Angiotensin II (Ang II) is a profoundly important signaling component of the renin-angiotensin system. Its principal functions are related to cardiovascular homeostasis, and its many targets include vascular myocytes, endothelium, cardiac myocytes, fibroblasts, diverse cells in the kidney and adrenal gland, and cells in the central and peripheral nervous systems. Ang II contributes to the physiological regulation in healthy states but can also induce or contribute to pathophysiological events seen in certain diseases.2,3

The cellular effects of Ang II are mediated by its interaction with membrane receptors, of which two subtypes have been identified.4 AT1 receptors are found in vascular tissue, heart, and brain, whereas AT2 receptors are found in adrenal and uterine cells. Many of the downstream events triggered by Ang II binding to the AT1 receptor have been found to involve signal transduction steps that require reactive oxygen species (ROS).5–9 The generation of ROS in mammalian systems begins with univalent transfer of an unpaired electron to O2, yielding superoxide. As an anion, superoxide does not easily cross cell membranes, so its signaling domain is generally restricted to the subcellular compartment where it is synthesized. Superoxide is well suited to act as a signaling molecule because it is not excessively reactive, but not excessively so. Dismutation of two superoxide anions yields hydrogen peroxide, an uncharged molecule that passes easily through cell membranes and can therefore transmit redox signals between subcellular compartments. Like superoxide, H2O2 behaves nicely as a signaling molecule because it is not overly reactive and can therefore diffuse toward its intended targets without excessively oxidizing other molecules along the way. Reduction of H2O2 with an electron yields hydroxyl radical, which is virtually useless as a signaling molecule because of its highly reactive nature and correspondingly short half-life. An exception to this limitation may occur under special circumstances where it is generated in close proximity to its intended target, thereby limiting its nonspecific effects.

In the cell, superoxide is dismutated into H2O2 by Cu, Zn-superoxide dismutase (Cu,Zn-SOD) in the cytosol or by Mn-SOD in the mitochondrial matrix. The former isozyme acts to detoxify superoxide released by various oxidase systems, whereas the latter functions to degrade superoxide generated by the mitochondrial electron transport system. The Mn-SOD encoded by nuclear DNA is localized to the mitochondrial matrix by virtue of a mitochondrial targeting sequence in its amino terminus. In signal transduction systems requiring H2O2, the SODs help to ensure efficient conversion of superoxide to H2O2. However, overexpression of SOD does not lead to excess peroxy formation, since the rate of H2O2 generation in the presence of normal levels of SOD is limited by the rate of superoxide formation.

Recent work points to the involvement of ROS in many of the responses elicited by Ang II in cardiovascular cells, with much evidence implicating superoxide production by members of the NAD(P)H oxidase family.6,9,10 Vascular myocytes possess an NAD(P)H oxidase that is similar but not identical to the neutrophil form of the enzyme.11 Both forms include the membrane-bound cytochrome b558-containing p22phox subunit, the cytosolic subunits p47phox and p67phox, and the small GTPase rac-1.9 Neutrophils and endothelial cells contain gp91phox, another b558 subunit, whereas vascular myocytes express nox1 and nox4, which are homologues of gp91phox.7 The mechanisms responsible for NAD(P)H oxidase activation by Ang II have not been identified, although evidence suggests that protein kinase C (PKC) activation is required. In neutrophils, PKC activation results in phosphorylation of the p47phox, which then binds to gp91phox.12 In rat aortas, superoxide generation and the associated upregulated expression of nox1, gp91phox, and p22phox subunits during long-term Ang II administration were prevented by simultaneous PKC inhibition with chelerythrine.5 Collectively, these findings support a role for PKC in the assembly of NAD(P)H oxidase, but the mechanism of PKC activation by Ang II is not known.

Although many of the cellular effects of Ang II appear to be mediated by ROS generated by NAD(P)H oxidase, some responses require superoxide whereas others are specific for H2O2. For example, Ang II–induced vascular myocyte hypertrophy can be blocked by transfection of antisense to p22phox and by catalase overexpression,13 thus implicating H2O2 derived from NAD(P)H oxidase in the response. Similarly, in cardiac fibroblasts, Ang II administration increased IL-6 mRNA expression, and the response was blocked by the H2O2 antioxidant N-acetylcysteine.8 On the other hand, Fukui et al.14 found that long-term administration of Ang II increased expression of p22phox, increased superoxide generation, and produced hypertension. However, the hypertensive response to long-term Ang II administration was later shown to be inhibited by liposomal SOD, which indicates a requirement for superoxide.14 The specificity for superoxide versus H2O2.
in these responses may relate to the access of these oxidant species to the cellular compartment containing the intended target. In either case, downstream targets of Ang II signaling that require superoxide should be inhibited by SOD, whereas others that can be activated by \( \text{H}_2\text{O}_2 \) should be insensitive to SOD overexpression.

Although much has been learned about the signaling pathways activated by Ang II in the cardiovascular system, comparatively less is known of its mechanism of action in the brain. Administration of Ang II into the CNS elicits hypertension by stimulating sympathetic tone, it triggers vasopressin release, and it promotes thirst through a receptor-mediated effect.\(^1\) In the accompanying article in this issue of Circulation Research, Zimmerman et al.\(^5\) show that overexpression of Mn-SOD in the CNS can abrogate the systemic response to intracerebroventricular (ICV) administration of Ang II but not the effects of another central pressor agent, carbachol. The Mn-SOD overexpression also abolished the dipsogenic response to ICV Ang II, without changing basal water intake. By contrast, the control adenovirus carrying the LacZ gene did not produce these changes. These findings clearly indicate that the neural responses to ICV Ang II require intracellular superoxide. To determine which cells were transduced by the adenoviral vector, these investigators performed immunohistochemical analyses and found widespread expression of human Mn-SOD in periventricular tissue in the CNS. Interestingly, they found intense immunofluorescence staining for human Mn-SOD in the subfornical organ (SFO), which is important in mediating the effects of intravascular or ventricular Ang II. The same region was also densely populated with AT\(_1\) receptors, consistent with their putative role in Ang II sensing. They also took the important step of confirming that the Mn-SOD protein was functional. Moreover, to assess superoxide generation in response to Ang II, Zimmerman et al. studied primary cultures of neurons from the periventricular region of rats. Using oxidation of dihydroethidium to detect intracellular superoxide, they found that responses to Ang II were specific to neurons, and that responses were blocked by the AT\(_1\) receptor inhibitor losartan or by previous infection with the AdMn-SOD but not blocked by the AdLacZ virus. Collectively, these results provide strong evidence specifically implicating superoxide in the intracellular signaling activated by the AT\(_1\) receptor in neurons and for the functional and behavioral responses in the intact animal.

The present study represents an important step forward in understanding the signaling pathways activated by Ang II in the brain. But, like many interesting studies, it also raises questions that will require additional effort to address. For example, although it is not surprising that ICV injection of the AdMn-SOD led to widespread expression of the transgene in periventricular tissue, it is remarkable that immunostaining was especially strong in the SFO cells that also exhibited strong AT\(_1\) receptor expression. The reason for the correspondence between basal AT\(_1\) expression and the strength of the transgene expression is not known, and it is surprising that the very cells relying on superoxide to signal important functional responses would be more effective in expressing an inhibitory protein than other nearby cells.

A more challenging question relates to their observation that Mn-SOD overexpression blocked the response to Ang II. This suggests that superoxide must have been generated in the mitochondria, because superoxide generated outside that organelle would not be affected by a matrix protein. This finding appears to contrast with the well-established role of NAD(P)H oxidase in the Ang II–induced superoxide response in vascular cells. Curiously, the responses to ICV Ang II were also abrogated by infection with an adenovirus encoding Cu,Zn-SOD. Assuming that the intended targeting of the Mn-SOD and Cu,Zn-SOD proteins to their respective destinations was successful, this finding indicates a dual requirement for cytosolic and mitochondrial superoxide in the Ang II response. They reasonably conclude that superoxide from both mitochondria and extramitochondrial sources may be involved or that mitochondrial superoxide may leak into the cytosol where it could act. An even more provocative hypothesis is that superoxide generation in one compartment triggers oxidant production by a different system in the other.

Given the established link between Ang II and NAD(P)H oxidase activity in other cell types, it is reasonable to postulate that this oxidase is also activated by Ang II in neurons. If so, their data raise the question of whether mitochondrial superoxide production might trigger subsequent activation of a cytosolic oxidase system, or vice versa. A signaling cascade involving oxidant generators in separate compartments could amplify the initiating signal across subcellular domains. In this regard, insight might be gained if they were to pursue additional studies examining the significance of superoxide generation in different cellular compartments during Ang II stimulation. Recent work reveals that multiple oxidases may be activated in T cells by receptor stimulation, suggesting that this may represent an interesting new chapter in the field of redox signaling.\(^6\) In either case, it is hoped that the journey down this road is just beginning.

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Keywords: superoxide ■ reactive oxygen species ■ NAD(P)H oxidase ■ oxidant signaling
Angiotensin II Signaling in the Brain: Compartmentalization of Redox Signaling?
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_Circ Res._ 2002;91:982-984
doi: 10.1161/01.RES.0000045655.34731.B6
_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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