

Connexins and the kidney

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Connexins and the kidney. Am J Physiol Regul Integr Comp Physiol 298: R1143–R1155, 2010. First published February 17, 2010; doi:10.1152/ajpregu.00808.2009.—Connexins (Cxs) are widely-expressed proteins that form gap junctions in most organs, including the kidney. In the renal vasculature, Cx37, Cx40, Cx43, and Cx45 are expressed, with predominant expression of Cx40 in the endothelial cells and Cx45 in the vascular smooth muscle cells. In the tubules, there is morphological evidence for the presence of gap junction plaques only in the proximal tubules. In the distal nephron, Cx30, Cx30.3, and Cx37 are expressed, but it is not known whether they form gap junctions connecting neighboring cells or whether they primarily act as hemichannels. As in other systems, the major function of Cxs in the kidney appears to be intercellular communication, although they may also form hemichannels that allow cellular secretion of large signaling molecules. Renal Cxs facilitate vascular conduction, juxtaglomerular apparatus calcium signaling, and tubular purinergic signaling. Accordingly, current evidence points to roles for these Cxs in several important regulatory mechanisms in the kidney, including the renin angiotensin system, tubuloglomerular feedback, and salt and water reabsorption. At the systemic level, renal Cxs may help regulate blood pressure and may be involved in hypertension and diabetes.

signal propagation; calcium wave; cell coupling; ATP; renal hemodynamics

Connexins (Cxs) are a multigene family of transmembrane proteins that form gap junctions, which are critical to intercellular communication in many tissues (99). Currently, the family comprises ~20 isoforms in humans and rodents. There is considerable homology between the different Cxs in humans, rats, and mice (99). The individual Cxs are named according to their molecular weight (for example Cx43 has a molecular weight of 43 kDa). The Cx protein consists of four membrane-spanning domains connected by two extracellular loops and one intracellular loop. In addition, there are cytoplasmic NH2- and COOH-terminal regions. The COOH-terminal region seems to play an important role in the regulation of channel conductance (99).

Morphologically gap junctions appear as plaques between cells. The junction has a pentalaminar structure due to the close juxtaposition of the cell membranes of the neighboring cells. A gap junction consists of a large number of channels that establishes connections between the cytoplasm of neighboring cells. In 1977, the structure of gap junction channels was elucidated from X-ray crystallographic data (11, 78). Six Cxs assemble to form a connexon, which docks with a connexon from a neighboring cell, creating a channel with a central aqueous pore. The two extracellular loops of the Cx molecules are essential elements in the docking process (99).

The gap junction channels allow the passage of inorganic ions, including Ca2+ and Na+ and secondary messengers such as cAMP and inositol 1,4,5-trisphosphate (71, 100, 115) and, therefore, enable the cells to communicate with one another. More recent data has pointed to the existence of functional, uncoupled connexon channels (116). These so-called hemichannels are thought to behave much like gap junctions, allowing molecular exchange between the intra- and extracellular environment (24).

Gap junction channels can be in an open or closed configuration, thereby controlling the passage of small molecules and secondary messengers between coupled cells (129). Along with this discovery, it was observed that the same small molecules (e.g., Ca2+) could alter gap junction conductance (129). Both gap junctions and hemichannels are regulated at several levels. In addition to the effects of small molecules on channel conductance, gating is also altered by metabolic inhibition, phosphorylation, transjunctional voltage, and mechanical stress (2, 15, 54, 93). The Cx proteins themselves undergo rapid turnover, with typical half-lives of a few hours (101). This constant renewal is thought to be an important aspect of gap junction regulation.

The connexon hexamer can be composed of one or more Cx isoforms, referred to as a homomeric or heteromeric connexon (27). Once synthesized and inserted into the cell membrane, connexons composed of similar or different Cx isoforms can

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dock to each other, forming either a homotypic or heterotypic gap junction. The many possible combinations can potentially lead to an incredible diversity in channel function, as each Cx isoform has different properties with regard to permeability, selectivity, conductance, and gating (8, 26, 31, 33, 94). The ability to regulate Cx expression and function are important (9, 22, 76, 127, 140), as gap junctions play a role in many physiological and pathophysiological functions, such as cell growth/hyperplasia (77), tissue regeneration, carcinogenesis (127), and glandular secretion (82–84).

In the cardiovascular system, several Cx isoforms play important roles in morphogenesis and electrical coupling (40, 63, 64, 119). Gap junctions transmit signals between specialized cell types, e.g., coordinating the contraction of myocytes. In the vascular system, gap junctions couple the vascular smooth muscle cells and the endothelial cells and also connect endothelial cells to underlying smooth muscle cells via myoendothelial gap junctions. The intercellular communication in the vasculature contributes to the regulation of resistance in different vascular beds according to metabolic needs and therefore ultimately participates in the regulation of blood pressure.

Pathological conditions, such as diabetes and hypertension, are associated with changes in Cx regulation and expression (38, 41, 144), while deletions of Cx genes can have deleterious effects on cardiac function (63). Cxs are of significance to normal development and physiology in a diverse range of organs, including the nervous system, reproductive organs, and the skin (14, 21, 113).

While the presence of gap junctions within the kidney has long been known (5, 124), it is only recently that the specific details of renal Cx expression have become apparent. In an excellent review published in 2008, Wagner (136) addressed, for the first time, Cx localization and function in the renal circulation. Since that time, new details have emerged that add to the issue of renal Cx expression and function within both the vasculature and the nephron. This review will provide an updated look at how Cx expression varies among different regions of the kidneys, including the renal tubules. We will also cover both established and proposed functions for Cxs in renal vascular conduction, signaling mechanisms, and the regulation of renal hemodynamics and salt/water handling. Finally, we examine what role Cxs play in renal organogenesis and pathophysiology in hypertension and diabetes.

Expression of Connexins in the Kidney

In 1966, Biava and West (5) demonstrated the presence of intercellular (gap) junctions between vascular smooth muscle cells and between vascular smooth muscle cells and endothelial cells in the juxtaglomerular part of afferent arterioles from humans. Subsequently, studies using thin-section and freeze-fracture electron microscopy have shown gap junctions in the tubular and vascular structures of the kidney (5, 6, 86, 91, 92, 123, 124). A series of early studies have shown expression of mRNA for nine different Cxs in the kidney, namely Cx26, Cx30.3, Cx31, Cx32, Cx37, Cx40, Cx43, Cx45, and Cx46 (3, 34, 37, 44, 46, 51, 88, 89, 128, 146).

Vascularature. Using freeze-fracture electron microscopy, Taugner and colleagues (123, 124) have shown the presence of numerous gap junctions (endothelial to endothelial cell, and smooth muscle to smooth muscle cell) in the renal vasculature. Upstream from the glomerulus, in the proximal afferent arteriole and the interlobular artery, fewer gap junctions were found between the vascular smooth muscle cells (30, 86, 122–124) compared with the juxtaglomerular part of the afferent arteriole. In addition, extensive myoendothelial coupling (junctions between endothelial cells and smooth muscle cells) was present in both rat and mouse afferent and efferent arterioles (30, 86, 123, 124).

In the renal vasculature, a number of Cx isoforms have been found as mRNA and protein, namely Cx37, Cx40, and Cx43 (1, 38, 144) (Table 1). Furthermore, studies in Cx45-deficient mice with replacement of the Cx45-coding region with the lacZ reporter gene suggest that Cx45 is expressed in renal arterioles (43, 64). Immunostaining of pregglomerular arterioles from rats and mice show gap junctions formed by Cx37, Cx40, and Cx43 primarily between the endothelial cells (1, 38, 51, 144), but some species’ variation seems to exist (see below). There are also some discrepancies between the different studies of Cx expression in the renal vasculature in the same species. These discrepancies most likely stem from the use of different antibodies or variation in the phosphorylation of the COOH terminus of the Cxs to which most of the antibodies bind.

Endothelial Cells. In both rats and mice, the endothelial cells of the entire pregglomerular vasculature from the renal artery to the afferent arteriole express Cx37, Cx40, and Cx43 (1, 7, 38, 39, 51, 56, 109, 121, 144) (Table 1). Cx40 shows the strongest expression throughout the endothelium of the pregglomerular vasculature (1, 51, 144), whereas the expression of Cx43 is weaker and more variable (1, 51, 144).

In the postglomerular vessels Cx43 was found to be expressed in the endothelial cells of the efferent arteriole in mice (144), but not in rats, where instead Cx37 was present in the endothelial cells (121). In rats the endothelial cells of the descending vasa recta has been found to express Cx37, Cx40, and Cx43. In contrast, a study in mice detected Cx37 and Cx40, but not Cx43 in the vascular bundles (144). It is not possible at this stage to decide whether the discrepancy with regard to Cx43 is due to technical issues or whether it represents a true species difference. Cx43 was also found in structures around the peritubular capillaries (144), and a single study has reported Cx43 in the endothelial cells of the renal veins (69).

Vascular Smooth Muscle Cells. In contrast to the situation in the endothelium, the data on the expression of Cxs in the smooth muscle cells is less clear. In an early study in rats, Barajas et al. (3) described the expression of Cx43 in the media of the preglomerular vessels. Although weak staining for Cx43 was also reported in murine afferent arterioles (51), most studies have failed to find evidence for Cx43 in the media of renal arteries and arterioles (1, 7, 118, 121, 144) (Table 1). While one study found staining for Cx37 in the media of arcuate and interlobular arteries (144), other studies in both mice and rats have failed to find any staining for Cx37 in the preglomerular vascular smooth muscle cells (1, 7, 51). A single study has reported the presence of Cx37 in pericytes in rat descending vasa recta (147). In contrast, all studies consistently show a lack of expression for Cx40 in the renal vascular smooth muscle cells in vivo (1, 7, 51, 121, 144).

Studies in extrarenal vessels suggest that Cx45 may be expressed in vascular smooth muscle cells (74, 97, 98). However, due to either the properties of the current Cx45 antibodies or the fact that gap junctions formed by Cx45 are small, only
three studies have been successful in demonstrating the presence of Cx45 in renal vessels (56, 69, 105). In these three studies, expression of Cx45 was found in vascular smooth muscle cells of both the afferent and efferent arterioles. To circumvent the potential problems with Cx45 antibodies, Krüger et al. (64) developed a transgenic mouse where the coding region of one of the Cx45 alleles is replaced by the coding region for LacZ, which can be visualized easily in cells and tissues. These mice showed staining corresponding to the media of interlobular arteries and afferent and efferent arterioles, suggesting that Cx45 is expressed in the vascular smooth muscle cells of these vessels (43, 64). The apparently low abundance of Cxs in the media of the interlobular artery and the afferent arteriole is in agreement with the ultrastructural data (see above) from mice, which also suggests that gap junctions are relatively scarce between the smooth muscle cells in this part of the renal vasculature (122).

In summary, there is abundant expression of Cx40, but also of Cx37, and, to a lesser extent, of Cx43 in the endothelial cells of the preglomerular vessels. Some studies have detected Cx43 and Cx37 in the media of the renal vessels, but the results are inconsistent between both studies and species. It remains an attractive possibility that Cx45 may be the predominant Cx expressed in renal vascular smooth muscle cells, but further studies are needed to finally settle this issue.

**Glomerulus.** Ultrastructural studies in both humans and rats have shown the presence of gap junctions within all the cells of the glomerulus (86, 124) (Table 1). In particular, many gap junctions are found between the intraglomerular mesangial cells (124), but also the podocytes and the endothelial cells showed the presence of gap junctions in freeze-fracture studies (65, 66, 123). There are no reports of gap junctions linking different cell types, e.g., mesangial and endothelial cells. Immunofluorescence studies in mice show strong staining for Cx40 in the entire intraglomerular mesangium, while staining for Cx37 is only found in the mesangial cells at the vascular pole of the glomerulus (51, 144). There is some discrepancy with regard to the glomerular endothelium, where one study reports the presence of Cx40 in the endothelial cells (51), while another fails to show Cx40 in the glomerular endothelium (144). As stated above, there are gap junctions between the podocytes, and these have been found to contain primarily Cx43 (103), although one study also suggests that Cx45 may be present in podocytes (56). Three studies have reported

**Table 1. Summary of the localization of connexin isoforms in the kidney**

<table>
<thead>
<tr>
<th>Cx</th>
<th>Renal Localization</th>
<th>Cell Type</th>
<th>Species</th>
<th>References</th>
</tr>
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<tr>
<td>26</td>
<td>Proximal tubule*</td>
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</tr>
<tr>
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<td>42, 148</td>
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<td>Epithelial</td>
<td>Mouse</td>
<td>10</td>
</tr>
<tr>
<td>37</td>
<td>Arcuate artery; IA*</td>
<td>VSMC</td>
<td>Mouse</td>
<td>144</td>
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<td>Rat</td>
<td>121</td>
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<td>Vasa recta</td>
<td>Endothelial</td>
<td>Rat</td>
<td>147</td>
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<td></td>
<td>Vascular bundles</td>
<td>Endothelial</td>
<td>Mouse</td>
<td>144</td>
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<tr>
<td></td>
<td>Glomerulus</td>
<td>Endothelial,*</td>
<td>mesangial</td>
<td>Mouse 52, 56, 144</td>
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<td>JGA*</td>
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<td>Mouse, rat</td>
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<td>CNT</td>
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<td>Mouse</td>
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<td>Endothelial</td>
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<td>Renal veins</td>
<td>Endothelial</td>
<td>Mouse</td>
<td>69</td>
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<td>Glomerulus</td>
<td>Podocytes</td>
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<td>103</td>
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<td>Epithelial</td>
<td>Human</td>
<td>133, 134</td>
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<td>Inner medullary CD*</td>
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<tr>
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<td>IA, AA</td>
<td>VSMC</td>
<td>Mouse</td>
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<tr>
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<td>VSMC</td>
<td>Mouse</td>
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<td></td>
<td>JGA</td>
<td>Granular,*</td>
<td>mesangial</td>
<td>Mouse 43</td>
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</table>

ATL, ascending thin limb of the loop of Henle; AA, afferent arteriole; CD, collecting duct; CNT, connecting tubule; DCT, distal convoluted tubule; EA, efferent arteriole; IA, interlobular artery. JGA, juxtaglomerular apparatus; TAL, thick ascending limb of the loop of Henle; VSMC, vascular smooth muscle cell. >, Stronger than. *Conflicting localization data.
staining for Cx45 in the glomerulus, but the cellular localization was not identified (10, 69, 105) (Table 1).

When LacZ and eGFP are used as reporters for the expression of Cx45 in transgenic mice, staining was found in the extra- and the intraglomerular mesangial cells, indicating that Cx45 may also be present in the mesangial cells (43).

Juxtaglomerular apparatus. The juxtaglomerular apparatus (JGA) constitutes a unique structure in the kidney consisting of the juxtaglomerular parts of the afferent and the efferent arterioles, the macula densa in the thick ascending limb of the loop of Henle, and the extraglomerular mesangium (122). Using freeze fracture, Taugner and colleagues (30, 124) demonstrated the presence of numerous gap junctions linking the various cell types in the JGA, with the exception of the macula densa where gap junctions were not found. There are many gap junctions between the cells of the extraglomerular mesangium, which is also connected by gap junctions to the cells in the intraglomerular mesangium. In addition, there are gap junctions between the extraglomerular mesangial cells and the granular cells (renin expressing cells) and smooth muscle cells of the afferent and efferent arterioles. Finally, the granular cells themselves are heavily interconnected by gap junctions (Table 1).

With the exception of the macula densa cells, which apparently lack gap junctions, the entire JGA seems to constitute a syncytium of cells interconnected by gap junctions. It is of interest to note that gap junctions are more numerous between the renin-containing granular cells than between the ordinary smooth muscle cells in the afferent arteriole (123). Interestingly, the ultrastructural studies indicated a relative lack or even absence of gap junctions between the endothelial cells in the part of the endothelium corresponding to the renin-containing part of the afferent arteriole (122, 123). Despite the lack of interendothelial gap junctions in the juxtaglomerular part of the afferent arteriole, numerous gap junctions between endothelial and granular cells were present in this part of the vessel (123).

Immunostaining studies in mice and rats have consistently shown that Cx40 has a high abundance in both the granular and the extraglomerular mesangial cells (1, 51, 69, 121, 144). Most studies also show the presence of Cx37 in the same cell types, although to a lesser extent compared with the levels of Cx40 (69, 118, 121, 144). However, a few studies have failed to show staining for Cx37 in the extraglomerular mesangial cells (51, 121) and in the granular cells (51) (Table 1). Despite the lack of evidence for morphological gap junctions in the macula densa (see above), a recent study has reported staining for Cx37 in the basolateral membrane of macula densa cells (118).

In agreement with the ultrastructural studies (see above) showing lack of interendothelial gap junctions in the juxtaglomerular part of the afferent arteriole, Hwan Seul and Beyer (51) also reported a specific absence of Cx40 staining in the endothelium of the juxtaglomerular parts of the afferent and efferent arterioles.

Hanner et al. (43) found the eGFP signal in the granular cells in Cx45fl/Nestin-Cre (eGFP) transgenic mice, suggesting that Cx45 may also be present in these cells. However, a recent study using an immunohistochemical approach failed to find evidence of Cx45 in granular cells in the adult mouse (69). The authors did, however, observe Cx45-renin colocalization during embryogenesis.

Tubules. Classical gap junctions have only been described in the proximal tubule. Ultrastructural studies using freeze-fracture found evidence for gap junctions between the proximal tubular cells in human (65) and in the rat kidney (91).

RT-PCR in situ and on microdissected tubules have shown expression of mRNA for many Cx isoforms including Cx30, Cx36, Cx37, Cx40, Cx43, Cx45, Cx46, and Cx50 in the tubules and collecting ducts (34, 110) (Table 1). Data from immunohistochemistry experiments have, in many cases, however, either failed to confirm these results or presented conflicting data. For example RT-PCR on microdissected tubules showed a particularly strong expression of Cx43 mRNA in rat inner medullary collecting ducts (34). Some studies using immunohistochemistry have been able to detect the Cx43 protein in the cells of the rat inner medullary collecting duct (3, 34), whereas other studies have failed to find any expression of the Cx43 protein in this segment of the tubular system (10, 144). The latter two studies were performed in mice, and the discrepancy with regard to the presence of Cx43 in the inner medullary collecting duct, may reflect species differences between mice and rats. A similar conflict exists over the expression of Cx45. Butterweck et al. (10) found staining for Cx45 in the distal tubule of the mouse, whereas Silverstein et al. (110) failed to find mRNA for Cx45 in the rat distal tubule using in situ RT-PCR. The former study also reported the presence of Cx26 and Cx32 in the proximal tubule (10), but this has not been confirmed by other studies (80). A recent study in mice and rats showed strong basolateral expression of Cx37 in the thick ascending limb of the loop of Henle and in the distal tubule, while the expression in the proximal tubule and the collecting duct was weaker (118).

Immunofluorescence studies in mice, rats, and rabbits showed that Cx30.3 is expressed in the thin, ascending limb of the loop of Henle and in the intercalated cells of the cortical collecting duct. The pattern of expression of Cx30.3 was in agreement with the expression of β-galactosidase in kidneys from transgenic Cx30.3+/LacZ mice (42, 148). A similar tubular localization was found for Cx30 (80). In a study in rats, mice, and rabbits, Cx30 was found to have the highest expression level in rats, where it was present along the entire distal nephron from the medullary thin ascending limb to the inner medullary collecting duct. In mice and rabbits, the expression level was lower, and Cx30 was primarily found in the intercalated cells of the connecting tubule and cortical collecting duct (80). Neither Cx30 nor Cx30.3 was expressed in the adjacent principal cells (Table 1, Fig. 1).

The strong expression of Cxs in the distal nephron is in contrast to the apparent lack of ultrastructural gap junctions between the tubular epithelial cells in this part of the nephron (see above). It is possible that the number of connexons in the gap junctions is so low that it is undetectable by freeze-fracture techniques. On the other hand, the observation that, in many cases, the staining for Cx30 and Cx30.3 was seen in the unopposed apical membrane of the cells suggests that these Cxs may not form gap junctions between the cells, but rather be present as hemichannels across the apical cell membrane (42, 80).

The possibility that both gap junctions and hemichannels may be present in tubular epithelial cells is supported by studies in cell cultures of renal origin. Studies in primary cultures of proximal tubule cells found Cx43 protein to be expressed in the cells, and functional Cx43 hemichannels were shown to be present (133, 134). A line of immortalized cells derived from human collecting duct cells also showed expres-
sion of Cx43, and intercellular coupling was documented by transfer of Lucifer yellow between the cells (45). The importance of these observations is, however, tempered by the fact that tubule cells are known to dedifferentiate in culture and dedifferentiated cells often express Cx43 (61, 85).

**Functional Role of Cxs in the Kidney**

**Vascular conducted responses.** When a small artery or arteriole is stimulated locally (e.g., with ACh or KCl), the response is propagated along the length of the vessel leading to a response several millimeters from the stimulation site. These vascular conducted responses have been shown in many different vascular beds and are believed to play a significant role in the regulation of blood flow in the microcirculation. The conduction of vascular responses is believed to travel through gap junctions (28, 35, 108) between the cells of the vascular wall. In several vascular beds, it has been shown that gap junctions participate in the propagation of vasodilation. In mesenteric arterioles, local application of ACh induces a local and a propagated response traveling up to 1,000 μm against the direction of the blood flow. This propagated vasodilation is significantly decreased in Cx40 knockout mice, indicating a role for Cx40 in the endothelial cells in the propagation (20). The propagated vasodilation can also be blocked by specific gap junction inhibitors, Cx mimetic peptides (12, 23, 50). These peptides are homologous to specific sequences on the extracellular loops of the Cxs responsible for the docking of two connexons. The mimetic peptides reduce the formation of new functional gap junctions between cells in a Cx-specific manner. Interestingly, in the Cx40 knockout mice, propagated vasoconstriction in response to local application of KCl was not inhibited, whereas a reduced conduction of KCl-induced vasoconstriction was seen in mesenteric arterioles from Cx37 knockout mice (81). This suggests that although both pathways involve Cxs, the signaling pathways for propagated vasoconstriction and dilation are not the same (19).

Propagated vasoconstriction can also be seen in afferent arterioles and interlobular arteries from rats (117, 135). Stimulation of the afferent arteriole close to the glomerulus elicits a propagated vasoconstriction that decays exponentially with a length constant of ~300–400 μm (117, 135). In isolated rat interlobular arteries, local electrical stimulation elicited an increase in intracellular calcium, which quickly spread (>100 μm/s) along the vessel (102). Currently, little is known of the role of gap junctions and the type of Cxs in this response. The high speed of propagation strongly suggests that electrotonic spread of a local membrane depolarization through intercellular gap junctions is involved in the propagation (36). The propagated calcium response in rat preglomerular arterioles was completely blocked by the nonspecific gap junction uncoupler carbenoxolone, whereas incubation with Cx mimetic peptides against Cx37/Cx43, Cx40, or Cx45 or a cocktail of all three peptides had no effect on the propagation (114). The reason for this failure is unclear. One possibility is that a Cx other than Cx37, Cx40, Cx43, or Cx45 mediates the response, or, more likely, that the peptides fail to gain access to the gap junctions between the vascular smooth muscle cells in an intact vessel wall.

As mentioned above, the endothelial cells of descending vasa recta also contain Cxs, and, in accordance with this, they have been shown to be highly coupled both electrically and metabolically (147). The coupling was abrogated by the unspecific gap junction uncouplers 18α-glycyrrhetinic acid and carbenoxolone (147). Although the surrounding pericytes contained Cx37, the coupling of pericytes to one another and to the endothelial cells was inconsistent (147).

The present evidence strongly suggests that the Cxs in endothelial and vascular smooth muscle cells are essential for the vascular conducted responses seen in many vessels, including renal vessels. By establishing gap junctions between the cells of the vascular wall, they allow the spread of vasomotor signals along the vasculature. The specific Cxs and the signal-

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**Fig. 1.** Schematic drawing of the localization of connexin isoforms in the kidney. JGA, juxtaglomerular apparatus; CNT, connecting tubule; PT, proximal tubule; CCD, cortical collecting duct; TAL, thick ascending limb of the loop of Henle; OMCD, outer medullary collecting duct; ATL, ascending thin limb of the loop of Henle; IMCD, inner medullary collecting duct.
ing pathways involved in the conduction process still needs to be determined.

**Myoendothelial coupling.** Gap junctions between the endothelial cells and the vascular smooth muscle cells (myoendothelial gap junctions) may be important for mediating endothelial-dependent vasomotor responses. It has been suggested that the myoendothelial gap junctions represent a so-called endothelial-derived hyperpolarizing factor (EDHF) (28, 32), by allowing an agonist-induced hyperpolarization of the endothelial cell membrane to spread to the vascular smooth muscle cells. The resultant hyperpolarization will close voltage-gated calcium channels and relax the smooth muscle cells. While little work has been done in renal vessels, one study showed that the unspecific gap junction blockers heptanol and 18α-glycyrrhetinic acid reduce the acetylcholine-induced nitric oxide (NO)- and prostaglandin-independent (EDHF) vasodilation in isolated rabbit renal arteries (57). A more recent study in rat renal arteries using Cx mimic peptides against Cx40 and Cx43 found a similar reduction in the EDHF-mediated vasodilation in response to carbachol, whereas the peptides had no effect on vasodilation induced by sodium nitroprusside (58). A similar effect was observed in vivo where intrarenal infusion of the same peptides reduced the EDHF-mediated vasodilation in response to intrarenal acetylcholine infusion (18). Whereas the Cx40 mimic peptide was found to be the most potent in vivo (18), the reverse was the case in the in vitro study (58). Together, these three studies suggest that, as in other vessels in the body, parts of the EDHF response in renal vessels are mediated by myoendothelial gap junctions.

**JGA signaling.** By monitoring the concentration of NaCl in the tubular fluid at the site of the macula densa, the JGA regulates the diameter of the afferent arteriole via the tubuloglomerular feedback (TGF) mechanism and the secretion of renin from the granular (renin-containing) cells in the media of the juxtaglomerular part of the afferent arteriole. However, since the macula densa only establishes contact to the most juxtaglomerular part of the afferent arteriole and the extraglomerular mesangial cells, an efficient control of preglomerular vascular tone and renin secretion requires that signals from macula densa reach the relevant cell population (renin-containing granular cells and/or vascular smooth muscle cells) through some signaling pathway. As described above, except for the macula densa cells, there appears to be extensive linking by gap junctions between all the cells of the JGA, and it is therefore an intriguing possibility that the gap junctions are central to signaling in the JGA and that the cells of the JGA form a functional syncytium.

In a recent study, Peti-Peterdi showed in the microdissected rabbit JGA that stimulation of the macula densa by increasing the tubular flow rate elicits a rapid increase in intracellular calcium in the extraglomerular mesangial cells and in the granular cells. Subsequently, a calcium wave propagating from cell to cell was observed upstream toward the proximal segments of the afferent arteriole and the adjacent glomeruli, as well as toward intraglomerular elements including the most distant podocytes (90). The calcium wave was associated with contraction of the afferent arteriole and the glomerular tuft (90).

Several studies suggest that increased tubular flow causes an increased secretion of ATP from the macula densa cells, which, after conversion to adenosine by the extracellular 5′-nucleotidase, initiates signaling within the JGA (104). It was therefore surprising that the calcium wave was not inhibited by adenosine A1 receptor blockers but by an ATP scavenger cocktail and inhibition of P2 purinergic receptors (90). Gap junction uncoupling by 18α-glycyrrhetinic acid or heptanol also inhibited the spread of the intracellular calcium wave. The exact mechanism of the calcium wave and the role of the Cxs still remain to be resolved. It is possible that the wave spreads through either current or diffusion of Ca2+ and/or second messengers like inositol 1,4,5-trisphosphate through the gap junctions. Alternatively, a calcium-induced secretion of ATP and UTP through hemichannels may lead to stimulation of purinergic receptors on neighboring cells, giving rise to a propagated calcium wave. In favor of the latter model is a study in a cultured glomerular endothelial cell line (126). Three lines of evidence from this study point toward ATP release via hemichannels as the mechanism behind the calcium wave. First, elimination of direct contact between cells failed to inhibit the spread of calcium. Second, the calcium wave could be blocked by either ATP scavengers or by purinergic receptor antagonists. Finally, an ATP biosensor technique, where dye-loaded ATP-sensitive cells are used to detect ATP, found evidence of its release from the cells after mechanical stimulation (126). Both nonspecific Cx antagonists, such as 18α-glycyrrhetinic acid and specific blockade with Cx40 siRNA, interfered with calcium wave propagation. Cx45 may also be involved in the propagation of the calcium wave. In vascular smooth muscle cells isolated from the afferent arterioles of mice with a conditional knockout of Cx45, a reduction in the propagation speed was observed. Pretreatment of wild-type cells with a Cx45 gap mimetic peptide produced the same results (43).

Stimulation of the macula densa by an increased NaCl concentration results not only in a calcium wave that propagates across the JGA, but also in a rapid depolarization of the vascular smooth muscle cells of the afferent arteriole (79). The depolarization spreads rapidly to the corresponding interlobular artery and from there into the afferent arterioles belonging to the neighboring nephrons (79). These data show that the JGA and the preglomerular vasculature form an electric syncytium, allowing the spread of signals between nephrons. This is in excellent agreement with a series of experimental studies that have shown the spread of TGF signals between nephrons and the presence of nephron-nephron interactions (13, 47, 48, 143).

In view of these results, how might Cx40 and Cx45 function together in the JGA? One potential explanation is that the two isoforms are functionally redundant. But, if the expression and function of Cx45 and Cx40 overlap, why do they not compensate for each other in their respective knockout models? The study of Cxs in the renal vasculature/JGA relies heavily on transgenic mouse models, which begs the question, whether genetic knockout of one Cx isoform may change the expression of another. This seems a likely possibility, since studies in the Cx40 knockout mouse have shown that despite no change in Cx37 or Cx43 mRNA expression (19, 62, 121, 136, 137), there is a reduced expression of the Cx37 protein in the vessels (19). Neither Cx45 expression in this model nor the expression of other JGA Cxs in the Cx45fl/fl:Nestin-Cre transgenic mouse has been examined. Could different Cx isoforms be directly interacting? Studies in other cell types have shown that Cx45 can form heterotypic channels with Cx40 and Cx43 and that
each combination has unique properties (8, 25, 33, 94). While there is currently no known overlap in Cx45 and Cx43 expression in the JGA, Cx45 and Cx40 are both found in the mesangium and likely the granular cells, making the formation of heterotypic channels a distinct possibility (43, 69, 94). Clearly more studies are needed to finally resolve the signaling mechanism(s) in the JGA and the specific role played by the Cxs in the signaling.

Regulation of renin secretion. In the normotensive WKY rat, it has been shown that intrarenal infusion of Cx mimetic peptides against Cx40 and Cx37/Cx43 increases blood pressure, decreases renal blood flow, and increases renin secretion (18, 121), suggesting that Cxs may play a role in the regulation of renin secretion. Cx40 knockout mice are viable, but hypertensive, and have a fourfold increase in plasma renin concentration (62, 137). Treatment with either an AT1 receptor blocker or an angiotensin converting enzyme inhibitor was found to normalize the blood pressure in one study (62) and significantly reduce it, although not to the control level, in another (137), showing that the increased blood pressure in the mice to a large extent is renin dependent. The Cx40 knockout animals have clear defects in the regulation of renin secretion in that they have lost the normal negative feedback effects of pressure and angiotensin II on secretion rate (62, 137). In contrast, beta adrenergic stimulation still increased renin secretion, and a high-salt diet suppressed renin secretion as in wild-type mice, showing that part of the mechanisms controlling renin secretion remain functional (62, 137). In isolated kidneys, infusion of bumetanide, which inhibits NaCl reabsorption in the macula densa cells, failed to stimulate renin secretion in kidneys from Cx40 knockout mice, indicating disruption of signaling through the JGA (70). The most striking morphological finding in the Cx40 knockout mice is a displacement of the renin-producing cells. Renin is normally synthesized in and secreted from the granular cells in the media of the juxtaglomerular part of the afferent arteriole. In the Cx40 knockout mice, renin-containing cells are no longer found in the media of the afferent arteriole, but are found in the periglomerular interstitium, the extraglomerular mesangium, and the glomerular tuft. The cells did not show the classical epithelioid appearance, but appeared mesenchyme-like (70). The abnormal localization of the renin-containing cells was not present in the embryo but appeared after birth of the mice, showing that the effect exerted by Cx40 is not of a developmental nature (70).

Substitution of Cx40 with Cx45 (Cx40KI45) results in an intermediate blood pressure between the wild-type and Cx40 knockout mice (105). The plasma renin concentration is normal and increases in response to angiotensin converting enzyme inhibition and blood pressure reductions. This indicates that the regulation of renin release might not depend on a specific type of Cx but more likely depends on the cells of the JGA being able to communicate with one another. The morphology of the JGA is normal in the Cx40KI45 mice when on a normal sodium diet, but when challenged by a low-sodium diet they show expression of renin in mesenchyme-like cells in the periglomerular interstitium and the extraglomerular mesangium. So, although Cx45 apparently could compensate for the regulatory deficiencies following Cx40 knockout, it was not able to fully restore the capacity to recruit renin-expressing cells in the media of the afferent arteriole (68).

Selective knockout of Cx45 in nestin-expressing cells, which includes cells of the afferent and efferent arteriolar smooth muscle cells, the renin producing granular cells, and the intra- and extraglomerular mesangial cells, resulted in a phenotype characterized by increased blood pressure and increased expression and secretion of renin (43). This resembles the phenotype seen in Cx40 mice and suggests that Cx45, like Cx40, is also involved in the regulation of renin secretion and thus of the arterial pressure.

Although Cx37 is also present in the renin-containing granular cells, a recent study in Cx37 knockout mice failed to find any effects of this deletion on the control of renin secretion and the morphology and position of the granular cells (138). The Cx37 KO animals responded normally to a low-sodium diet, and in isolated perfused kidneys, the responses to pressure and angiotensin II were similar to that in the wild-type kidney (138). So, in striking contrast to the situation for Cx40, Cx37 does not appear to be necessary for a normally functioning renin system.

Also Cx43 appears to influence renin secretion. The Cx43 knockout mice are not viable but conditional knockouts lacking Cx43 in endothelial cells, and mice where Cx43 has been replaced with Cx32 are available (Cx43KI32). Cx43KI32 mice have reduced plasma renin concentration and do not seem to respond to stimuli that usually change renin secretion. High-salt diet and clipping of one kidney does not change the plasma renin concentration in Cx43KI32 mice (39). The fact that Cx43 is not present within the granular cells, but only appears to be expressed in the endothelial cells, makes these observations difficult to explain. Studies in mice with conditional knockout lacking Cx43 in the endothelial cells have been contradictory. One strain of knockout mice has hypotension, increased plasma NO concentration, and increased plasma angiotensin I and II (75), whereas another strain was reported to be normotensive, and the effect of NO synthase (NOS) inhibition with Nω-nitro-1-arginine methyl ester was not different from the control strain, indicating a normal NO production (125). The increased plasma concentration of angiotensin I and II seen in the former study was interpreted as being secondary to the hypotension (75). Thus, there is no indication of any changes in the control of renin secretion in mice with a conditional knockout of Cx43 in the endothelial cells. Clearly, more studies are needed to establish the role of Cx43 in the regulation of renin secretion.

Renal hemodynamics. Intrarenal infusion of Cx mimetic peptides (Cx37,43Gap27 or Cx40Gap27) causes vasoconstriction, increased blood pressure, and reduced renal blood flow (18, 121). The mechanism(s) behind the vasoconstriction and the reduced renal blood flow is unknown, but increased levels of plasma angiotensin II due to increased renin secretion (see Regulation of renin secretion), reduced conduction of vasodilation (see Vascular conducted responses), or a reduced activity of EDHF (see Myoendothelial coupling) could play a role.

Cx40 knockout mice have an impaired steady-state autoregulatory response to a step increase in renal perfusion pressure (56). When the pressure is acutely increased, renal blood flow increases to the same extent. This reduced autoregulatory response was attributed to a marked reduction in the activity of the TGF response. Transgenic mice in which Cx40 was replaced with Cx45 showed an intermediate phenotype with an autoregulatory efficiency between that of the wild-type and the Cx40 knockouts (56). Blocking Cx37 and Cx40 by infusion of
Cx mimetic peptides in rats was likewise associated with decreased autoregulatory efficiency, resulting in a less efficient autoregulation of both renal blood flow and glomerular filtration rate. Infusion of Cx43-Gap26, which specifically blocks Cx43, was without effect on autoregulatory efficiency (120).

TGF is a major contributor to renal autoregulation cooperating with the myogenic response to maintain renal and glomerular blood flow during acute blood pressure changes (for recent reviews see Refs. 17 and 55). As described above, Cx37 and Cx40 are the major Cxs expressed in the JGA and in the afferent arteriole, and the observed impairment of autoregulation in Cx40 knockout mice and in rats where Cx37 and Cx40 are blocked is therefore consistent with a major role of these Cxs in the signaling between the macula densa and the afferent arteriole (see JGA signaling).

Tubular function. Although Cxs are expressed in tubular cells, nearly nothing is known of their physiological role in the tubular system. Morphologic gap junctions are only found between the proximal tubule cells despite the fact that all cells of the tubular system have been found to express Cxs to a smaller or larger extent (Fig. 1). At present, it cannot be excluded that these Cxs may form gap junctions small enough to be undetectable by conventional electron microscopy or freeze-fracture techniques. It can be speculated that in parallel to their function in other tissues, tubular gap junctions may play a role in coordinating cellular function and transferring information along the tubule. More information on the physiological role of tubular gap junctions is clearly needed.

A physiological role for Cxs in tubular function is suggested by the recent observation that expression of Cx30 and Cx37 in tubular cells are influenced by dietary salt levels (80, 118). A high-salt diet was associated with an increased expression of Cx30 in the apical membrane of the rat inner medullary collecting duct cells (80). On the other hand, Cx37 that is present in the basolateral membrane of the tubule cells was found to be upregulated in rat cortical membrane fractions following a high-salt diet, whereas there was no change in the expression level in the medullary membrane fractions (118). In contrast, Hanner et al. (42) found no changes in the tubular expression of Cx30.3 in response to changes in dietary sodium content. The above observations may point to a role of tubular Cxs in regulating renal sodium excretion, but more studies are clearly needed within this important area.

Purinergic signaling in the tubular system. Recent data suggest that the major function of Cxs in tubular cells may not be the formation of gap junctions, but rather the formation of hemichannels that can act as secretory pathways for purinergic signals. In purinergic signaling, ATP is released from the cell and binds to a local purinergic receptor, thereby increasing intracellular levels of secondary messengers, such as calcium and cAMP. Activation of purinergic receptors along the nephron can stimulate or inhibit various renal transport mechanisms and therefore regulate renal sodium, potassium, and water handling (73, 107, 131).

A wide variety of P2 receptor subtypes have been identified along the nephron and collecting duct system, along with nucleotidases that rapidly degrade ATP nearby (72, 130, 131). In the proximal tubules, Vekaria et al. (132) found local concentrations of ATP that were well above the threshold for receptor activation. Likewise, a study by Woda et al. (142) identified functional P2Y2 receptors on the apical surface of the collecting duct.

Despite the well-established occurrence of purinergic signaling in regulating renal epithelial transport, a major question remains: how does ATP move across the cell membrane? Many transport mechanisms have been hypothesized to play a role (4, 87, 106), but no consensus has been reached. One possibility is that ATP is secreted into the lumen via hemichannels, a function that is documented in other cell types, such as glial cells (15, 16). Since purinergic signaling occurs in an autocrine/paracrine manner, due to the activity of local nucleotidases, the site of ATP release must be close to P2 receptors. It is therefore of interest that Cx30, Cx30.3, and P2Y2 receptors all appear to be expressed on the apical membrane of the collecting duct cells (42, 80).

New evidence from a study by Sipos et al. (112) points toward a role for distal nephron hemichannels in salt and water handling. Using the ATP biosensor technique, the authors were able to detect significant ATP release from the luminal membrane of isolated cortical collecting ducts upon mechanical stimulation. A loss of ATP release in Cx30 knockout mice shows that ATP release in the cortical collecting duct is dependent on Cx30 hemichannels. Cx30 knockout mice also failed to show an increase in salt and water excretion in response to an acute blood pressure increase, indicating that Cx30 hemichannels play a role in the pressure-natriuresis phenomenon. These data suggest that increased pressure and tubular flow triggers Cx30 hemichannel opening. ATP is then released into the lumen, where it binds P2Y2 receptors in an autocrine/paracrine manner, inhibiting salt and water reabsorption.

A recent publication examining gap junctions in astrocytes proposed that gap junction proteins and P2 receptors form a functional unit, with their expression coregulated (52). P2Y2 receptors in the collecting duct are known to be regulated by hydration states (60), and it would be interesting to see whether Cx30 expression may be changed under similar circumstances. Finally, how might Cx30 hemichannel opening be regulated? Sipos et al. (112) proposed that hemichannel gating may involve a flow sensor, suggested to be a cilium or microvilli, on the apical surface of either the principal or intercalated cells of the collecting duct. Other studies would seem to support this hypothesis (49).

Role of gap junctions in kidney pathology

Gap junctions have been implicated in many cellular processes, but the lack of specific blockers has limited the study of their physiological role in vivo. New information about the physiological roles of vertebrate Cxs has emerged from genetic studies. Mutations in Cx genes correlate with a variety of human diseases, including demyelinating neuropathies, deafness, epidermal diseases, and lens cataracts (139). Likewise, primary disease processes may also elicit secondary changes in gap junction function, which may contribute to the pathophysiology of the disease. At present little is known about the role of renal Cxs in the etiology and pathophysiology of various diseases.

Organogenesis. Although gap junctions are present in most kidney cells, deletions of individual Cx genes have only minor effects on kidney development (139, 141). Cx43 knockout
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mice die shortly after birth due to a malformation of the right ventricular outflow tract (96), but do not seem to have a specific renal phenotype despite the presence of Cx-43 within renal vessels and tubules (111). Likewise, although Cx-37 is expressed in several cells throughout the kidney, Cx-37 knockout mice have no renal phenotype, but female mice are infertile due to follicular dysfunction (139). Knockout of Cx-45 constitutes a lethal mutant characterized by the impairment of differentiation of vascular smooth muscle throughout the arterial system (64). This is consistent with the observations reviewed above, which suggest that Cx-45 may be the dominant Cx present in vascular smooth muscle cells of arteries and arterioles. As already reviewed above, Cx-40 knockout mice show postnatal aberrant localization of renin-producing cells in the periglomerular interstitium and the extraglomerular mesangium, but apart from this, renal development appears to be normal (70). The remarkable lack of developmental and functional effects of knockout of Cxs may reflect redundancy between the different Cxs. Most cells express more than one Cx isoform, and as reviewed above, they may in some cases substitute for each other.

Hypertension. Primary hypertension does not seem to change the expression of Cxs in the preglomerular vasculature. In spontaneously hypertensive rats (SHR), the expression of Cx-40 and Cx-43 in renal preglomerular arterioles was similar to the expression seen in normotensive Sprague-Dawley (SD) rats and Wistar-Kyoto (WKY) rats (7). Cx-37 expression was increased in WKY rats, whereas the expression was similar in SHR and SD rats (7). It is important to note that in this study a comparison of Cx-37 expression between SHR and WKY rats would have shown a decreased expression of Cx-37 in the SHR.

Hypertension induced by clipping one kidney (2K1C) increases Cx-43 mRNA in the unclipped kidney, which is exposed to the increased blood pressure (38). Cx-40 mRNA and protein expression was increased in both kidneys, suggesting sensitivity to increased angiotensin II (38). It should be noted that these measurements were performed on homogenized kidney tissue, and it is therefore not possible to distinguish between increases in vascular and tubular Cx expression.

At present, there is some evidence to support that altered Cx expression may be involved in the etiology and pathogenesis of hypertension. That an altered Cx expression may be causally linked to high blood pressure is supported by the observation that deletion of the Cx-40 or the Cx-45 gene in mice results in sustained hypertension (20, 43). Although not finally settled, an important pathophysiological mechanism appears to be increased renin secretion from the kidneys, although other mechanisms may also be involved (62, 70, 105, 121, 137).

Deletion of Cx-43 in endothelial cells results in hypertension in mice (75), although other studies have failed to confirm this finding (125). Replacement of Cx-43 with Cx-32 was associated with decreased expression and secretion of renin and appeared to protect the animals against the development of renin-dependent hypertension (2K1C hypertension) (39). In contrast, a coexpression study failed to show an association between blood pressure and the Cx-43 locus in the F2 generation of SHR and WKY rats (59).

Intrarenal infusion of Cx mimetic peptides against Cx-40 or Cx-37/Cx-43 in rats increased the blood pressure, and this could be prevented by cotreatment with the angiotensin receptor blocker losartan (121). Cx-30 knockout mice was found to have a blunted pressure natriuresis, but despite this, had a normal blood pressure (80). Taken together these studies suggest that the primary mechanism whereby altered function of renal Cxs increases blood pressure is through alterations in the renin-angiotensin system.

Altered Cx function may also be relevant in human hypertension, as shown in a recent study where a polymorphism in the Cx-40 promoter was associated with an increased risk of hypertension (29). The polymorphism is associated with differences in promoter activity and therefore the amount of Cx-40 in the cells. Men with the less active variant of the promoter had an increased risk of hypertension; and in the normal population women, with the less active variant had higher systolic blood pressure compared with the noncarriers (29). Whether alterations in renin secretion (see above) are involved in the pathophysiological mechanism is not known.

Diabetes. Diabetes changes the expression of Cxs in the murine renal vasculature. In control mice, Cx-37 was the primary Cx found in the vascular smooth muscle cells, but in diabetic mice, Cx-40 was also found in the smooth muscle cells in afferent arterioles (144). Cx-43 is expressed in renin-secreting cells during diabetes, whereas the efferent arteriole looses the expression of Cx-43 in endothelial cells (144). As NO availability is changed during diabetes, the expression of Cxs in transgenic mice overexpressing endothelial NOS (eNOS) and eNOS knockout mice was examined (145). In transgenic eNOS mice, the expression of Cx-40 in afferent smooth muscle cells was increased, whereas the expression of Cx-43 in efferent endothelial cells was abolished, resembling the Cx expression in diabetic mice. In eNOS knockout mice, the expression of Cx-40 and Cx-43 was similar to wild-type mice, and induction of diabetes did not change Cx expression, indicating that increased availability of NO is causing the changed expression of Cxs in the renal vasculature during diabetes. The functional significance of the changes in Cx expression in diabetes is unknown. It may be speculated that the increased expression of Cxs may be a compensation for reduced gap junction permeability. It is well known that elevated glucose levels inhibit intercellular communication in endothelial and vascular smooth muscle cells (53, 67). This is associated with reduced conducted vasoconstriction in the mouse cremaster microcirculation, and it was suggested that the decrease in conducted vasoconstriction could be associated with the hyperperfusion seen in various tissues during the early stages of diabetes (95). Whether this is relevant in the renal microcirculation remains to be established.

Summary

Current localization studies present a complex, and at times contradictory, picture of Cx expression in the kidney. The renal vasculature, glomerulus, and JGA express the classic vascular Cxs in the renal microvasculature. In control mice, Cx-37, Cx-40, Cx-43, and Cx-45. However, there are notable discrepancies between studies as to which cells within these regions specifically express each isoform. This is likely due to both species variation and limitations in the specificity of current Cx antibodies. Similarly, the identity of all tubular Cxs remains unresolved, with the strongest evidence favoring Cx-30, Cx-30.3, and Cx-43.

As with Cx localization in the kidney, the functional data about Cxs in renal physiology points in many directions. Cxs almost certainly play some role in the conduction of vascular
responses in the kidney, with different isoforms likely responsible for vasoconstriction and dilation. Nonspecific blockade of gap junctions points to their potential in mediating myoendothelial coupling. Also apparent is the required expression of certain vascular Cx isoforms for normal cellular localization and regulation of renin secretion, implicating renal Cxs in the pathology of hypertension. Roles for classical gap junctions and Cx hemichannels in calcium wave propagation and purinergic signaling in the kidney are emerging as well. Finally, there is limited data supporting a connection between diabetes and renal expression of Cx37, Cx40, and Cx43.

Despite decades of awareness of their existence, the functional significance of Cxs in the kidney is still a new field of interest for integrative physiology. As such, there are many interesting areas of research that need to be addressed in future studies, which include reaching a consensus regarding renal localization of Cx isoforms, establishing factors that regulate Cx expression and function in the kidney, and the continued evaluation of how renal Cxs effect homeostasis at a systemic level.

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DISCLOSURES
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REFERENCES
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