Restoring a Critical Element in Renin-Producing Cells
Connexin40 Hits the Brakes on Renin Release

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See related article, pp 1198–1204

The kidney is crucial for arterial pressure control, and this key function is exerted in part by 1 of its endocrine functions—the release of the protease renin. It is released by specialized juxtaglomerular cells in afferent arterioles (renin-producing cells, RPCs), and the release is governed mainly by the activity of the sympathetic system, by the chloride content in the fluid of the distal tubule at the macula densa, and by renal perfusion pressure. Appropriate secretion of renin into the blood stream requires a close interaction of many cells, possibly even different cell types, and therefore intercellular communication is a prerequisite. This can be achieved by the release of mediators but also by direct communication through gap junction channels composed of connexins. In recent years, it has emerged that of this protein family, specifically connexin40 (Cx40) is crucially involved. Global deletion of Cx40 (Cx40-null mice) induces hypertension and enhances renin release by disrupting the negative feedback of pressure on RPCs. However, cell-selective deletion demonstrated that Cx40 is necessary in RPC but not in ECs to exert this feedback. Only mice deficient for Cx40 in RPCs were hypertensive and exhibited elevated renin levels, whereas EC-deficient mice were normotensive. Despite this seemingly good evidence, doubts remain as hypertension itself or the deficiency of a single gene on RPCs and may also interconnect these cell types. However, despite the presence of a Cx40 promoter (human renin), and both transgenes were introduced into Cx40-null mice. Thus, they generated 2 new mouse lines that expressed Cx40 either only in ECs (EC-Cx40 mice) or only in RPCs (RPC-Cx40 mice). The restoration of Cx40 expression in ECs, nicely verified by immunostaining in different organs and by Western blot in the kidney, was, however, ineffective to reduce enhanced kidney renin levels. Renin protein remained at the same level (3-fold elevated compared with wild type) as observed in Cx40-null mice. In contrast, selective restoration of Cx40 in RPC reduced renin protein in the kidney, albeit it remained elevated (2-fold) compared with wild-type mice.

The arterial pressure reflected kidney renin levels (suggesting congeneric renin plasma levels): Cx40-null and EC-Cx40 mice were similarly hypertensive, whereas pressure was reduced in RPC-Cx40 but still elevated compared with wild type. Although this verifies a crucial role of Cx40 in RPCs to hit the brakes on renin release (Figure), the question arises as to how much of the critical element Cx40 was restored in this setting. The authors suggest that moderate changes of Cx40 expression may alter the physiological feedback mechanism. It is rather difficult to assess whether Cx40 was expressed in RPC-Cx40 mice up to the same level as in wild type. Le Gal et al. certainly found Cx40 protein in the kidney of RPC-Cx40 mice, but it amounted to only 11% of wild-type level (for comparison, EC-Cx40 mice exhibited 50% of wild-type kidney levels). However, are these normal Cx40 levels in RPCs, and can Cx40 expression titrate renin release and arterial pressure? How much communication is enough? It should be kept in mind that mice carrying a single Cx40 gene (heterozygous Cx40-null mice) as well as mice carrying heterozygously a nonconducting mutated Cx40 are normotensive. Likewise, substitution of Cx40 with Cx45 protein, which forms lower conductance channels, prevented excessive renin release in mice. Even more interesting, but also far more difficult to examine, is the question whether Cx40 expression in RPC-Cx40 mice is heterogeneous and thereby renders some RPCs nonresponsive to pressure feedback. Whether a conceivable nonresponsiveness coincides with the previously described displacement of RPC from the juxtaglomerular part of the afferent arteriole in Cx40-null mice is currently unknown. Unfortunately, the altered distribution of RPC observed even in RPC-Cx40 mice does not provide further clues, because it may result from a heterogeneous Cx40 expression pattern. It was shown, however, that a conducting Cx40 channel is required for normal localization of RPC. Thus, the quantity (number of channels and their conductivity) and quality (displacement and loss of contact) of communication required to distribute the inhibitory signal remains not well defined.

Restoring Cx40 in EC rescued the downregulation of Cx37 and eNOS but did not change kidney renin levels or pressure. Moreover, restored Cx40 was assembled in such a way that the tight local interaction with eNOS protein was again evident. The role of this interaction is currently elusive, but eNOS may be just 1 protein among others and Cx40 acts as a scaffolding domain. This may also underlie the downregulation of Cx37 in ECs if Cx40 is lacking as was also reported previously. At least, Cx37 is not downregulated at the transcriptional level,
and even a nonconducting Cx40 rescues this scaffolding function, however, without modifying renin release, hypertension, or displacement of RPC.\textsuperscript{10} The failure to hit the brakes on renin by restoring endothelial Cx40 expression, while sole restoration in RPC is able to do so, strongly suggests that RPCs and ECs do not require Cx40-dependent channels to communicate to each other, which is consistent with the data gathered in the previous findings that selective deletion of Cx40, led to the same conclusion, namely that Cx40 expression (because nonconducting Cx40 channels are likewise insufficient) communicate signals between RPCs. Can the file be closed and considered settled? Only in part, because now the race is on to identify the nature of the signal that is communicated to inhibit renin release. The most likely candidate is Ca\textsuperscript{2+}, but the membrane potential should also be considered because both Ca\textsuperscript{2+} increase and depolarization inhibit renin release. The renal baroreceptor that initiates the negative feedback remains obscure. However, the crucial role of Cx40 suggests that only Ca\textsuperscript{2+} increase is crucial for conducted dilations irrespective of hypertension. Hypertension. 2012;60:1422–1429.

Le Gal et al created 2 new mouse lines, crossed the bridge, and provided a view from the other side of the river bank. Their data complete the previous findings that selective deletion of Cx40 in RPC unleashed renin release and arterial pressure but not deletion of Cx40 in ECs.\textsuperscript{3} Both approaches, deletion and restoration of Cx40, led to the same conclusion, namely that Cx40 expression in RPCs is crucial for the negative feedback of pressure on renin secretion. This strengthens the notion that Cx40 channels (because nonconducting Cx40 channels are likewise insufficient) communicate signals between RPCs. Can the file be closed and considered settled? Only in part, because now the race is on to identify the nature of the signal that is communicated to inhibit renin release. The most likely candidate is Ca\textsuperscript{2+}, but the membrane potential should also be considered because both Ca\textsuperscript{2+} increase and depolarization inhibit renin release. The renal baroreceptor that initiates the negative feedback remains obscure. However, the crucial role of Cx40 suggests that only Ca\textsuperscript{2+} increase is crucial for conducted dilations irrespective of hypertension. Hypertension. 2012;60:1422–1429.

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References
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