Comment on: Thallas-Bonke et al. (2008) Inhibition of NADPH Oxidase Prevents Advanced Glycation End Product–Mediated Damage in Diabetic Nephropathy Through a Protein Kinase C-α–Dependent Pathway: *Diabetes* 57:460–469, 2008

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I read with interest the recent study by Thallas-Bonke et al. (1), which reported that inhibition of NADPH oxidase prevents advanced glycation end product (AGE)-mediated damage in diabetic nephropathy through a protein kinase C-α (PKC-α)-dependent pathway. As far as I know, this is the first study that clearly demonstrated the interaction of NADPH oxidase with PKC-α and AGEs in established diabetic nephropathy. In the schema of Fig. 7 of their article, the authors claimed that NADPH oxidase was a downstream molecule of the AGE/receptor for AGE (RAGE)-mediated PKC-α activation based on the evidence that NADPH oxidase is activated via PKC-mediated phosphorylation of the $p47^{phox}$ subunit in neutrophils (2). However, the authors did not show data supporting their claim that inhibition of PKC-α actually suppressed superoxide generation and reduced the levels of phosphorylated $p47^{phox}$ in the kidneys of their diabetic models. On the contrary, they showed in Fig. 3 of their article that apocynin, an inhibitor of NADPH oxidase, blocked PKC activity and phosphorylated PKC-α expression in the kidneys of diabetic rats (1). These observations suggest that PKC-α activation is downstream of the AGE/RAGE-elicited NADPH oxidase–derived reactive oxygen species (ROS) generation, which could lead to vascular endothelial growth factor overexpression and accumulation of basement membrane proteins in diabetic kidneys. As the authors claimed, sustained activation of PKC-α is also mediated by ROS (3). Therefore, it is conceivable that the AGE/RAGE-mediated NADPH oxidase–derived ROS generation stimulates PKC activity, which could form a positive feedback loop to activate NADPH oxidase activity again via increased phosphorylation of $p47^{phox}$ levels. This is one possible explanation of why Ro-32-0432, an inhibitor of PKC-α, ameliorated the elevations in cytosolic ROS generated by AGE exposure (1). The data presented in Fig. 6*E* of the study by Thallas-Bonke et al. do not necessarily mean that PKC-α is an upstream molecule of NADPH oxidase-derived ROS in cultured mesangial cells exposed to AGE. The reason why Ro-32-0432 inhibited the RAGE expression in AGE-exposed mesangial cells is the same as above: inhibition of PKC-α by Ro-32-0432 could block the positive feedback loop to shut down further ROS generation. In support of this, we have recently found that the AGE/RAGE interaction upregulates RAGE expression via the NADPH oxidase–derived ROS generation (4,5). Taken together, this finding and the data presented by Thallas-Bonke et al. suggest that PKC-α is a downstream molecule of NADPH oxidase and that the former is a more important therapeutic target for the treatment of progressive diabetic nephropathy.

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