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Editorial Commentary

Angiotensin II, Oxidant Signaling, and Hypertension
Down to a T?

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It has been 30 years since landmark studies by Johnson and Brody[1] revealed a pivotal role for the forebrain circumventricular organs (CVOs) in experimental hypertension. We have since come to understand that these small midline structures, mounted along the cerebral ventricles and lacking well-formed blood-brain barriers, shoulder a huge responsibility for maintaining the delicate balance of cardiovascular and body fluid homeostases. With their exotic cytology and morphology, including "neuron-like" cells lying free on the ependymal surface and unusually dense and complex fenestrated capillary networks, the CVOs are involved in a remarkable array of homeostatic functions ranging from thirst and salt appetite to vasopressin release and sympathetic outflow.[2] With the study by Lob et al in this issue of *Hypertension,*[3] we must also consider adding to this list the key role that CVOs play in linking central and peripheral mechanisms of hypertension through activation of peripheral T lymphocytes. If proven true, these findings could have broad implications for a unifying hypothesis of how the central nervous system, the vasculature, and possibly other peripheral organs, including the kidney, are involved in the etiology of hypertension.

The first of 2 major findings in the study by Lob et al[3] supports and extends previous reports that reactive oxygen species signaling in the subfornical organ (SFO), a key forebrain CVO, is critical in angiotensin II (Ang II)–mediated regulation of blood pressure and hypertension.[4,5] Using an established viral gene transfer approach that induces robust transgene expression nearly exclusively in the SFO,[6] Lob et al[3] show that SFO-targeted ablation of endogenous extracellular superoxide dismutase (SOD3), 1 of 3 isozymes in mammals that catalyzes dismutation of superoxide ($O_2^{\cdot-}$), causes a significant elevation in basal blood pressure. In addition, deletion of SOD3 in the SFO increases the sensitivity to systemic Ang II at a dose that does not normally affect blood pressure in mice. These studies using gene deletion lead to a similar general conclusion as was made

earlier using gene overexpression, namely, that elevated levels of $O_2^{\cdot-}$ in the SFO lead to hypertension. What is new and important here is the unmasking of a key role for the extracellular form of SOD. In previous studies, we showed that adenoviral-mediated overexpression of cytoplasmic Cu/Zn SOD or mitochondrial SOD but not SOD3 in the SFO interfered with the pressor effects of Ang II.[4,5] However, as shown earlier and reiterated in the study by Lob et al,[3] SOD3 is expressed at high basal levels in SFO. This may explain why additional overexpression of this form of the enzyme failed to inhibit the pressor effects of Ang II in our earlier studies. The use of Cre-loxP technology and selective deletion of endogenous SOD3 in the present study elegantly reveal that extracellular $O_2^{\cdot-}$ signaling in SFO is also important. Future analysis of the relative expression, distribution, and functional role of these 3 SOD isozymes in SFO will be important in understanding the mechanisms of central redox signaling and how it regulates hemodynamics.

The second and potentially groundbreaking finding in the study by Lob et al[3] is that the SFO, at least in part through SOD3, may couple central and peripheral oxidant systems through activation of peripheral T cells, thereby providing a possible common underlying mechanism of hypertension that spans multiple organ systems. These investigators show that SOD3 ablation in the SFO is sufficient to increase oxidants in both the SFO and the aorta, and this is accompanied by an elevation in sympathetic output and an increase in the number of circulating CD69$^+$ T lymphocytes. The addition of low-dose Ang II infusion does not have further effects on these end points in the SOD3-ablated mice. In contrast, it does enhance the number of inflammatory CD45$^+$, CD3$^+$, and CD69$^+$ cells and expression of T-cell recruitment molecules in peripheral aortic tissue. Broadly interpreted, these results suggest that elevating extracellular $O_2^{\cdot-}$ levels in the SFO through deletion of SOD3 causes significant alterations in peripheral T-cell activation and vascular $O_2^{\cdot-}$ regulation and inflammation, leading to hypertension.

As with all potentially important discoveries, this study raises as many questions as it answers. The first and perhaps most important is the sequence of interplay (and, therefore, the exact cause-effect relationships) between the multiple signaling systems invoked, including $O_2^{\cdot-}$ in the SFO, sympathetic outflow, T-cell activation, vascular $O_2^{\cdot-}$ regulation, vascular infiltration, and blood pressure. Given the increase in basal sympathetic outflow with SOD3 deletion in SFO and the known effect of oxidant stress to induce sympathoexcitation, it is highly plausible that this is a key initiating mechanism linking the central and peripheral responses observed in this study. Indeed, increased sympathetic activity could lead to each of the responses observed after
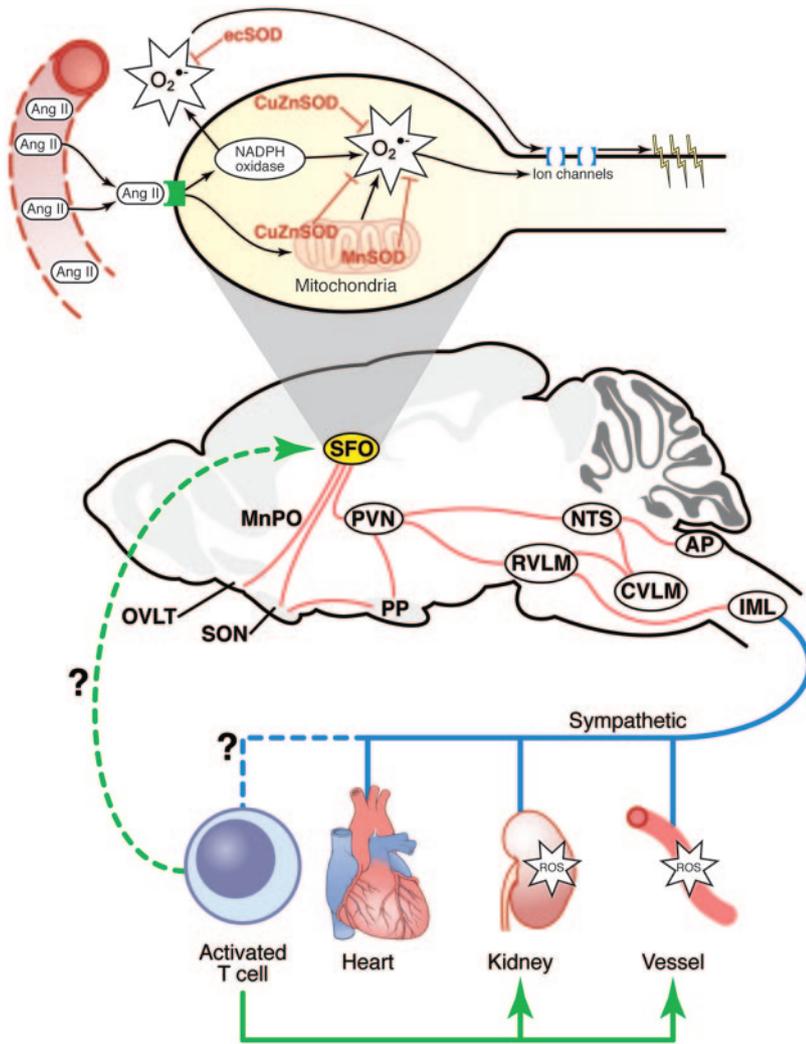
Figure. Schematic of how the SFO may link central and peripheral mechanisms of Ang II–dependent hypertension through activation of peripheral T cells. Perturbations in SOD (cytoplasmic SOD [CuZn SOD], mitochondrial SOD [MnSOD], or extracellular SOD [ecSOD]) and/or Ang II–mediated activation of NADPH oxidase in the SFO lead to increased oxidant formation and stimulation of downstream sympathoexcitatory circuits. Increased sympathetic nerve firing and/or circulating Ang II may activate peripheral T cells and expression of homing signals in the vasculature and the kidney. This could promote infiltration of T lymphocytes and oxidant stress–mediated effects on vascular and renal tissues, leading to hypertension. Activated circulating T cells may also act back at the SFO to further promote this cycle.

SOD3 ablation, including T-cell activation and aortic $O_2^{.-}$ increases, in addition to direct actions on the vasculature and other peripheral organs. It will be critical to establish these causal links definitively and determine their relative roles. It will also be important to determine whether one or all of these responses, along with the elevated basal blood pressure, are necessary and/or sufficient for the effects observed after superimposition of Ang II, including increased circulating $CD4^-/CD8^-$ T cells, vascular infiltration, and further augmentation of hypertension. Related to this is the question of whether Ang II stimulates immune activation directly or through sympathetic nerve firing, or both, and to what relative extent these mechanisms are involved. Moreover, because the aorta is a conduction vessel and contributes little to vascular resistance, one wonders what an increase in T cells in the aorta means and, more importantly, whether Ang II stimulates this response in resistance vessels. Finally, because the vascular inflammation and homing marker data were collected only at the end of the Ang II infusion period when blood pressure was at its maximum, it is impossible with this experimental paradigm to tease out whether the inflammatory effects of Ang II are the cause or the result of hypertension. However, if the studies are repeated at earlier

time points before maximum Ang II–induced hypertension is reached, and it is revealed that inflammation plays a causative role in the severe hypertension of this model, it will be important to determine the mechanism by which this occurs. Although there is no further augmentation of vascular $O_2^{.-}$ with Ang II treatment in this study, increased oxidative stress is a likely culprit and should be examined in other target end organs, particularly the kidney.

Another important series of questions raised by the Lob et al[3] study concerns the local cellular mechanisms at play with SOD3 ablation and extracellular $O_2^{.-}$ formation in the SFO, particularly as it relates to Ang II–mediated intracellular signaling mechanisms. Certainly more work will be required to fully address this, but previous studies may provide a few clues. For example, increased scavenging of intracellular $O_2^{.-}$ via overexpression of Cu/Zn SOD significantly, but not completely, inhibits Ang II–induced influx of extracellular Ca^{2+}.[7] In contrast, inhibition of NADPH oxidase virtually abolishes this Ang II response,[7–9] as well as the Ang II–induced inhibition of K^+ current.[10] We surmised that the differential effects of Cu/Zn SOD overexpression and NADPH oxidase inhibition had to do with differences in efficiencies of increased $O_2^{.-}$ scavenging versus suppression

of $O_2^{\cdot-}$ production. However, given the study by Lob et al,[3] it is tempting to speculate that a complete Ang II–evoked response depends on both intracellular and extracellular $O_2^{\cdot-}$. One could imagine that NADPH oxidase may be the source of $O_2^{\cdot-}$ both intracellularly and extracellularly. However, even if this is true, we will still need to come to terms with how extracellular $O_2^{\cdot-}$ evokes intracellular signaling in SFO neurons. There are a number of possibilities as outlined by the authors, including extracellular $O_2^{\cdot-}$-mediated decreases in NO·, increases in peroxynitrite, alterations of cell surface proteins via an extracellular redox-sensitive domain, or modification of ionic currents. In support of these latter possibilities, Sun et al[10] reported that treatment of primary neurons with exogenous xanthine-xanthine oxidase, an oxidant-generating system, decreases the K^+ current and increases neuronal firing. Considering that the membrane permeability of the large ($\approx$300-kDa) xanthine oxidase and its generated $O_2^{\cdot-}$ remains unclear, it is possible that these results are in keeping with the idea that extracellular $O_2^{\cdot-}$ mediates its effects on the K^+ current and neuronal firing by acting on an extracellular redox-sensitive domain of a transmembrane protein, such as a receptor or ion channel.

One of the greatest challenges facing the hypertension research community is how to reconcile very convincing findings that the CNS, vasculature, and kidney each appears to play a major role in the etiology of this disease. There is no better example of this than that of the Ang II/oxidant signaling system, which can explain much of the pathophysiology of high blood pressure when studied in the context of these individual organs. The study by Lob et al[3] requires us to consider that T-cell activation via the CVOs may just provide the unifying answer that we have been looking for as to how these organ systems are linked, and each contributes to the origin of this disease (see Figure).

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Disclosures

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