Role of Reactive Oxygen Species in Angiotensin II Signaling

The Plot Thickens

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Angiotensin II (Ang II) is the dominant effector of the renin-angiotensin system. In addition to its well-known hemodynamic and endocrine effects, Ang II regulates the cardiovascular remodeling associated with hypertension, atherosclerosis, heart failure, and diabetes mellitus. The importance of Ang II in cardiovascular disease states is highlighted by the prominent role that ACE inhibitors and angiotensin receptor blockers play in cardiovascular medicine. These drugs reduce clinical events and improve survival in patients with vascular disease or congestive heart failure and are among the most widely used medications in the world.1,2

There is accumulating evidence that Ang II has direct effects on smooth muscle cells (SMCs) that contribute to abnormalities ranging from subtle vascular dysfunction to severe atherosclerosis, ischemia, and necrosis. Ang II stimulates proliferation, hypertrophy, and migration of cultured vascular SMCs (VSMCs) via binding to the angiotensin type 1 (AT$_1$) receptor.3 This receptor is a member of the superfamily of 7 transmembrane-spanning, G protein–coupled cell surface receptors (GPCRs). Traditionally, these receptors were thought to elicit intracellular signaling solely via activation of heterotrimeric G proteins, but more recent evidence has demonstrated the importance of tyrosine phosphorylation of various signaling proteins, including tyrosine kinase receptors.

Transactivated epidermal growth factor (EGF) receptor (EGFR) serves as a scaffold for the assembly of protein-signaling complexes in VSMCs. EGFR is transactivated by many GPCRs in various cell types, suggesting an important role in GPCR signaling. In VSMCs, activation of AT$_1$ induces calcium-dependent transactivation of EGFR, which serves as a scaffold for c-src and downstream adaptors Shc/GRB2.4 Transactivation of EGFR is necessary for maximal Ang II–induced extracellular signal–related kinase 1/2 (ERK 1/2) activation,5 c-fos protein expression, and hypertrophy.6

AT$_1$ lacks intrinsic tyrosine kinase activity; thus, an important question is which secondary messengers mediate the transactivation of EGFRs. Ushio-Fukai et al.7 in the present issue of Arteriosclerosis, Thrombosis, and Vascular Biology, present evidence that reactive oxygen species (ROS) are involved in this process. They found that Ang II stimulated rapid transient tyrosine phosphorylation of EGFRs in VSMCs. The maximal effect was observed at 1 minute, which is probably not coincidentally the earliest time point at which hydrogen peroxide is detected after exposure to Ang II.5 Treatment with exogenous hydrogen peroxide or a superoxide-generating compound also elicited tyrosine phosphorylation of EGFRs in a time-dependent manner, albeit with different kinetics (peak effect at >15 minutes). More important, treatment with various antioxidants inhibited Ang II–induced, but not EGF-induced, tyrosine phosphorylation of EGFRs by 75% to 93%.

In addition to delineating the functional importance of ROS, Ushio-Fukai et al7 provide insight into how ROS regulate the tyrosine phosphorylation of EGFRs in VSMCs. Their data support the notion that ROS probably do not have a direct effect on EGFR and that the intermediary is not Janus kinase-2 or phosphatidylinositol-3 kinase. Nor is it likely that secreted EGFR ligands are involved.5 The mechanism supported by the findings of Ushio-Fukai et al7 is that c-Src is involved in Ang II–induced EGFR tyrosine phosphorylation in a redox-sensitive manner. Thus, in addition to identifying an important mediator of the effects of ROS, these results provide further evidence that c-Src acts upstream of EGFR tyrosine phosphorylation in Ang II–treated VSMCs.

Studies of ROS may shed some light on pathways involved in Ang II–induced ERK 1/2 activation in VSMCs. The finding that antioxidants inhibited Ang II–induced EGFR transactivation,7 together with previous data8 that inhibition of EGFR transactivation blocked Ang II–induced ERK 1/2 activation, would seem to suggest that ROS are an essential intermediary in Ang II–induced ERK 1/2 activation. However, this has not been a consistent finding. Frank et al9 have recently reported that diphenylene iodonium, an inhibitor of flavin-containing oxidases including NAD(P)H, almost completely inhibits Ang II–induced ERK 1/2 activation. They have also found that exogenous hydrogen peroxide (200 &mu;mol/L) stimulates ERK 1/2 activation. In contrast, Ushio-Fukai et al10 in a prior study found that diphenylene iodonium had no effect on Ang II–induced ERK 1/2 activation and that exogenous hydrogen peroxide inhibited ERK 1/2 activation. All of these studies were performed in growth-arrested aortic SMCs from Sprague-Dawley rats, ruling out a species effect but leaving open the possibility that the effects of ROS on ERK 1/2 activation in rat aortic SMCs may vary under different phenotypic influences or under other conditions that have yet to be understood. Together, these studies indicate

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that Ang II is able to elicit ERK 1/2 activation via ROS-dependent and ROS-independent pathways.

There is growing evidence that ROS play a major role in VSMC proliferation, protein synthesis, and survival. The study of Ushio-Fukai et al further emphasizes the pleiotropic effects of ROS and demonstrates an important, and potentially wide-ranging, role as a secondary messenger system. Furthermore, in addition to mediating EGFR transactivation, ROS may have additional roles in signals downstream from EGFR or in Ang II–induced events that are independent of EGFR activation. Further work is needed to define the different points at which ROS participate in GPCR-mediated signaling events.

Given these integral ROS-mediated signals in vascular cells and the tight regulation of ROS production and inactivation, it is then less surprising that the oral administration of vitamins has not reduced clinical cardiovascular events. Although antioxidants can modulate growth-signaling responses in cultured cells, as demonstrated by Ushio-Fukai et al and many others, the complexities of achieving the same local concentrations after systemic administration have yet to be understood. Antioxidants have great potential to mitigate cardiovascular disease, but effective agents will have to be cleverly designed to compete with the cellular defenses that maintain redox homeostasis.

References


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