Volume Matters

Novel Roles of the Volume-Regulated ClC-3 Channels in Hypertension-Induced Cerebrovascular Remodeling

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The adaptive remodeling of cerebral arteries, including hypertrophy (medial thickening) and eutrophy (luminal narrowing), is a prominent feature of hypertension and a major risk factor for stroke.1–3 Multiple mechanisms, such as arterial smooth muscle cell (SMC) proliferation, migration and apoptosis, endothelial cell dysfunction, inflammation, and fibrosis, have been implicated in structural remodeling in response to chronic hypertension.4 Although the hypertrophic increase in cell volume of arterial SMCs is the primary mechanism for the thickening of mesenteric arterial media in spontaneously hypertensive rats1 and of cerebral arteries in the 2-kidney, 2-clip renal hypertensive rats,5 the impact of the hypertension-induced changes in cell volume of the arterial SMCs and its clinical significance are still unknown.

Alterations in cell volume are common adaptive mechanisms of most mammalian cells, including arterial SMCs, in response to metabolic, osmotic, and/or static pressure perturbations.5 Cells are able to precisely maintain their size through the regulated loss or gain of intracellular ions or other osmolytes to avoid excessive alterations of cell volume that may jeopardize structural integrity and a variety of cellular functions.6 Acute increase in cell volume will initiate the regulatory volume decrease process to bring the cells back to their initial volume, which is achieved by the opening of volume-regulated Cl– channels (VRCCs) and other channels and transporters mediating Cl–, K+, and taurine efflux.5 As one of the most important mechanisms for cell volume homeostasis, activation of VRCCs has been implicated in a number of vital cellular functions involved in hypertension-induced vascular remodeling, including the regulation of membrane potentials, vascular myogenic tone, cell proliferation, migration, and apoptosis (Figure).6–8 For example, high blood pressure–induced depolarization and contraction of cerebral artery smooth muscle may be partially mediated by VRCCs.7 There is evidence that the magnitude of VRCC currents in actively growing vascular SMCs is higher than in growth-arrested or differentiated SMCs, suggesting that VRCCs may be important for SMC proliferation.7 Therefore, hypertension-induced increase in cell volume and activation of VRCCs may contribute to the structural and functional remodeling through an integrated regulation of multiple cellular functions.

The short isoform of CIC-3, a member of the CIC superfamily of voltage-dependent Cl– channels, has been proposed to be the molecular correlate of a key component of the native VRCCs in cardiac myocytes and vascular SMCs.7,9 A series of recent independent studies from many laboratories further strongly corroborated this hypothesis (please see recent reviews by Duan6 and Hume et al7). It has been demonstrated that CIC-3 is expressed in aortic, pulmonary, and cerebral artery SMCs of many species, including humans. Knockdown of CIC-3 by small interfering RNA, short hairpin RNA, and antisense and intracellular dialysis of anti-CIC-3 antibody all consistently eliminated VRCC currents in many types of cells. Recent accumulating evidence suggests an important role for CIC-3 and VRCCs in the regulation of cell proliferation induced by hypertrophic alternations in cell volume. A recent study found that static pressure increased VRCCs and CIC-3 expression and promoted rat aortic vascular SMC proliferation and cell cycle progression. Inhibition of VRCCs with pharmacological blockers (eg, diphenyleneiodonium) or knockdown of CIC-3 with CIC-3 antisense oligonucleotide dramatically inhibited pressure evoked cell proliferation and cell cycle progression of rat aortic SMCs. These data suggest that CIC-3 and VRCCs may play a critical role in static pressure–induced cell proliferation and cell cycle progression. Because arterial SMC proliferation is a key event in the development of hypertension-associated vascular disease, CIC-3 and VRCCs may be of unique therapeutic importance for the treatment of hypertension-attendant vascular complications.

Recent studies have demonstrated that statins are effective in attenuating vascular remodeling, although the underlying mechanisms are still not determined. In this issue of Hypertension, Liu et al10 used integrated, multiple approaches and performed a thorough investigation on the effects of simvastatin on the hypertension-induced cerebrovascular remodeling and VRCCs in basilar SMCs. They first demonstrated that simvastatin improved the hypertension-caused cerebrovascular remodeling in 2-kidney, 2-clip renal hypertensive rats. Then they used cultured rat basilar SMCs to further study the effects of simvastatin on cell proliferation and the whole-cell VRCC current and volume-regulated Cl– movement. They found that simvastatin inhibited cell proliferation and also the volume-regulated chloride movement and VRCCs, which could be abolished by pretreatment of the cells with meval-
Figure. Schematic representation of CIC-3 Cl⁻ channels in vascular SMCs. CIC-3, a member of the voltage-gated CIC Cl⁻ channel family, encodes Cl⁻ channels in vascular SMCs that are volume regulated (l<sub>Cl,vol</sub>) and can be activated by cell swelling (l<sub>Cl,swell</sub>) induced by exposure to hypotonic extracellular solutions or possibly membrane stretch. l<sub>Cl,b</sub> is a basally activated CIC-3 Cl⁻ current. α-Helices of CIC-3 are shown as α-τ (see review by Duan). CIC-3 proteins are expressed on both the sarcolemmal membrane and intracellular organelles including mitochondria (mito) and endosomes. The proposed model of endosome ion flux and function of Nox1 and CIC-3 in the signaling endosome is adapted from Miller et al. Binding of interleukin (IL)-1β or tumor necrosis factor (TNF)-α to the cell membrane initiates endocytosis and formation of an early endosome (EEA1 and Rab5), which also contains Nox subunits Nox1 and p22phox, in addition to CIC-3. Nox1 is electrogenic, moving electrons from intracellular NADPH through a redox chain within the membrane initiates endocytosis and formation of an early endosome (EEA1 and Rab5), which also contains Nox subunits Nox1 and p22phox, in addition to CIC-3. Nox1 is electrogenic, moving electrons from intracellular NADPH through a redox chain within the enzyme into the endosome to reduce oxygen to superoxide. CIC-3 functions as a chloride-proton exchanger, required for charge neutralization of the electron flow generated by Nox1. The ROS generated by Nox1 result in nuclear factor (NF)-κB activation. Both CIC-3 and Nox1 are necessary for generation of endosomal ROS and subsequent NF-κB activation by IL-1β or TNF-α in vascular SMCs. Statins block CIC-3 channels, which causes hyperpolarization of the cell membrane, closure of Ca²⁺ channels and vasorelaxation, and inhibition of cell proliferation. PKC indicates protein kinase C; PP, serine-threonine protein phosphatases; α-AR, α-adrenergic receptor; Gi, heterodimeric inhibitory G protein.

In addition, they found that both Rho A inhibitor C3 exoenzyme and Rho kinase inhibitor Y-27632 reduced the cell proliferation and inhibited the volume-regulated chloride channel. The authors went on to examine the expression of the CIC-3 gene in vascular smooth muscles and many other cell types in the basilar arteries; they found that the expression of CIC-3 was increased during hypertension, and simvastatin treatment reduced the upregulation of CIC-3 expression. Finally, the authors used a gain-of-function approach to examine whether CIC-3 overexpression would antagonize the inhibitory effect of simvastatin on cell proliferation. Indeed, they found that increased CIC-3 activity diminished the inhibitory effect of simvastatin on cell proliferation. A positive correlation between cell proliferation and activation of the CIC-3 channels was revealed.

Therefore, this study provided novel and convincing experimental evidence that simvastatin improves cerebrovascular remodeling in 2-kidney, 2-clip hypertensive rats through inhibition of the vascular SMC proliferation by suppression of volume-regulated CIC-3 channels. These results provided novel mechanistic insight into the beneficial effects of statins in the treatment of hypertension and stroke.

In addition to its important role in cell volume regulation, CIC-3 may also regulate the redox signaling pathway through interaction with NADPH oxidase (Nox) and/or transport of superoxide to improve myocyte viability against oxidative damage. It has been reported that activation of CIC-3 may improve the resistance of vascular SMCs to reactive oxygen species (ROS) in an environment of elevated inflammatory cytokines in hypertensive pulmonary arteries (please see recent reviews by Hume et al). ROS has been implicated in cellular signaling processes, as well as a cause of oxidative stress-induced cell proliferation. One of the major sources of ROS in the vasculature is through one or more isoforms of the phagocytic enzyme Nox, a membrane-localized protein that generates the superoxide anion on the extracellular surface of the plasma membrane (Figure).

As a charged and short-lived anion, it is believed that superoxide flux is insufficient to initiate intracellular signaling because of the combination of poor permeability through the phospholipid bilayer and a rapid dismutation to its uncharged and more stable derivative, hydrogen peroxide. Recent studies have also shown that CIC-3 may also function as an antiapoptotic mechanism through regulation of cell volume and intracellular pH and as a regulator of other transport functions involved in the etiology of hypertension (Figure).

Whether the beneficial effects of statins could be attributed also to their effects on these cellular functions of CIC-3 in cerebrovascular SMCs during hypertension is still an unanswered question. Nevertheless, regulation of CIC-3 functions...
in the cardiovascular system is emerging as a novel and important mechanism for the structural remodeling of the vasculature and may provide a novel therapeutic approach for the treatment of many vascular diseases, such as hypertension and stroke.

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None.

**References**


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