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Who and where is the renal baroreceptor?: the connexin hypothesis

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Gap junctions are emerging as a fundamental mechanism for the control of renin synthesis and release. Connexin40 is prominent in juxtaglomerular cells. When missing, it results in hyperreninemia and hypertension. Schweda *et al.* offer exciting data demonstrating that connexin45, a connexin with different biophysical properties, can replace connexin40 functions related to the control of renin.

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Gap junctions allow groups of cells to inform each other about their physiological status and facilitate the coordinated activity of tissues and functional units, a crucial homeostatic mechanism in multicellular organisms.[1] Gap junctions are formed when two adjacent cells each contribute a hemichannel, called a connexon. Each connexon is composed of six membrane connexins, a family of proteins containing 21 members in humans. Aqueous channels within gap junctions enable cells to share ions, second messengers, small metabolites, and other signaling molecules. In addition, hemichannels mediate the spread of calcium waves across cells. Besides intercellular communication, connexins also support adherent junctions and stabilize migratory pathways.[2,3]

The juxtaglomerular apparatus (JGA) is a structural and functional unit, strategically located to sense changes in perfusion pressure and fluid and electrolyte status, among other things. This information is transmitted to the juxtaglomerular (JG) cells that release renin, in order to maintain fluid–electrolyte and blood pressure homeostasis. It is not surprising, therefore, that the different cells of the JGA (renin-secreting cells, smooth muscle cells, mesangial cells, endothelial cells, and macula densa cells) are all interconnected by gap junctions allowing them to communicate with each other and coordinate the vasomotor, endocrine, and paracrine functions of the JGA. Several connexins are found in the kidney, including connexin32 (Cx32), Cx37, Cx40, Cx43, and Cx45,[4] and gap junctions of Cx37, Cx40, and Cx43 interconnect the cells of the JGA.[5]

Cx40 is the most prominent connexin in renin cells; it forms gap junctions among the JG cells themselves as well as between JG cells and neighboring endothelial and mesangial cells.[5–7] Cx40 deletion in mice results in the expression of renin by interstitial cells about the periglomerular space rather than in the classical JG localization.[8] Functionally, this is accompanied by hypertension and abolition of the classical inverse relation between blood pressure and renin release.[5,9] In addition, the inhibition of renin release by angiotensin II is markedly attenuated by Cx40 deletion.[5]

To determine whether control of renin secretion is uniquely dependent on Cx40 or whether its function can be replaced by other connexins with different biophysical properties, Schweda *et al.*[7] (this issue) studied the regulation of renin in mice in which the coding region of Cx40 had been replaced by Cx45. In this interesting experiment, the genetic substitution resulted in a near-normalization of the functional regulation of renin secretion. Thus, circulating renin and kidney renin mRNA levels were similar in wild-type and 'substituted' mice at baseline and in response to renal artery clipping and enalapril treatment, two manipulations known to increase renin. The authors conclude that Cx45 can functionally replace Cx40 for the control of renin release and restores the normal location of renin cells, implying that the regulation of renin release is not dependent on unique – biophysical – properties of Cx40, since a connexin with lower conductivity can substitute for it. It remains to be determined whether other connexins can function in a similar fashion. However, as is mentioned above, $Cx40^{-/-}$ mice are markedly hypertensive, and mice carrying the substitution do not fully normalize their blood pressure to wild-type levels in spite of normalizing their plasma renin concentration; this suggests that additional factors besides renin may contribute to arterial hypertension in $Cx40^{-/-}$ mice. It seems obvious that lack of Cx40 prevents the appropriate 'sensing' of the baroreceptor mechanism for renin release. Whether this is due to the loss of a baroreceptor function 'intrinsic' to renin cells or whether their location impairs this function remains to be determined. The fact is that the cells continue to operate as if they were continuously exposed to a low perfusion pressure even though the systemic pressure is high. The work of Wagner and collaborators[9] is illuminating in this regard: Not only are $Cx40^{-/-}$ mice less sensitive to angiotensin II suppression of renin release (Figure 1), but they also have an abnormal, almost inverted relationship between perfusion pressure and renin secretory rate (Figure 1); thus the renal baroceptor is almost unresponsive at low perfusion pressures with negligible change in renin release, whereas at high pressures, renin release paradoxically increases with each increment in pressure, aggravating the hypertension. Thus, a dysfunctional baroreceptor and insensitivity to angiotensin II both cooperate to create hyperreninemia and hypertension, which is further aggravated by inappropriate transmission of vasodilatory responses (Figure 1).

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A similarly elegant approach was used by Haefliger *et al.*,[10] who studied mice in which Cx43 (prominently expressed in endothelial cells of renal arterioles and glomeruli) was replaced by Cx32 under the control of the regulatory regions of Cx43. In these knock-in mice, the levels of renin and its mRNA were decreased by about half of the wild-type levels. Homozygous mice did not downregulate renin in response to high-salt diet and were unable to increase renin and blood pressure in response to renal artery clipping in a 2K1C model. Not only were the animals protected against hypertension, they also featured a normal cardiac weight index, which was altered in the wild-type animals subjected to chronic hypertension induced by the 2K1C model. It remains to be determined whether the effects observed were due to the lack of Cx43 or the addition of Cx32. What is interesting is that endogenous Cx40 under these conditions seems unable to compensate for or maintain the normal relationship between renin secretion and perfusion pressure. The pioneering studies mentioned above suggest an intricate complexity in the regulation of renin release by various connexins, which are also likely to cross-talk among each other to sustain the tight control of renin release that is so vital for homeostasis. An exciting question whose answer may have eluded investigators since Tobian[11] first proposed the baroreceptor hypothesis is perhaps within reach now: who is the renal baroreceptor and where is it located? Is it a connexin or a combination of connexins? Is it located exclusively in JG cells? Or – linked by these wonderful communication devices – does it reside in and require the participation of endothelial and other cells of the JGA? Perhaps, future experiments using cell- and time-specific deletions of particular connexins together with cell ablation experiments may help unravel the relative contribution of each connexin and cell types of the JGA to the baroreceptor mechanism for renin release. In this regard, the availability of mice bearing cre recombinase in various cell types should allow the selective conditional deletion of connexins in particular cells of the nephron, including those of the renin lineage.[12]

In addition to the hemodynamic effects mentioned above, in Cx40$^{-/-}$ mice the appearance, location, and number of renin-expressing cells seem to be disturbed.[5,8] Mice with the deletion have more renin-containing cells. The cells are smaller and have fewer and rounder secretory granules.[5] In addition, Kurtz *et al.*[8]

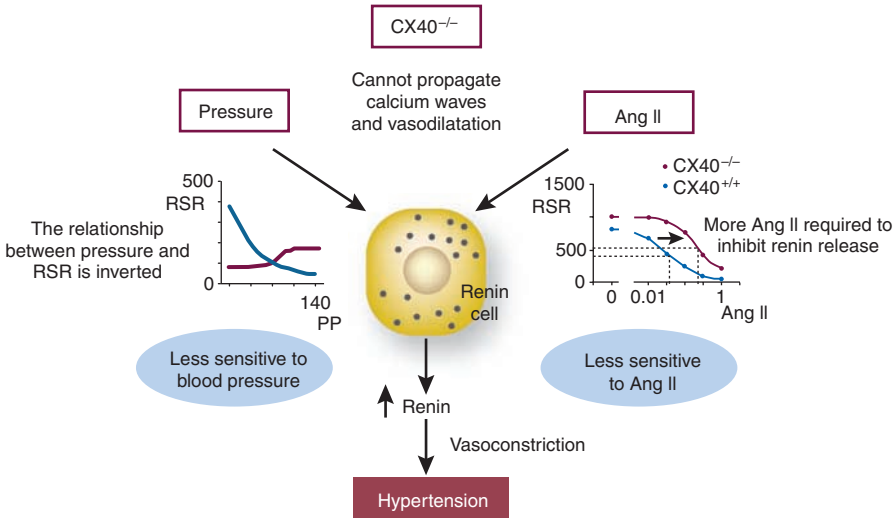

Figure 1 | Response of renin cells to changes in perfusion pressure and angiotensin II in Cx40$^{-/-}$ mice. Graphs relating renin-secreting rate (RSR) to perfusion pressure (PP) and to angiotensin II (Ang II) are adapted from Wagner *et al.*[9] For comparison, the normal curves in wild-type animals (Cx40$^{+/+}$, blue) are also depicted. Cx40$^{-/-}$ animals are hyperreninemic and hypertensive. Although plasma renin concentration and the response to several manipulations are normalized when Cx40 is replaced by Cx45, blood pressure improves, but it does not reach normal wild-type levels.[7]

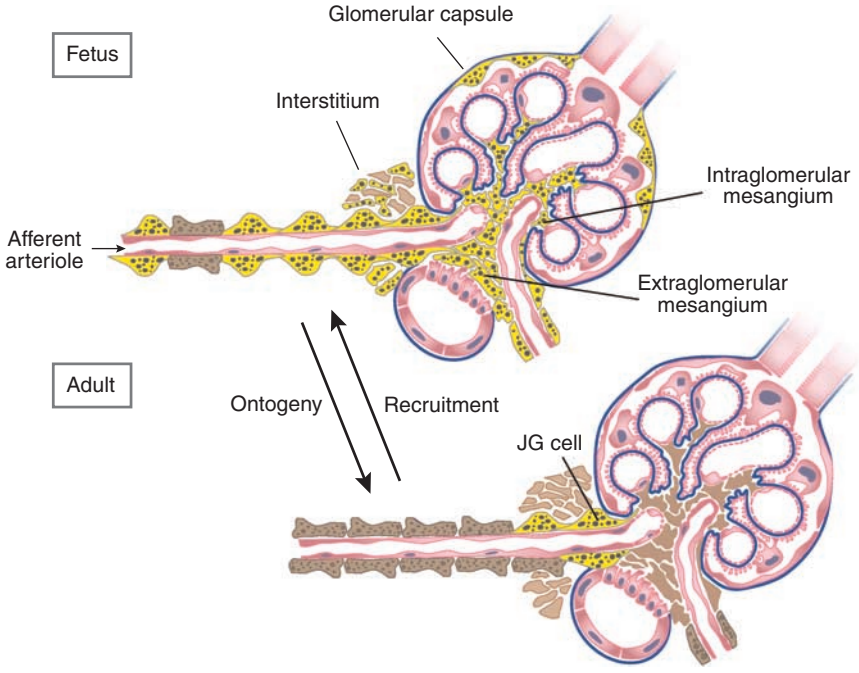

Figure 2 | Renin distribution during ontogeny and recruitment. During embryonic development, renin cells (yellow, with black dots representing granules) are broadly distributed along intrarenal arteries, inside the glomeruli, and in the renal interstitium. With maturation the number of renin cells is progressively restricted to a few cells in the juxtaglomerular (JG) area of the adult kidney. The extension and localization of renin cells during recruitment in the adult vary depending on the physiological manipulation and the intensity and duration of the stimulus. Usually, the increase in the number of renin cells occurs along and upstream of the afferent arteriole. Recruited cells can also be observed in the intraglomerular mesangium, extraglomerular mesangium, interstitium, and occasionally the glomerular capsule and efferent arteriole.

described the presence of renin cells in a periglomerular interstitial location rather than in the classical JG localization. The paper by Schweda *et al.*[7] shows that the replacement of Cx40 with Cx45 re-establishes the location of JG cells. The relocation of cells in the $Cx40^{-/-}$ mice can be interpreted to suggest that the presence of either connexin provides the necessary cues for appropriate positional information. In this regard, gap junctions are known to regulate migration, proliferation, and adhesion during embryonic development.[2,3] Because the distribution of renin cells is normal during embryonic life in $C40^{-/-}$ mice and becomes disrupted only later in postnatal life, this may argue against an inadequate assembly of renin cells into the vasculature during early development. It is more likely that the observed presence of renin cells in the periglomerular interstitial area and in the extraglomerular and glomerular mesangium is a return to the embryonic and fetal pattern of renin expression. It should be noted that in fetal life, renin precursors are present in the interstitium, and the appearance of renin cells in the periglomerular area may reflect the capability of those cells of re-expressing or retaining expression of the renin gene when the demands of extrauterine life so require it to maintain homeostasis. In fact, interstitial cells can and do re-express renin in response to manipulations that in the adult animal threaten homeostasis.[13,14]

Notably, deletion or mutations of several genes of the renin–angiotensin system in mice and humans, pharmacological inhibition of angiotensin actions, as well as threats to homeostasis such as hypotension or dehydration result in the reappearance of renin-expressing cells in several sites, including the interstitium, the glomerulus, periglomerular areas, and along and around afferent arterioles (Figure 2).[12–14] This response – called recruitment – does not involve cell migration or replication of cells, and it is a reacquisition of cell identity along a developmentally and lineage-restricted pathway.[12] Whether recruitment upstream of the afferent arteriole occurs in $Cx40^{-/-}$ mice will require further experimentation with different manipulations.

It would be of interest to explore which connexins are involved in the reacquisition of renin-cell identity known to occur in animals and humans with renin–angiotensin system gene mutations resulting in kidney abnormalities and/or hypertension.

DISCLOSURE
The authors declared no competing interests.

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Engineering parathyroid cells to treat secondary hyperparathyroidism

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Kanai *et al.* used antisense technology to reduce excessive PTH production. The authors have overcome technical difficulties to demonstrate that, by strategies of RNA interference, a steady reduction of PTH secretion can be induced in cultured parathyroid-cell spheroids and in athymic nude mice with hyperplastic parathyroid cells transplanted from patients with secondary hyperparathyroidism.

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This commentary focuses on two important methodological topics that have been addressed in the paper by Kanai *et al.*[1] (this issue) to demonstrate that, by strategies of RNA interference, a steady reduction of PTH secretion can be induced: first, the use of an innovative parathyroid tissue culture system, 'spheroids,' which provide a three-dimensional structured culture that retains a long-term calcium responsiveness; and second, the use of small interfering RNA (siRNA) to drive the specific suppression of parathyroid

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