some features in common with lysosomes; myeloid bodies and vesicular membranes are found as inclusions within the granules, and they contain proteolytic enzymes, including cathepsin B for processing prorenin, cathepsin D, and other acid hydrolases (26). To date, however, many aspects of the renin secretory pathway remain poorly understood; for instance, it is not known how prorenin is sorted and packaged into immature granules at the level of the trans-Golgi network (TGN). One approach to further investigating renin secretion is to compare the sorting and trafficking of renin with that of other proteins trafficked in the same, or in distinct, regulated pathways in granular cells.

Glucose transporters are widely and variably distributed in different cells, where they reside constitutively at the cell surface to mediate the facilitative uptake of glucose into cells (1). One member of this family, the insulin-regulated glucose transporter, GLUT-4, is sequestered within the cell and is recruited to the cell surface to augment glucose uptake in response to insulin (reviewed in Ref. 13). GLUT-4 is expressed in adipocytes and muscle cells, which have acute and frequent requirements for increased glucose uptake (19, 20). In the unstimulated state, GLUT-4 in these cells is concentrated in a specialized tubulovesicular compartment located just beneath the plasma membrane (19, 20). This reservoir of GLUT-4 is then available for rapid recycling to and from the cell surface upon insulin stimulation (21). GLUT-4 is thus trafficked, within specialized cells, in an insulin-responsive, customized, recycling, regulated pathway. The exact nature of the tubulovesicular compartment and the GLUT-4 recycling pathway has not yet been fully defined. It is not yet clear, for instance, whether the GLUT-4 pathway is unique or whether it is a subset of...
other regulated trafficking pathways. Similarly, is the sorting of GLUT-4 handled by unique mechanisms or is it sorted by mechanisms common to other regulated secretory proteins?

To address these questions, GLUT-4 distribution has been studied in cells with multiple regulated secretory pathways. In two separate studies, GLUT-4 was expressed in PC12 neuroendocrine cells, and its distribution was compared with that of other proteins serving as markers for defined secretory pathways in these cells. One of these studies (12), but not the other (11), found evidence for GLUT-4 packaging into large, dense core granules of the regulated secretory pathway, in addition to its presence in smaller vesicles. Recently, localization of GLUT-4 in atrial cardiomyocytes showed that GLUT-4 was partitioned into both regulated secretory granules and into tubulovesicular elements (17). Questions about the sorting and trafficking of GLUT-4 and about the origins of the tubulovesicular compartment in different cell types therefore still persist.

GLUT-4 is expressed at relatively low levels in a variety of other tissues, including the kidney (1), where its intracellular distribution and modes of trafficking are not yet known. Members of the glucose transporter family are expressed throughout the kidney, where their distribution varies with glucose transport activity and metabolic requirements of cells along the length of the nephron (5, 6, 9, 22). Previous studies, using a combination of immunocytochemical techniques to detect GLUT-4 mRNA or protein at the light microscopy level, have demonstrated relatively high expression of GLUT-4 in smooth muscle cells of the afferent arterioles (5) or in cells of the thick ascending limb (TAL) of the nephron (6, 9). These studies vary in detection of GLUT-4 in the glomerulus, although GLUT-4 has been shown to be expressed by cultured mesangial cells (5, 27). The ultrastructural localization of GLUT-4 within different renal cells has not yet been determined, yet such information is key for understanding the possible physiological role of GLUT-4 in the normal kidney and also in the diabetic condition.

The intracellular distribution of GLUT-4 in kidney cells, particularly those with regulated secretory pathways, is of interest in further defining the pathway(s) for GLUT-4 trafficking. In this study, we investigated the distribution of renin and GLUT-4 in granular cells of the JGA. Our aim was to compare the sorting and packaging of these two proteins in this unique cell type, which has at least one established regulated pathway for renin secretion.

**MATERIALS AND METHODS**

Fixation and tissue processing. BALB/c mice weighing 15–20 g were perfused with phosphate-buffered saline (PBS) followed by fixative (4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4) for 5 min. Both kidneys were dissected out, and 1-mm³ cubes of cortex were immersed in fixative for a further 1–2 h, then stored at 4°C.

Immunogold labeling. Tissue blocks were washed in PBS, infiltrated with 2.3 M sucrose in PBS overnight, mounted on cryostubs, and frozen in liquid nitrogen. Thin sections were cut using a Reichert Ultracut S cryoultramicrotome and labeled with antibodies specific for GLUT-4 and renin. The polyclonal GLUT-4 antibody was raised against a carboxy-terminal peptide of GLUT-4 and has been shown to give specific labeling of this protein in a variety of cells (19, 20). A polyclonal rabbit anti-mouse renin serum has been shown to recognize renin in mouse and rat tissues (4). This renin antibody recognizes both prorenin and active renin, lacking all or part of the propeptide. All primary antisera were diluted to 5 μg/ml with PBS-1% bovine serum albumin (BSA). Protein A-10-nm gold and protein A-5-nm gold conjugates were produced by the method of Slot et al. (18). Ultrathin cryosections on grids were immunolabeled by successive flotation on drops of PBS containing the following major additives: 2% gelatin, 0.02% glycine, 1% BSA, primary antiserum, protein A-gold (5- or 10-nm particles), and finally 1% glutaraldehyde. Sections were then stained and mounted in 1.8% methyl cellulose containing 0.3% uranyl acetate and viewed in a JEOL 1010 electron microscope.

**RESULTS**

Examination of immunolabeled, serial cryosections of mouse kidney cortex revealed that renin labeling was present only in granular cells of the JGA or the afferent arteriole. GLUT-4 labeling was present at several sites in the mouse renal cortex, including 1) cells of the JGA, 2) smooth muscle cells along the length of the afferent arteriole wall, and 3) distal tubule epithelial cells. No GLUT-4 labeling was seen in proximal tubule epithelial cells in this study. Within the glomerulus, we found no specific labeling over the mesangium.

Colocalization of GLUT-4 and renin in juxtaglomerular cells. Serial ultrathin cryosections of the JGA in renal cortical tissue were single or double labeled with renin and GLUT-4 antibodies. Renin labeling was confined to a small group of cells, identifiable even at low magnification as granular cells by the presence of prominent secretory granules (Fig. 1). At higher magnification, it was evident that gold particles representing renin labeling were concentrated in the lumens of secretory granules (Fig. 2). Labeling was found in a range of granules with different sizes and densities, representing sequential stages of maturation (Figs. 2 and 3, A–C). In these sections, there was also labeling of (pro)renin in newly forming granules associated with the TGN, but no labeling of prorenin in earlier parts of the Golgi or in the endoplasmic reticulum was seen.

Within the JGA, GLUT-4 labeling was present in granular cells, in adjacent cells of the afferent arteriole without prominent renin granules, and in cells of the adjacent macula densa (described below). Over the extraglomerular mesangium there were occasional sparse gold particles, at the level of background labeling and not indicative of specific GLUT-4 labeling. The intracellular distribution of GLUT-4 within granular cells is shown in Fig. 3. The labeling was concentrated in distinct clusters of vesicles, including cistern-coated vesicles and interconnected ramifications, which were often adjacent to the TGN. The TGN was identified in these sections by its position adjacent to stacked Golgi cisternae and its proximity to the centriole (Fig. 3). These GLUT-4-labeled structures had the appearance of tubulovesicular elements found in...
other cells for the specialized sequestration and recycling of GLUT-4 (20, 19, 17). There was no labeling of GLUT-4 on the plasma membranes of granular cells.

Double labeling of sections with antibodies to both GLUT-4 and renin confirmed that both proteins were found in separate locations within granular cells. Most notable was the complete absence of GLUT-4 labeling in the renin-containing secretory granules (Fig. 3, A–C). Gold labeling representing GLUT-4 or renin was in separate post-Golgi compartments, either the tubulovesicular elements or the secretory granules, respectively. Even within the TGN, the labeling for GLUT-4 and (pro)renin was found in separate areas (Fig. 3C), consistent with the sorting and segregation of the two proteins into distinct pathways or compartments.

Distribution of GLUT-4 and renin along the afferent arteriole. Granular cells, identified by the presence of electron dense granules, were found along the wall of the afferent arteriole, even at a distance from the JGA. These cells contained multiple secretory granules labeled for renin (Fig. 4). GLUT-4 labeling was also found in these granular cells, and additionally, in other non-renin-labeled, smooth muscle cells in the walls of the afferent arteriole and interlobular arteries. The labeling of GLUT-4 in cells of the arteriolar walls was thus more widespread than that of renin. Both proteins exhibited intracellular distributions in these cells similar to those seen in the JGA cells. GLUT-4 was abundant on tubulovesicular elements near the TGN (Fig. 4), and renin labeling was seen in typical secretory granules. Thus the distributions of renin and GLUT-4 remain separate and consistent with respect to one another in cells along the length of the arterioles.

Localization of GLUT-4 in epithelial cells of the distal tubule. Significant concentrations of GLUT-4 labeling were also found in renal tubule epithelial cells within the cortex (Figs. 5 and 6). There was no GLUT-4 labeling in proximal tubule cells nor in cells of cortical collecting ducts. Viewing of several tissue blocks, serially sectioned through this region of cortex, showed GLUT-4 labeling in macula densa cells, providing a potentially interesting link to the GLUT-4 in the renin-secreting granular cells of the JGA. A cluster of macula densa cells with a typical compressed columnar appearance was identified by its position adjacent to the JGA (see Fig. 4).
Fig. 1. GLUT-4 was localized in a perinuclear position, near the Golgi complex in these cells (Fig. 5A). The labeling was concentrated in classic tubulovesicular elements at this position (Fig. 5B). Prominent staining of GLUT-4 was seen in the cells at the outer edges of the macula densa, whereas the equivalent peri-Golgi areas of the more central macula densa cells were not in these sections.

Specific GLUT-4 labeling was also present in cells along the length of the TAL of the distal tubule, outside the area of the macula densa (Fig. 6). In contrast to all of the other cells labeled in these mouse kidney sec-

Fig. 3. Double labeling of renin and the insulin-responsive glucose transporter, GLUT-4, in JGA cells. Serial sections of a granular cell are shown in A, B, and C. These frozen sections appear somewhat overstretched as a result of the weak fixative used to preserve antigenicity; this often causes the Golgi stack to split. A large granule is intensely labeled for renin and smaller, newly forming granules are less densely labeled for renin (r) (arrows). A small amount of labeling for renin is also found associated with the trans-Golgi network (TGN) adjacent to the stacked Golgi (g). Clusters of GLUT-4-positive tubulovesicular elements (arrowheads) are adjacent to stacked Golgi cisternae in the area of the TGN (t) which can be identified by its relationship to the centriole (c). Scattered tubulovesicular elements and clathrin-coated vesicles in this region are labeled for GLUT-4 (arrowheads). C: a slightly reduced field of view shows that, although tubulovesicular elements and the TGN directly abut the renin granule at this level, there is no overlap in renin and GLUT-4 labeling; n, nucleus. Bars = 100 nm.
tions, these TAL epithelial cells showed prominent labeling of GLUT-4 in tubulovesicular elements close to the cell surface (Fig. 6). GLUT-4 was also found in tubulovesicular elements at the level of the TGN in a supranuclear position. GLUT-4 was also present among the infoldings of the basolateral plasma membrane, where there was labeling on the basolateral plasma membrane itself; however, no specific labeling was found on the apical (luminal) plasma membrane (Fig. 6B). The peripherally located clusters of GLUT-4 in TAL cells are more typical of GLUT-4 distribution in cells such as adipocytes, which most actively deploy and recycle this transporter.

DISCUSSION

We have demonstrated that the insulin-regulated glucose transporter, GLUT-4, is expressed in smooth muscle cells and in restricted populations of epithelial cells in the mouse renal cortex. The most prominent concentrations of GLUT-4 we detected were in afferent arteriole cells and smooth muscle cells of the larger arterioles. Both GLUT-4 and renin were localized in the granular cells of the JGA. The presence of GLUT-4 in the same cells that secrete renin is significant, because it shows that these unique cells, the granular cells, contain two, distinct secretory compartments for the separate sequestration of renin and GLUT-4.

The intracellular processing and storage of renin in distinctive secretory granules in granular cells of the JGA and distal afferent arteriole has been well documented in the literature (reviewed in Ref. 24). Our data showing localization of renin in secretory granules at different stages of maturation in granular cells is consistent with previous observations at the electron microscopy level (4, 23). The antibody used here for labeling recognizes both prorenin and renin. The concentrated labeling seen in mature renin granules most likely represents labeling of the cleaved, active form of renin, whereas smaller amounts of labeling detected in the TGN represent prepackaged prorenin. The sections of granular cells observed in this study were from untreated mouse kidneys and thus had little evidence of active renin secretion. Morphological features associated with active secretion, such as discharging granules, widespread labeling of renin and prorenin in a variety of post-Golgi structures, and concentrations of secreted renin in extracellular interstitium, are usually only seen following treatment with angiotensin converting enzyme inhibitors or in hydronephrotic kidney cells, where renin secretion is greatly enhanced (2–4). Interestingly, under stimulated conditions, extra cells in the afferent arteriole wall can be recruited for de

Fig. 4. Arteriolar renin and GLUT-4 labeling distal to the JGA. A: a renin-secreting cell in the wall of the afferent arteriole at a distance from the JGA is double labeled for GLUT-4 (10-nm gold) and renin (5-nm gold). GLUT-4 labeling (arrowheads) is on coated vesicles and tubulovesicular elements adjacent to the TGN. Renin labeling (arrows) is shown here in one fully formed granule and one newly formed granule (top). Bar = 0.25 µm.

Fig. 5. GLUT-4 labeling in the macula densa. A: low-power view showing a cross section of the macula densa. One of the cells on the outer edge of the macula densa is shown at higher magnification in B. B: area outlined in A shows gold labeling of GLUT-4 in perinuclear tubulovesicular elements. There was no labeling near the cell surface. Golgi areas are not observed in the more central macula densa cells in these sections. Bars: 2 µm (A) and 0.25 µm (B).
novo renin production, seen as the emergence of renin granules in increased numbers of cells in this region (2, 8). Renin can also be produced by other cortical cells, particularly, it seems, under stimulated conditions or in cell culture where gene expression is turned on. Proximal tubule cells in culture for instance express renin (10, 22) and have also been reported to express GLUT-4 (6), although the mouse kidney sections showed no labeling for either of these proteins. Thus arteriole smooth muscle cells continuously express GLUT-4, as shown both previously (5) and in the present study, but only initiate the expression and trafficking of renin under stimulated conditions.

In this study we found that the only significant concentrations of GLUT-4 in mouse kidney cortex were seen in cells of the JGA, in arterioles, and in epithelial cells of the TAL. Previous studies have also reported the presence of GLUT-4 mRNA or protein in each of these cell types (5, 6, 9). In addition, GLUT-4 mRNA has been detected in situ in glomerular cells and in cultured mesangial cells (5, 27). We found no significant labeling over areas of the intra- and extraglomerular mesangial cells. The relative sensitivity of techniques used in different studies or differences in expression between species or differences in the state of physiological stimulation may account for the inconsistent detection of GLUT-4 in glomeruli. Similar explanations may apply to the absence of GLUT-4 labeling in proximal tubule cells, which is at odds with some of the previous reports showing GLUT-4 expression in these cells (9). Although the presence of very low amounts of GLUT-4 in other cortical cells cannot be ruled out, the consensus of this study, together with previous data, suggests that the major sites of GLUT-4 expression in the kidney cortex are in the renal arterioles and in distal tubule TAL epithelial cells.

We found that GLUT-4 in TAL epithelial cells was localized in the region of the TGN and was also found more peripherally in the cells in tubulovesicular elements and on the basolateral plasma membrane. This distribution resembles the pattern of insulin-responsive GLUT-4 accumulation in adipocytes and muscle cells (19, 20). GLUT-4 has previously been described in cells of the medullary TAL, where it was noted that the same cells express insulin-like growth factor-I (IGF-I) (6). This fact, along with the finding that GLUT-4 mRNA expression increases with stimulation of TAL metabolic activity, led to the speculation that IGF-I-responsive GLUT-4 might be involved in providing the high levels of glucose typically utilized by

![Image](http://altprenal.physiology.org/)

**Fig. 6.** GLUT-4 labeling in an epithelial cell of the thick ascending limb (TAL) of the distal tubule. A: GLUT-4 is present on tubulovesicular elements in the region of the TGN in a supranuclear (n) position within this TAL cell. There is no labeling along the apical plasma membrane (ap); sporadic gold particles can be seen near the lateral plasma membrane below the level of the tight junction. B: clusters of gold particles representing GLUT-4 labeling on small cross sections of tubulovesicular elements and scattered membrane staining can be seen among the basal infoldings of the TAL cells. Base of this cell is denoted by the adjacent basement membrane (bm). Bars: 1 µm (A) and 0.5 µm (B).
TAL cells for oxidative metabolism (6). Our data showing clusters of GLUT-4 near the cell surface are consistent with such an active utilization of GLUT-4 for glucose uptake in TAL cells. The distribution of GLUT-4 in these TAL cells is similar to that found in adipocytes and muscle cells, where GLUT-4 is typically concentrated in a prominent tubulovesicular compartment from which it is recruited to the cell surface upon insulin stimulation (19).

The other contents of the tubulovesicular elements and the nature of the recycling pathway for GLUT-4 have not yet been fully characterized, even in adipocytes (13). It is not clear, for instance, to what degree the GLUT-4 pathway overlaps with the trafficking of other secreted proteins. There is now evidence to suggest that GLUT-4 may be trafficked by divergent pathways in some cell types (11, 12, 17). Heterologous expression of GLUT-4 in PC12 neuroendocrine cells resulted in sorting of some of the GLUT-4 into dense core secretory granules where it colocalized with secretogranin (12). Another study in PC12 cells, however, reports that the expressed GLUT-4 was excluded from the regulated secretory pathways but was concentrated in a unique, small vesicle population, which is likened to the tubulovesicular compartment in adipocytes (11). These somewhat conflicting results may be due in part to the heterologous expression of the GLUT-4.

Endogenous GLUT-4 has also recently been localized in atrial cardiomyocytes, which have a major regulated secretory pathway for the release of atrial natriuretic factor (ANF) from stored secretory granules (17). In atrial cardiomyocytes, GLUT-4 was found in tubulovesicular elements that were often adjacent to the TGN, similar to the distribution we report here in granular cells. However, in the cardiomyocytes, a significant proportion of GLUT-4 was also found within the dense core secretory granules, where it is packaged along with the soluble ANF (17). The pool of GLUT-4 in ANF granules was derived from a recycling pathway and was not responsive to insulin, whereas the GLUT-4 found in the tubulovesicular elements was translocated upon insulin challenge (17). Thus atrial cardiomyocytes have two distinctly regulated pools of GLUT-4 in separate, regulated secretory pathways.

In the current study our approach was to compare the localization of endogenous GLUT-4 with that of an endogenous regulated secretory protein, renin, in JGA cells. Our findings in granular cells show that GLUT-4 is completely excluded from the regulated secretory pathway and is sequestered in the tubulovesicular compartment. Granular cells in the kidney, while having an active regulated secretory pathway for renin release, do not package any GLUT-4 into regulated secretory granules. Thus the sorting and trafficking of endogenous GLUT-4 is different in two types of modified smooth muscle cells, granular cells and atrial cardiomyocytes. Our results suggest that granular cells have a highly efficient sorting mechanism for routing GLUT-4 into its own compartment, whereas the overall sorting of GLUT-4 in atrial cardiomyocytes is simply less efficient or occurs by different mechanisms.

The studies here describe the steady-state distribution of GLUT-4 in tubulovesicular compartment in granular cells. It is not currently known how GLUT-4 in granular cells is regulated. There was no significant labeling of GLUT-4 on the plasma membrane in granular cells in the kidneys of unstimulated mice, which is consistent with the intracellular sequestration of GLUT-4 in other cells in the absence of insulin. By comparison with analogous pools of GLUT-4 in other cells (17, 20), it is likely that this pool of GLUT-4 (i.e., the whole complement of GLUT-4 in granular cells) is available for translocation to the cell surface in response to a stimulus such as insulin. Further studies are required to test the trafficking of GLUT-4 in granular cells in response to a range of stimuli. In diabetes mellitus, GLUT-4 expression and translocation in adipocytes are defective, resulting in decreased glucose uptake (14). Similarly, GLUT-4 expression and resulting glucose uptake are also decreased in glomerular and vascular cells from rats with streptozotocin diabetes (15). The coordinate presence but distinct compartmentalization of GLUT-4 and renin in granular cells raises the possibility that these cells have multiple hormonally responsive pathways that may contribute to the pathophysiology of diseases such as diabetes and hypertension.

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REFERENCES


