Angiotensin (Ang) II is an octapeptide in the blood that causes vasoconstriction, the release of aldosterone from the adrenal cortex, drives thirst behaviour in the brain, and initiates cell signalling by means of various intracellular mechanisms, notably in the endothelium. The endothelium modulates vascular tone by producing vasodilator and vasoconstrictor substances. Of these, the best characterized and potentially most important are nitric oxide (NO) and superoxide (O2). These small molecules are free radicals, meaning that they have one or more unpaired electrons in their outer orbitals. NO and O2 exhibit opposing effects on vascular tone and chemically react with each other in a fashion that negates their individual effects and leads to the production of potentially toxic substances, such as peroxynitrite (ONOO−). Peroxynitrite is an unstable valence isomer of nitrate and is also a free radical, resulting from the action of superoxide, a reactive oxygen species (ROS), on NO. These dynamic interactions have important implications, altering not only tissue perfusion but also contributing to the process of atherosclerosis. A superficial outline of peroxynitrite-related effects is shown in Figure 1.

In endothelial cells and in vascular smooth muscle cells, a membrane-associated nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase is the most important O2− source. This oxidase is activated by stimulation with Ang II, suggesting that, under all conditions of an activated circulating and/or local renin–angiotensin system, endothelial dysfunction related to increased vascular O2− production would be expected. The fact that Ang II stimulates the NADPH oxidase has been known for a decade. Many studies have shown that Ang II activates the enzyme and in turn how NADPH oxidases regulate numerous key Ang II-mediated effects. The topic has recently been reviewed by one of its originators [2].

All nucleated cells produce ROS, including superoxide anion, hydrogen peroxide (H2O2), and peroxynitrite. The NADPH oxidase enzyme complex is first worked out in neutrophils, where it plays an antimicrobial role. The enzyme complex has a series of subunits that vary to some degree between cell types. Precisely how Ang II activates the enzyme complex remains incompletely understood; however, Ang II, an AT1 receptor, mediates the process. In vascular smooth muscle cells (VSMC), the pivotal Nox1 subunit is activated, thereby beginning the ROS production–signalling cascade. ROS derived from NADPH oxidases serves a signalling function by inducing specific biochemical changes in numerous molecular targets. For example, the resultantly produced H2O2 can oxidize the thio group of protein cysteine residues that can inhibit protein tyrosine phosphatase activity. Figure 2 shows an abbreviated schema using VSMC as a model.

However, Ang II has many more targets than VSMC. The AT1 receptor appears essentially ubiquitous. As a result, new functions and new mechanisms of action for Ang II crop up all the time, with surprising findings and results. Recently, Loot et al. explored a novel mechanism to show how Ang II results in impaired endothelial function [3]. The fact that Ang II does so is not new; however, the mechanism involved, namely the shutting down of the endothelial nitric oxide synthase (eNOS), is novel and introduces further twists into our knowledge of Ang II signalling that surely have ramifications above and beyond endothelial dysfunction.

The signalling mechanisms involve the proline-rich tyrosine kinase 2 (PYK2). The group had observed earlier that fluid shear stress elicits the tyrosine phosphorylation of eNOS; however, the consequences of this modification on enzyme activity and the mechanisms were unclear. Fisslthaler and colleagues found that fluid shear stress induces the association of eNOS with PYK2 [4]. Furthermore, they were able to immunoprecipitate eNOS and PKY2 from PYK2-overexpressing HEK293 cells and observed that eNOS was tyrosine phosphorylated on Tyr657. They then performed a variety of functional studies in carotid arteries and other systems and showed that PYK2-induced phosphorylation at Tyr657 effectively shuts down the enzyme. Cleverly, they then produced a non-phosphorylatable mutant eNOS that was impervious to PYK2 phosphorylation at Tyr657. Their data indicated that PYK2 mediates the tyrosine phosphorylation of eNOS on Tyr657 in response to fluid shear stress and that this modific-
tion attenuates the activity of the enzyme. The PYK2-dependent inhibition of NO production could serve to keep eNOS activity low and to limit the detrimental consequences of maintained high NO output, namely the generation of peroxynitrite, mentioned earlier.

In the follow-up study by Loot et al. [3], the team explored the potential role of Ang II in these findings. Loot et al. report that, in endothelial cells, Ang II enhances the tyrosine phosphorylation of eNOS in an H2O2- and PYK2-dependent manner. They used low concentrations down to 1–100 μmol/L of H2O2 and with these doses stimulated the phosphorylation of eNOS Tyr657 without affecting that of another phosphorylation site, namely Ser1177. They concomitantly showed an attenuated basal and agonist-induced NO production by eNOS. The group next used mouse aortas and showed that 30 μmol/L H2O2 induced phosphorylation of eNOS on Tyr657 and impaired acetylcholine-induced relaxation. Endothelial overexpression of a dominant-negative PYK2 mutant protected against H2O2-induced endothelial dysfunction. Furthermore, carotid arteries from eNOS gene-deleted mice overexpressing the non-phosphorylatable eNOS Y657F mutant were also protected against H2O2. Telmisartan blocked the Ang II-related effect, implicating the AT1 receptor. The group next checked on the relevance of NADPH. They obtained Nox2 gene-deleted mice. Three weeks of Ang II infused by the authors considerably increased levels of Tyr657-phosphorylated eNOS in the aortas of wild-type but not in Nox2-deleted mice. Moreover, they showed a clear impairment in endothelium-dependent vasodilatation in the wild-type mice, but not in the Nox2 gene-deleted mice.

Thus, we have learned that PYK2 activation by Ang II and H2O2 causes the phosphorylation of eNOS at the Tyr657 residue [3]. As a result, eNOS activity is diminished, less NO is produced and endothelial function is impaired.
ceptor blockade, for example, would influence both pathways by maintaining eNOS activity while diminishing superoxide production.

Conflict of interest statement. None declared.

References


Glomerular scarring: can we delay or even reverse glomerulosclerosis by RAAS inhibition?

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Summary

In a recent publication, Macconi et al. show that reversal of focal and segmental glomerulosclerosis (FSGS) lesions in aged Munich Wistar Frömter (MWF) rats treated with high-dose angiotensin-converting enzyme inhibitors (ACE) inhibitors is associated with a reconstitution of the number of podocytes [1].

Aged MWF rats spontaneously develop hypertension, proteinuria, and focal and segmental glomerulosclerosis due to an unknown genetic defect. In 2006, the Remuzzi group has shown that treatment of aged MWF rats with very high doses of the ACE inhibitor lisinopril (~10 mg/kg/day) could revert proteinuria, and additional loss of renal function could be prevented effectively [2]. A detailed histological analysis showed that glomerular scarring was effectively reduced by this treatment suggesting that regression of glomerular scars could be achieved in these animals. In a recent paper, Macconi et al. followed up on this important finding and investigated changes in resident glomerular cells. They showed that the absolute podocyte number was decreased from 159 to 109 podocytes per glomerulus in aged MWF rats and that lisinopril treatment could restore the podocyte number to 144 (P < 0.01). As a mechanism for this cell renewal, the authors show that ~10% of podocytes co-expressed the proliferation marker Ki-67 in the treated rats suggesting that podocytes could undergo cell divisions in situ or that these cells could be regenerated from parietal cells. To support the latter notion, the authors show that a significantly higher proportion of parietal podocytes were present in the treated animals. This raises the interesting possibility for the existence of a glomerular regenerative mechanism.

Discussion

Although many groups (including the ones of Remuzzi, Fogo, Ritz, Zat, Weening or Chatziantoniou) agree that regression of glomerulosclerosis is possible [3,4], the mechanism by which this occurs is not clear. Regression of a glomerular scar may occur through various mechanisms. The balance between collagen synthesis could change versus proteolytic mediators. Glomerular capillaries are reorganized and multiplied by longitudinal splitting ('intussusception') [5]. The fate of the fibrocellular infiltrate is largely unknown. As a net result, the glomerular scar appears to shrink—but most investigators conclude that a glomerular scar never disappears entirely.