COMMENT

How does endothelin induce vascular oxidative stress in mineralocorticoid hypertension?

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ABSTRACT

Endothelin and reactive oxygen species have been identified as important mediators in the pathogenesis of hypertension and associated end-organ damage. In the present issue of *Clinical Science*, Callera and co-workers have provided new evidence that endothelin stimulates mitochondria to generate reactive oxygen species in the vascular wall during mineralocorticoid-induced hypertension in the rat. These studies open a new line of investigation that could be important for the development of therapeutic strategies; however, there still remains a great deal of uncertainty about the mechanisms that define the relationship between endothelin and oxidative stress in hypertension.

A major focus within the basic science community studying hypertension is related to the role of ROS (reactive oxygen species). The broad definition of ROS includes oxygen-containing species that are all capable of reacting with proteins and lipids to produce abnormal cellular responses. ROS include molecules such as superoxide, hydroxyl radical, H₂O₂, peroxynitrite and nitric oxide. Oxidative stress is defined as an imbalance of oxidant and antioxidant mechanisms and is generally thought to contribute to the development of hypertension and end-organ damage.

It has become clear that angiotensin II stimulates ROS in various tissues during hypertension, most likely through activation of NADPH oxidase [1]. Similarly, several studies have suggested that ET (endothelin)-1 increases oxidative stress, although the mechanisms are less well developed. Previously, it has been established that: (i) mineralocorticoid-induced hypertension [i.e. DOCA (deoxycorticosterone acetate)-salt] is associated with increased oxidative stress, which is dependent upon NADPH oxidase [2], (ii) ET-1 through activation of the ETA receptor can stimulate NADPH oxidase-dependent superoxide production [3–5], and (iii) ETA receptor blockade can reduce oxidative stress and blood pressure in mineralocorticoid-induced hypertension [6]. This has led to the general conclusion that ET-1 increases oxidative stress by activation of NADPH oxidase. However, until now, the evidence that ET-induced activation of NADPH oxidase contributes to oxidative stress has been circumstantial in this model. The study by Callera and co-workers [7] in the present issue of *Clinical Science* provides important new information on the relationship between ET and ROS generation in this model to include mitochondrial-derived ROS. This is the first study to demonstrate involvement of mitochondria in ET-dependent oxidative stress.

The most widely studied of the ROS-generating enzymes in hypertension is NADPH oxidase, but xanthine oxidase, cytochrome P450, uncoupled NOS (nitric oxide synthase) and enzymes of the mitochondrial electron transport chain are also potentially important. The major impact of the studies by Callera et al. [7] is that they provide strong evidence that mitochondria are an important source of ROS produced by ET in the DOCA-salt hypertensive rat. In fact, they confirmed that expression of NADPH oxidase subunits are increased in the vascular wall of DOCA-salt-treated rats, yet blockade of ETA receptors did not influence changes in NADPH

Key words: deoxycorticosterone acetate (DOCA), endothelin, hypertension, mitochondria, NADPH oxidase, reactive oxygen species (ROS).

Abbreviations: DOCA, deoxycorticosterone acetate; ET, endothelin; NOS, nitric oxide synthase; ROS, reactive oxygen species.

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oxidase expression or activity in vascular tissue, despite reducing ROS. These findings provide a clear rationale for a new line of investigation into the mechanisms by which ET influences cellular respiration and mitochondrial electron transfer. These pathways could lead to novel approaches towards reducing vascular dysfunction and injury in disease models associated with elevations in local ET expression and activity.

As with every important new discovery, there are also many new questions that arise. In this case, the question remains that, despite reductions in overall measures of oxidative stress, NADPH oxidase activity and translocation is still elevated. These findings suggest that changes in vascular NADPH oxidase do not account for the hypertension produced in this model. This conclusion would seem to contradict the studies of Beswick et al. [2], who demonstrated that the NADPH oxidase inhibitor, apocynin, can significantly attenuate hypertension in the DOCA-salt model. One possible explanation for these apparently discrepant findings is that, although oxidative stress may be reduced in large vessels, as in the study by Callera et al. [7], this may not be reflective of mechanisms that regulate blood pressure. For example, most studies have not specifically identified the degree of localized oxidative stress within the kidney in the DOCA-salt or other models hypertension. Since neither apocynin nor ETA receptor blockade completely normalized blood pressure in DOCA-salt-treated rats, it is quite possible that there are multiple sources of ROS generation in this model and that ROS are derived from both mitochondrial, NADPH oxidase and perhaps even other sources depending on the specific tissue and cell type.

Other remaining questions are related to the significance of previous studies indicating that ET-1 can stimulate NADPH oxidase to generate superoxide. Li and co-workers [3] showed that in vitro incubation of carotid arteries with apocynin inhibits ET-1-induced increases in superoxide production, demonstrating that ET-1 activates NADPH oxidase. Furthermore, in vivo studies showed that treating DOCA-salt rats with an ETA antagonist lowered blood pressure and vascular superoxide production. The results by Callera et al. [7] suggest that the stimulation of NADPH oxidase is unrelated to in vivo generation of superoxide and blood-pressure-lowering actions of the ETA antagonist. Consistent with this conclusion, Elmarakby et al. [5] recently published results from another set of experiments that dissociated ET-dependent hypertension from ROS generated by NADPH oxidase. In that study [5], hypertension was induced by chronic ET-1 infusion into rats on a high-salt diet. Blockade of NADPH oxidase with apocynin reduced aortic superoxide, yet the hypertension persisted.

In addition to mitochondrial enzymes and NADPH oxidase, a recent study by Loomis et al. [4] provided in vitro evidence that ET-1 can directly stimulate both NADPH oxidase and uncoupled NOS to produce superoxide in the thoracic aorta and alter vascular reactivity. The study by Callera et al. [7] showed that ETA receptor blockade in DOCA-salt rats had no effect on NOS expression. However, these results do not assess enzyme activity or whether the enzyme is making nitric oxide, or whether NOS is uncoupled and is producing superoxide. Further studies are still needed to discern whether or not the stimulation of uncoupled NOS by ET is of functional significance in hypertension.

In conclusion, Callera and co-workers [7] have provided an important new step towards our understanding of the complex nature of ET-induced increases in ROS and how these effects may or may not be related to the degree of hypertension in an ET-dependent model. The contribution of ET-dependent superoxide from various sources to the degree of blood pressure elevation remains to be resolved. We are left with several major unanswered questions that will require further investigation. These include (i) whether ET stimulates superoxide production from different enzymatic sources in different tissues and/or cell types, (ii) whether the resulting superoxide is a cause or consequence of hypertension, and (iii) what role, if any, do changes in antioxidant capacity contribute to the myriad of results from various laboratories.

REFERENCES


Received 1 December 2005; accepted 5 December 2005
Published as Immediate Publication 5 December 2005, doi:10.1042/CS20050359