

## Activation of the renal renin–angiotensin system in diabetes—new concepts

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### Background

The incidence of diabetes mellitus, obesity and the metabolic syndrome is rapidly rising to epidemic levels worldwide. Hyperglycemia, the metabolic hallmark of the pathology, is a significant causative factor for the complications of diabetes mellitus which result in significant morbidity and mortality for millions. Hyperglycemia is clearly associated with microvascular complications in many organs including the kidney, and diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) in developed countries [1,2]. In addition, diabetes may lead to other vascular complications, including systemic hypertension [1,2]. Recent advances on the cellular and kidney-specific effects of hyperglycemia place activation of the local, intra-renal renin–angiotensin system (RAS) as a strong candidate for the core abnormality that leads to renal tissue injury [1–3].

The nature of RAS activation in diabetes is, however, controversial [3]. It has been difficult to isolate the acute and direct actions of hyperglycemia *per se* from the many other systemic factors and intra-renal, macula densa-mediated feedback mechanisms that can indirectly activate the intra-renal RAS. Therefore, the primary cause and exact mechanism of RAS activation in early diabetes have been unknown. The prevailing paradigm, the ‘tubular hypothesis of glomerular filtration’ [4], argues that the two hallmarks of early changes, glomerular hyperfiltration and renin activation, originate from the primary effects of glucose on proximal tubule salt reabsorption that secondarily activate macula densa-mediated feedback mechanisms. However, the development of diabetes-induced glomerular hyperfiltration was intact, or even augmented, in mice that lacked tubuloglomerular feedback (TGF), demonstrating that the TGF mechanism could not be the major cause of the development of hyperfiltration [5,6].

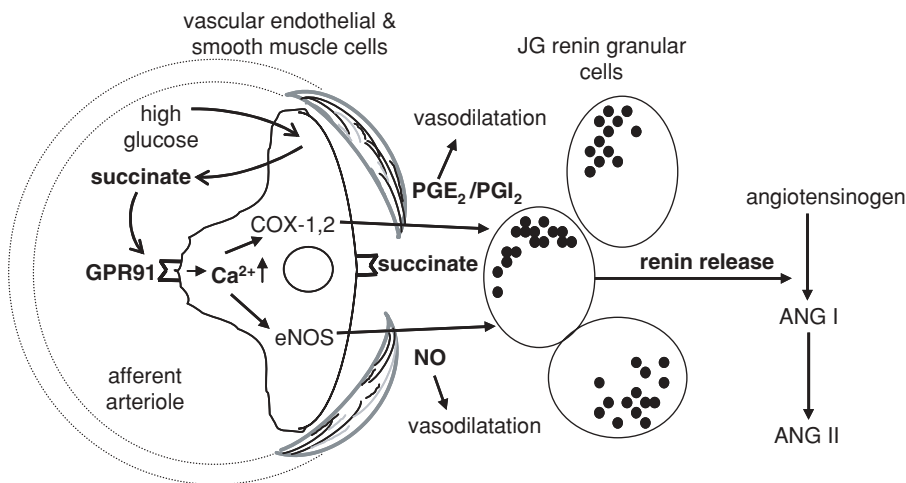
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The recent discovery of the G-protein-coupled receptor GPR91 [7] which is activated by the citric acid cycle intermediate succinate prompted the discovery of a new, direct link between high glucose levels and renin release from the juxtaglomerular apparatus (JGA) in the kidney [8].

### Succinate and GPR91 directly link high glucose levels to JGA renin release

GPR91, a metabolic receptor which is highly expressed in the kidney, can lead to renin-dependent activation of RAS and increased systemic blood pressure [7]. Its ligand, the citric acid cycle intermediate succinate, has long been known to cause renin release from the JGA [9]. However, the importance of the local interstitial accumulation of succinate as a sign of ischemic organ damage in sites like the brain [10] or its role as an indicator of the imbalance between tissue energy supply and demand was only recently recognized [8,11,12]. At least in the liver and the kidneys [8,11,12], succinate triggers paracrine signaling through GPR91 leading to (patho)physiological alterations in organ function.

In fact, new data demonstrate that localized succinate accumulation occurs in the intact diabetic kidney as well as in the dissected, *in vitro* microperfused JGA preparation acutely subjected to high glucose levels [8]. High glucose and succinate-induced GPR91 activation trigger paracrine signaling (summarized in Figure 1) from the (juxta)glomerular endothelium to the adjacent renin-producing JG cells to increase renin synthesis and release [8], the rate-limiting step of RAS activation. Elements of the signal transduction cascade involve succinate and GPR91-dependent elevations in vascular endothelial  $[Ca^{2+}]_i$ ; as well as the synthesis and release of NO and PGE<sub>2</sub>, classic mediators of renin release [13]. Endothelial NO and prostaglandin production also directly causes vasodilatation of the afferent arteriole, which may be important in the development of glomerular hyperfiltration. In summary, this GPR91-mediated paracrine signaling pathway provides an alternative to the ‘tubular hypothesis’ and offers a direct mechanism for the development of both hallmarks of diabetes: glomerular hyperfiltration and JGA renin activation.



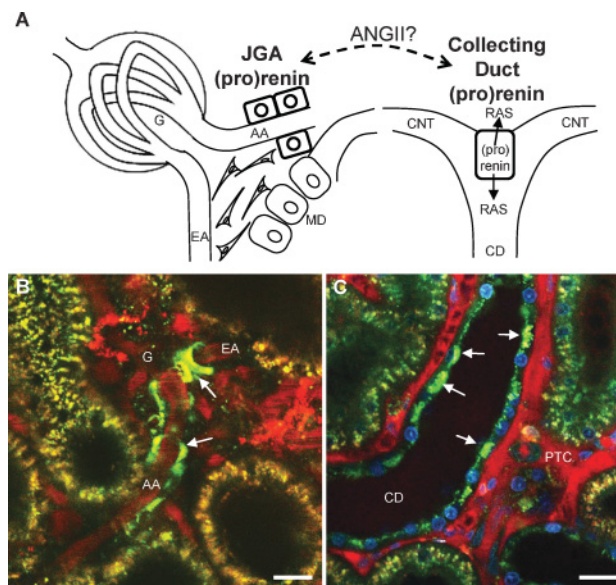
**Fig. 1.** Schematic illustration of the newly described (juxta)glomerular paracrine signaling cascade involving succinate activation of GPR91, leading to renin synthesis and release. High glucose levels result in the accumulation of the metabolic intermediate succinate, which acts directly on the vascular endothelium and triggers cell-to-cell signaling to culminate in renin release from the juxtaglomerular apparatus. The chain of events involves elevating glucose levels, succinate accumulation in the plasma and local interstitium, GPR91 activation, increases in endothelial cytosolic calcium, nitric oxide (NO) and prostaglandin (at least PGE<sub>2</sub>) production and release from endothelium, PG actions on renin-producing JG cells, renin release, angiotensin (Ang I and Ang II) synthesis and RAS activation. Endothelial NO and PG production also causes vasodilatation that may be important in the development of glomerular hyperfiltration.

### *In vivo* imaging of (pro)renin

Characterization of this novel GPR91-mediated renin release pathway was made possible, at least in part, by the recent development of a quantitative *in vivo* imaging model based on multi-photon excitation fluorescence confocal microscopy [14,15]. Using this new imaging approach, it is now possible to directly visualize JGA renin granular content, release, and tissue activity in the intact living kidney with high temporal and spatial resolution [16–18]. A recent study provided quantitative, functional and *in vivo* visual analysis of (pro)renin (a term denoting both renin and its precursor prorenin) in the diabetic rat kidney [19]. In addition to the JGA, which is the classic site of (pro)renin synthesis, significant amounts of (pro)renin were present in principal cells of the collecting duct (CD), especially in diabetes. The two most important intra-renal locations of (pro)renin synthesis and release and the *in vivo* imaging of these two sites by multi-photon microscopy are shown in Figure 2.

### The distal nephron is the major source of (pro)renin in diabetes

While the JGA is recognized as the primary source of renin and GPR91-mediated renin release in early diabetes could serve as the gatekeeper responsible for early RAS activation, its negative feedback function dictates that elevating Ang II levels eventually suppress JGA renin [13]. At the same time when Ang II suppresses JGA renin, it activates CD (pro)renin production in a number of disease models including high Ang II states, renovascular hypertension and diabetes [19–21]. Significant levels of *de novo* renin synthesis in the connecting segment (CNT) and CD were con-



**Fig. 2.** The two major sites of intra-renal (pro)renin synthesis: the juxtaglomerular apparatus (JGA) and the collecting duct (CD). Schematic drawing (A) and *in vivo* multi-photon fluorescence images (B, C) of these two sites. Direct *in vivo* visualization of quinacrine-labeled renin granules (green) in the JGA (B) and CD (C) in the intact kidney using multi-photon confocal microscopy. Control rat (B) and diabetic mouse kidney (C) are shown. A dextran-rhodamine B conjugate (70 kDa) labeled the intravascular space (plasma) red. (B) Quinacrine identified JGA renin granules in the terminal afferent arteriole (AA). The efferent arteriole (EA) is seen leaving the glomerulus (G). (C) Note the abundance of quinacrine staining in the bulging apical aspects of CD principal cells in diabetes (arrows). Nuclei are labeled blue with Hoechst 33342. Bars are 20  $\mu$ m. MD: macula densa; CNT: connecting tubule; PTC: peritubular capillaries.

firmed earlier [22]. The vast CD (pro)renin upregulation in diabetes is most likely mediated by Ang II, which appears to promote the buildup and release of CD prorenin [19]. CD

prorenin may be released to cause systemic or local pathological actions at the recently identified and characterized (pro)renin receptor [23–26], or may be cleaved to serve as a source of active renin in the face of JG renin suppression. This would be consistent with the existence of a local distal tubular RAS [22] and its possible regulatory effects on salt reabsorption [27,28].

Another thus far unrecognized and potentially very important feature of the diabetic kidney is significant proliferation of the CNT [19]. While Ang II up-regulates (pro)renin synthesis on the individual cellular level in this part of the nephron, proliferation of the entire CNT has larger scale effects on RAS activation by increasing the entire population of the (pro)renin-producing principal cells.

## Conclusion

GPR91-induced JGA renin release and the identification of the CD as the major site of intrarenal (pro)renin synthesis in diabetes are two innovative, potentially paradigm-shifting discoveries. Physiologically, GPR91-mediated paracrine signaling pathway in the JGA may serve to modulate glomerular filtration rate and RAS activity in relation to changes in whole body metabolism (especially in the post-prandial phase). Pathologically, it could link metabolic diseases (diabetes, metabolic syndrome) with RAS over-activation, systemic hypertension and organ injury. In diabetes, the proximity of CDprorenin synthesis to the (pro)renin receptor [24] establishes a volatile setting for persistent, local RAS activation in the kidney, and end-organ damage. GPR91 and CD (pro)renin are critical new sites to target in the treatment of diabetic complications.

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**Conflict of interest statement.** The results presented in this paper have not been published previously in whole or part, except in abstract format.

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