Connexin 40 mediates tubuloglomerular feedback paracrine signaling by coupling tubular and vascular cells in the renal juxtaglomerular apparatus

William J. Arendshorst
Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

This commentary highlights recent advances about the functional significance of connexins (Cx) in signal transduction in the renal cortex, with particular focus on the recent work of Sorensen and colleagues (24). Their results provide insight into the role of Cx40 in the juxtaglomerular apparatus (JGA), a unique structural and functional nephrovascular unit of multiple cell types. Signaling within the JGA functional unit occurs among epithelial macula densa cells at the end of the thick ascending limb of Henle’s loop, extraglomerular mesangial cells, and terminal portions of the afferent arteriole before entering a glomerulus. This specialized apparatus has important homeostatic functions in matching sodium excretion to glomerular filtration rate (GFR) to control extracellular fluid volume and arterial pressure, regulating activity of the renin-angiotensin-aldosterone system, and an autoregulatory role in maintaining renal blood flow (RBF) and GFR stable during acute changes in renal perfusion pressure. A classical tubuloglomerular feedback (TGF) circuit couples NaCl reabsorption by the macula densa plaque to afferent arteriolar tone such that increased NaCl delivery from Henle’s loop reduces GFR and the filtered NaCl load.

Cell-cell signaling in the JGA is integrative and highly dependent on specific Cx hemichannels and/or gap junctions. In mice and rats, Cx40 is strongly expressed in the endothelium of preglomerular vessels and glomerulae as well as in renin-containing granular cells at the end of the afferent arteriole and glomerular mesangial cells (4, 26, 28). In contrast, little to no Cx40 is present in vascular smooth muscle or macula densa cells. Cx45 predominates in smooth muscle. Cx40 is also abundant in human mesangial cells and renin-producing cells (12). It is important to recognize that no Cx has been identified on macula densa dells.

Cx are involved in regulating renin release by juxtaglomerular (JG) granular cells, with Cx40 channels, but not Cx37 or Cx45, being critical (10, 20, 29, 31). Genetic deletion of Cx40 results in displacement of renin-producing cells to the extraglomerular mesangium and hyperplasia. High renal renin mRNA, high plasma renin concentration, and hypertension result from the loss of Cx40-dependent afferent arteriolar baroreceptor regulation and of ANG II inhibition. This phenotype is replicated by selective deletion of Cx40 from renin-producing cells (30). Global replacement of Cx40 on JG granular cells with Cx45, a channel with lower conductivity, prevents ectopic mislocalization and hyperreninemia and attenuates hypertension (22).

The functional roles of Cx channels and gap junctions in vascular contractility and macula densa signaling in the JGA are less well-characterized. Nonspecific destruction of gap junctions using heptanol negates TGF responses in an isolated rabbit JGA (17). More recent pharmacological studies utilizing Gap27 peptides intended to inhibit Cx37 + Cx43 or Cx40 report that each peptide impairs steady-state RBF autoregulation in Wistar-Kyoto and Zucker lean rats, reducing autoregulatory efficiency by ~50% (26, 27). Although individual mechanisms were not analyzed, this degree of inhibition is consistent with Cx mediating TGF.

The work of Sorensen et al. (24) provides important insight into the physiological role of Cx40 in renal autoregulatory responses of the preglomerular vasculature and the contribution of TGF. Utilizing the isolated perfused juxtamedullary nephron preparation from gene-targeted mice challenged with an input forcing of acute changes in perfusion pressure above 75 mmHg, they convincingly show that the presence of Cx40 is essential for normal operation of TGF mediated by transmission of macula densa signaling to the afferent arteriole. Use of papillectomy to interrupt tubular flow to the macula densa-sensing segment and to effectively eliminate TGF allowed determination of the relative contributions of TGF and the myogenic mechanisms to overall autoregulatory responses. The absence of TGF in Cx40 null nephrons was evidenced by the lack of a papillectomy effect on vascular responsiveness in gene-targeted vs. a demonstrable effect in wild-type preparations. Due to the absence of the TGF contribution, the overall afferent arteriole autoregulatory response was weaker in Cx40-/- vs. control nephrons. Nevertheless, the renal myogenic response intrinsic to arteriolar smooth muscle cells was normal and unaffected by Cx40 deletion. In the absence of TGF, residual myogenic tone was, as expected, inhibited by removal of extracellular Ca2+ and by antagonism of L-type Ca2+ channels.

The in vitro single nephron study of Sorensen et al. provides solid evidence reinforcing the earlier whole kidney in vivo studies of Cx40 mutant mice by Just et al. (8), who assessed autoregulatory mechanisms based on the dynamic time-dependent response of renal vascular resistance of all nephrons to a rapid step increase in perfusion pressure. Just et al. found that overall steady-state RBF autoregulation was weaker in Cx40-deficient mice than in wild-type controls, as was seen by Sorensen et al. at the single nephron level. Dynamic responses indicated that the most rapid myogenic response was normal as was the case for the slowest ill-defined third mechanism. In marked contrast, the classical TGF component with an intermediate response time was markedly attenuated. Just et al. concluded that Cx40 mediates JGA cell coupling responsible for TGF and its partial contribution to RBF autoregulation, with Cx40 mediating signal transduction from macula densa cells through extraglomerular mesangial cells to afferent arteriolar target cells. Similar conclusions are reached by Sorensen et al. (24). Just et al. (8) also studied mice with Cx40 replaced by Cx45 and found that steady-state RBF autoregulation and TGF were improved in part, but still weaker than in wild-type
controls, suggesting some restoration of signal transduction by Cx45 substitution. Therefore, selective gene targeting has helped us define which specific Cx is critical to renal autoregulatory mechanisms, especially TGF signaling.

An unresolved question is what particular signal(s) traverses through Cx40 joining extraglomerular mesangial cells. Cx40 gap junctions appear to allow Ca\(^{2+}\) influx that mediates the effect of vascular pressure on JG cells where increased cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) inhibits renin secretion (29, 30). Ca\(^{2+}\) permeability of Cx40 may explain intercellular spreading of a Ca\(^{2+}\) wave in the JGA initiated by stimulation of the macula densa plaque as propagation of the Ca\(^{2+}\) wave (16). However, as noted earlier, there appear to be no specialized Cx connections or junctions between macula densa cells and adjacent mesangial cells. The trigger signal is thought to be paracrine, with either ATP or its breakdown product adenosine stimulating vasoconstriction (2, 15). ATP is known to be released across the basolateral membrane of mitochondrial-rich macula densa cells through a maxi-anion channel when NaCl transport increases. The released ATP may activate purinergic P2X receptors on adjacent extraglomerular mesangial cells and more distant arteriolar smooth muscle cells, with this Ca\(^{2+}\)-permeable nonselective cation channel increasing [Ca\(^{2+}\)]\(_i\) (1, 6, 19). In support of this notion, an ATP scavenger enzyme cocktail (apyrase + hexokinase) and pharmacological antagonism of P2 purinergic receptors (suramin) interrupt propagation of a Ca\(^{2+}\) wave in the JGA (16). Alternatively, extracellular ATP may be enzymatically converted by nucleotidases to adenosine to act on A1 receptors to increase [Ca\(^{2+}\)]\(_i\) in mesangial and smooth muscle cells (2, 5, 15). Indeed, a concerted interaction of A1 and P2 receptors may be involved.

Few studies have tested a role of Cx in the intrinsic myogenic response of an artery or arteriole. The results of Sorensen et al. (24) and Just et al. (8) agree that Cx40 is not required for an efficient pressure-induced myogenic response of the renal preglomerular vasculature. Thus, this is the case for an arteriolar arteriole tested in vitro or the entire renal vascular bed analyzed in vivo. In contrast, gap junction inhibitors (e.g., heptanol) are reported to attenuate myogenic tone in rat cerebral or mesenteric arteries (3, 13) and the putative inhibitory peptide Gap27 implicates Cx37 and/or Cx43 in myogenic tone of mesenteric arteries that express Cx37 (3). Whether the myogenic response in the renal vasculature is unique, perhaps related to the expression patterns of specific Cx, or the actions of the pharmacological inhibitors used on nonrenal vessels are more nonselective than deletion of specific Cx awaits further investigation.

TGF signaling elicits a vascular response conducted upstream along the parent afferent arteriole and also down adjacent arterioles originating from a common interlobular artery (9). The highlighted Sorensen study shows that Cx40 is required for conduction of [Ca\(^{2+}\)]\(_i\) changes in response to local electrical stimulation of an interlobular artery (24), implicating endothelial cell Cx40 in the Ca\(^{2+}\) wave. This contrasts with an earlier study by the same group showing that Cx mimetic peptides directed against Cx40, Cx37/43, or Cx45 do not impede electrically induced conducted Ca\(^{2+}\) responses along the same vessel (25). Specificity of Cx mimetic peptides may be suspect.

Another consideration is the possible role of Cx in vascular responses to circulating agents. Whole kidney RBF responses to norepinephrine (NE) or acetylcholine administered systemically are not affected by deletion of the Cx40 gene (8). Sorensen et al. (24) find this is also the case for constriction of the afferent arteriole when NE is administered in the superfusion solution.

In addition to the discussed issues and gaps in our knowledge, future directions might consider whether the expression and permeability of Cx in the JGA participate in the regulation of TGF reactivity that varies with extracellular fluid volume and activity of the renin-angiotensin system. In this regard, ANG II is known to enhance TGF activity, with nitric oxide (NO) attenuating TGF strength at high tubular fluid flow rates (14, 32). To the extent that NO originating from macula densa cells attenuates the strength and speed of the myogenic response (7, 23), it is noteworthy that Cx40 is not critical for an action of NO on myogenic tone of renal arterioles (8). Other areas of intrigue include the role of specific Cx in different cell types as they are involved in signal transduction mediating macula densa regulation of renin release from JG granular cells (11) and the role of Cx, if any, in a second TGF system and in paracrine signaling linking NaCl transport by the cortical connecting tubule to modulation of afferent arteriolar vasomotor tone (18).

DISCLOSURES
No conflict of interest is declared by the author.

REFERENCES