Acta Pharmacologica Sinica (2013) 34: 17–23
 1671-4083/13 $32.00
www.nature.com/aps

Review

Chloride channels in stroke

Ya-ping ZHANG[1, 3], Hao ZHANG[2, *], Dayue Darrel DUAN[3, *]

[1]The Department of Cardiology, the Third Xiang-Ya Hospital of Central South University, Changsha 450013, China; [2] The Surgery Department, Huashan Hospital, Fudan University, Shanghai 200040, China; [3]The Laboratory of Cardiovascular Phenomics, the Department of Pharmacology, University of Nevada School of Medicine, Reno, Nevada 89557, USA

Vascular remodeling of cerebral arterioles, including proliferation, migration, and apoptosis of vascular smooth muscle cells (VSMCs), is the major cause of changes in the cross-sectional area and diameter of the arteries and sudden interruption of blood flow or hemorrhage in the brain, ie, stroke. Accumulating evidence strongly supports an important role for chloride (Cl^-) channels in vascular remodeling and stroke. At least three Cl^- channel genes are expressed in VSMCs: 1) the TMEM16A (or Ano1), which may encode the calcium-activated Cl^- channels (CACCs); 2) the *CLC-3* Cl^- channel and Cl^-/H^+ antiporter, which is closely related to the volume-regulated Cl^- channels (VRCCs); and 3) the cystic fibrosis transmembrane conductance regulator (CFTR), which encodes the PKA- and PKC-activated Cl^- channels. Activation of the CACCs by agonist-induced increase in intracellular Ca^{2+} causes membrane depolarization, vasoconstriction, and inhibition of VSMC proliferation. Activation of VRCCs by cell volume increase or membrane stretch promotes the production of reactive oxygen species, induces proliferation and inhibits apoptosis of VSMCs. Activation of CFTR inhibits oxidative stress and may prevent the development of hypertension. In addition, Cl^- current mediated by gamma-aminobutyric acid (GABA) receptor has also been implicated a role in ischemic neuron death. This review focuses on the functional roles of Cl^- channels in the development of stroke and provides a perspective on the future directions for research and the potential to develop Cl^- channels as new targets for the prevention and treatment of stroke.

Keywords: chloride channel; stroke; hypertension; vascular remodeling; oxidative stress

Acta Pharmacologica Sinica (2013) 34: 17–23; doi: 10.1038/aps.2012.140; published online 29 Oct 2012

Introduction

Stroke, or cerebrovascular accident, is the second leading cause of death and a foremost cause of serious, long-term disability in the world. It represents a major economic burden with considerable public health impact. A stroke is defined as a rapid loss of brain function(s) due to neuron death and tissue infarction in brain caused by blockage or rupture of blood vessel[1]. Depending on the extent and location of damage in brain, stroke could differently affect physical and mental functions of human being[2, 3]. There are two types of stroke: ischemic stroke and hemorrhagic stroke. The former is more prevalent than the latter, comprising approximately 87% of all stroke cases. The risk factors of stroke mainly include old age, hypertension, atrial fibrillation, dyslipidemia, diabetes, atherosclerosis, carotid stenosis, and previous stroke[4]. Hypertension is the most important risk factor of stroke. A sudden increase in blood pressure can make vessel rupture and result in hemorrhagic stroke[5]. The pathophysiological process of stroke refers to cellular and vascular mechanisms. Vascular remodeling of cerebral arterioles, including proliferation, migration, and apoptosis of vascular smooth muscle cells (VSMCs), is the major cause of changes in the cross-sectional area and diameter of the arteries and sudden interruption of blood flow or hemorrhage in the brain[6–8]. Oxidative stress due to excessive production of reactive oxygen species (ROS) promotes VSMC proliferation, migration and apoptosis, matrix remodeling, and neointimal hyperplasia in vascular structure[9]. Meanwhile, oxidative stress results in endothelial dysfunction which is an important mechanism of cerebrovascular damage, or stroke[10, 11].

Recent studies have accumulated compelling evidence that Cl^- channels are closely associated with some risk factors of stroke and may play important roles in the pathophysiological mechanism of vascular remodeling. At least three Cl^- channel genes are expressed in VSMCs: 1) the TMEM16A (anoctamin 1 or Ano1), which may encode the calcium-activated Cl^- channels (CACCs)[12–14]; 2) the *CLC-3* Cl^- channel and Cl^-/H^+ antiporter, which is closely related to the volume-regulated Cl^- channels (VRCCs)[15–21]; and 3) the cystic fibrosis trans-

* To whom correspondence should be addressed.
E-mail dduan@medicine.nevada.edu (Dayue Darrel DUAN);
drhao@hotmail.com (Hao ZHANG)
Received 2012-08-19 Accepted 2012-09-06

membrane conductance regulator (CFTR), which encodes the protein kinase A (PKA)- and protein kinase C (PKC)-activated Cl^- channels[22–24]. These Cl^- channels are involved in the regulation of many cellular functions of the VSMCs, including the membrane potentials, vascular tone, cell proliferation, migration, and apoptosis (Figure 1)[17, 18, 23, 25, 26]. They are linked to hypertension in many ways. For example, some new studies showed that in hypertensive animal models the expression of VRCCs was increased in VSMCs and activation of VRCCs promoted proliferation and inhibited apoptosis of VSMCs[17, 27], whereas the expression of CACCs was decreased in VSMCs and activation of CACCs was a negative regulator for proliferation of VSMCs[14]. Atherosclerosis, a chronic inflammatory disease[28], also resulted in cerebrovascular remodeling in which proliferation of VSMCs was fundamental[29, 30]. The latest study reported VRCC attended atherosclerotic plaque

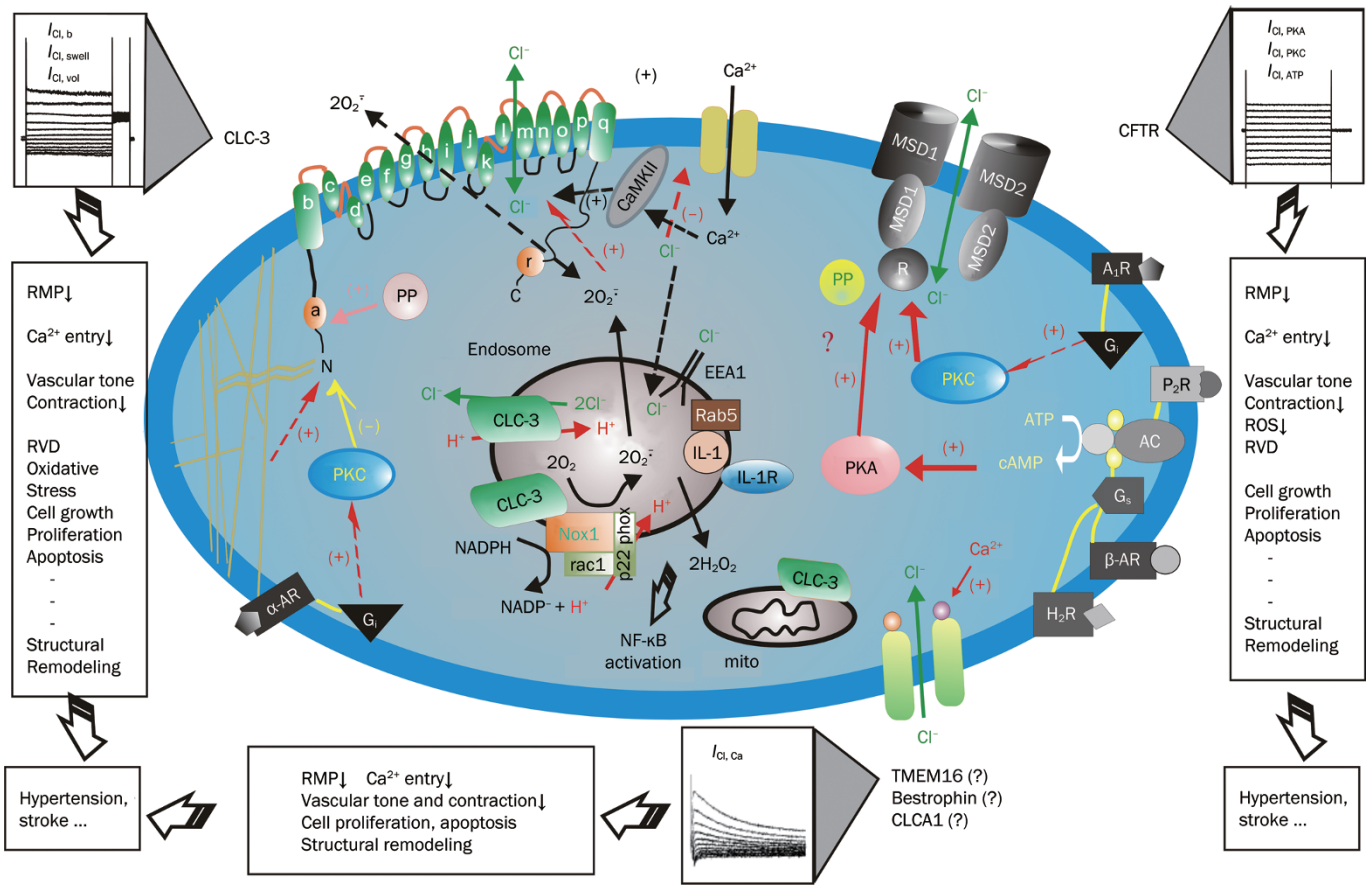

Figure 1. Cl^- channels and proposed functions in vascular smooth muscle cells. Cl^- channels and their corresponding molecular candidate genes and cellular functions are indicated. *CFTR*, cystic fibrosis transmembrane conductance regulator, encodes Cl^- channels activated by stimulation of cAMP-protein kinase A (PKA) pathway ($I_{Cl, PKA}$), protein kinase C (PKC) ($I_{Cl, PKC}$), or extracellular ATP through purinergic receptors ($I_{Cl, ATP}$). *CFTR* is composed by two membrane spanning domains (MSD1 and MSD2), two nucleotide binding domains (NBD1 and NBD2) and a regulatory subunit (R). Gi, heterodimeric inhibitory G protein; A_1R, adenosine type 1 receptor; AC, adenylyl cyclase; H_2R, histamine type II receptor; Gs, heterodimeric stimulatory G protein; α-AR, α-adrenergic receptor; β-AR, β-adrenergic receptor; P_2R, purinergic type 2 receptor; P, phosphorylation sites for PKA and PKC; PP, serine-threonine protein phosphatases. $I_{Cl, Ca}$ is a Cl^- current activated by increased intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$); Molecular candidates for $I_{Cl, Ca}$ include TMEM16A (transmembrane protein 16A), the Bestrophin gene family and CLCA1, a member of a Ca^{2+}-sensitive Cl^- channel family (CLCA). *CLC-3*, a member of voltage-gated CLC Cl^- channel family, encodes Cl^- channels that are volume-regulated ($I_{Cl, vol}$) and can be activated by cell swelling ($I_{Cl, swell}$) induced by exposure to hypotonic extracellular solutions or possibly membrane stretch. $I_{Cl, b}$ is a basally-activated *CLC-3* Cl^- current. Membrane topology model (α-helices a-r) for *CLC-3* is modified from Dutzler *et al*[85] *CLC-3* proteins are expressed on both sarcolemmal membrane and intracellular organelles including mitochondria (mito) and endosomes. The proposed model of endosome ion flux and function of Nox1 and *CLC-3* in the signaling endosome is adapted from Miller Jr *et al*[86]. Binding of IL-1β or TNF-α to the cell membrane initiates endocytosis and formation of an early endosome (EEA1 and Rab5), which also contains NADPH oxidase subunits Nox1 and p22phox, in addition to *CLC-3*. Nox1 is electrogenic, moving electrons from intracellular NADPH through a redox chain within the enzyme into the endosome to reduce oxygen to superoxide. *CLC-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 NF-κB activation. Both *CLC-3* and Nox1 are necessary for generation of endosomal ROS and subsequent NF-κB activation by IL-1β or TNF-α in VSMCs. Statins block *CLC-3* channels, which causes hyperpolarization of the cell membrane, closure of Ca^{2+} channels and vasorelaxation, and inhibition of cell proliferation. Nox: NADPH oxidase.

formation. VRCC may also play important roles in ischemic neuron apoptosis. In addition, gamma-aminobutyric acid (GABA) receptor-mediated Cl^- current has also been implicated a role in ischemic neuron death. This review focuses on the functional role of Cl^- channels in stroke and provides a perspective on the future directions for research and the potential to develop Cl^- channels as novel therapeutic targets for the prevention and treatment of stroke.

Calcium-activated Cl^- channels (CACCs) and stroke

The Cl^- current evoked by a rise in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and free Ca^{2+}, or Ca^{2+}-activated Cl^- current ($I_{Cl, Ca}$), was first described in *Xenopus* oocytes in 1982[31, 32]. Later studies found that similar $I_{Cl, Ca}$ is present widely in various cell types, such as endothelial cells and VSMCs of different organs, of many species, including human[33–35]. In the last two decades CACCs in VSMCs have received extensive attention due to the important roles of CACCs in multiple cellular functions of VSMCs, ranging from the regulation of the resting membrane potential (RMP), intracellular Ca^{2+} ($[Ca^{2+}]_i$), and vascular tone to the control of cell proliferation and apoptosis[25, 35–37]. But, the molecular identity of CACCs in VSMCs has not been fully defined[38, 39]. Several genes, including CLCA, Tweety, and bestrophin, have been proposed to encode the pore forming channel protein of CACCs[40–43]. Using siRNA technique, however, Wang *et al* excluded the contribution of bestrophin 3 to $I_{Cl, Ca}$ in rat basal artery smooth muscle cells (BASMCs)[14]. Recent independent studies from three laboratories have provided compelling evidence that the TMEM16/Anoctamin proteins, a family of transmembrane proteins, may be the pore forming channel protein of CACCs[44–46]. Heterogenous expression of TMEM16A/Ano1 or TMEM16B/Ano2 produced a Cl^- current with characteristics, including Ca^{2+} and voltage-dependence, outward rectification and ion selectivity, similar to the endogenous $I_{Cl, Ca}$ in many types of native cells[12, 44–49]. Thomas-Gatewood *et al* have found that TMEM16A channel proteins are expressed and inserted into the plasma membrane of rat cerebral arterial smooth muscle cells (CASMCs)[13]. Whole-cell $I_{Cl, Ca}$ in the CASMCs displayed properties similar to those generated by rTMEM16A channels, including the Ca^{2+}-dependent activation, current-voltage (I–V) relationship linearization by an elevation in $[Ca^{2+}]_i$, and an $I^->Cl^-$ permeability sequence. A pore-targeting TMEM16A antibody that effectively blocked rTMEM16A currents also inhibited $I_{Cl, Ca}$ in the cerebral arterial SMCs. TMEM16A knockdown using small interfering RNA (siRNA) also inhibited arterial $I_{Cl, Ca}$ in the CASMCs. These data strongly support the notion that $I_{Cl, Ca}$ in the CASMCs is encoded by TMEM16A. More recently, Wang *et al* have found that TMEM16A, TMEM16C, TMEM16E-F, and TMEM16K are expressed at high levels endogenously in rat BASMCs, while TMEM16B and TMEM16D were not detected in these cells. Knockdown of TMEM16A with siRNA remarkably attenuated endogenous $I_{Cl, Ca}$ in BASMCs, suggesting that TMEM16A encodes the CACCs in BASMCs[14].

Several studies have provided strong evidence for a key role of CACCs in the stroke-related vascular remodeling. In BASMCs isolated from 2k2c hypertensive rats, the expression of TMEM16A and functional $I_{Cl, Ca}$ was significantly reduced. The activity of $I_{Cl, Ca}$ negatively correlated with the blood pressure and the medial cross sectional area of basilar artery during the development of hypertension[14]. $I_{Cl, Ca}$ began to drop within 1 week after 2k2c operation and the expression of TMEM16A was reduced to a significant level at week 4 after surgery. It is currently not clear why down-regulation of TMEM16A expression lagged behind the decreased activity of CACCs. Wang *et al* found that increased activity of CaMK II inhibited $I_{Cl, Ca}$ and CaMK II activity was up-regulated at week 1 after 2k2c surgery. Therefore, the increased CaMK II activity in the 2K2c rats may be one of the major molecular mechanisms for the down-regulation of functional CACCs in the hypertensive rat[14]. Wang *et al* further demonstrated CACC is a negative regulator of cell proliferation of BASMCs. TMEM16A-mediated CACCs inhibited cell proliferation by arresting the cell cycle at G_0/G_1 phase through reduction of cyclin D1 and cyclin E expression. Therefore, down-regulation of CACCs in VSMCs may play an important role in hypertension-induced structural remodeling of cerebral arterials[14]. It has been demonstrated that CACCs may be a critical regulator of cell proliferation in Ehrlich lettre ascite cells[50]. The important role of CaCCs and TMEM16A in the regulation of proliferation suggests that CACCs may be new molecular targets for the prevention and treatment of hypertension-induced vascular remodeling and stroke.

Volume-regulated Cl^- channels (VRCCs) and stroke

VRCCs are ubiquitously distributed in mammalian cells including neurons, VSMCs, and endothelial cells. VRCCs are involved in many pathophysiological functions such as cell volume regulation, proliferation, differentiation and apoptosis. Although recent studies on the molecular identity of VRCCs are inconsistent, *CLC-3*, a member of voltage-gated *CLC* Cl^- channel family, is thought to be responsible for VRCCs and mediate volume regulation in many cell types[16, 51–53]. A study in A10 VSMCs strongly supported that *CLC-3* was the molecular component responsible for the activation and regulation of VRCCs[54]. Although CLC-2 channels, another member of the *CLC* family, are also volume-regulated Cl^- channels and involved in cell volume regulation[55] they differ significantly from native VRCCs in voltage sensitivity, anion selectivity, and pharmacology[56].

Several recent studies suggest that VRCCs and *CLC-3* are closely associated with blood pressure regulation and may play an important role in hypertension-induced cerebrovascular remodeling[15, 20, 21, 27, 57]. Shi *et al* found that functional VRCCs and *CLC-3* expression were increased in hypertensive rat BASMCs and the increment of *CLC-3* expression was correlated with the severity of hypertension, suggesting that VRCCs and *CLC-3* were involved in vascular remodeling during chronic hypertension[27]. Later study from Qian *et al* found that static pressure increased VRCCs and *CLC-3* expression in rat aortic SMCs, suggesting that elevation in blood pressure may directly up-regulate the expression of *CLC-3* in

BASMCs[58]. Inhibition of VRCCs with pharmacological blockers or knockdown of *CLC-3* with *CLC-3* antisense oligonucleotide dramatically inhibited the static pressure-induced cell proliferation and cell cycle progression of rat aortic SMCs[58]. VRCC currents in actively growing VSMCs were higher than that in growth-arrested or differentiated SMCs, suggesting that VRCCs may be important for SMC proliferation[18]. Silencing endogenous *CLC-3* by antisense oligonucleotide significantly inhibited endothelin-1 (ET-1) induced cell proliferation in BASMCs, which is consistent with previous studies in rat aortic SMCs[20, 59]. *CLC-3* contributed to phosphorylation of the Akt and glycogen synthase kinase-3β (GSK-3β), resulting in up-regulation of cyclin D1 and cyclin E which pushed forward cell cycle from G1 to S phase, whereas silencing *CLC-3* protein effectively up-regulated cyclin-dependent kinase inhibitors (CDKIs) $p27^{KIP}$ and $p21^{CIP}$ and inhibited cell cycle[20]. The beneficial effects of statins on cerebrovascular remodeling during hypertension may be due to the inhibition of *CLC-3* via modifying Rho activity and prevent the incidence of ischemic stroke[57]. Chu *et al* found that *CLC-3* was necessary for the activation of SMCs by TNF-α and neointimal hyperplasia[15]. TNF-α induced a NADPH-dependent generation of intracellular reactive ROS and *CLC-3* provided charge neutralization for the NADPH oxidase electron current[60–63]. ERK1/2 activation is linked to ROS production and ERK1/2 is upstream of MMP-9 activation and proliferation[64]. ROS activation of the transcription factor NF-κB is widely recognized as a key regulatory step in vascular inflammation[65]. Therefore, *CLC-3* is very critical for TNF-α induced cell proliferation and inflammation response. These findings identify *CLC-3* as a novel target for the prevention of inflammatory and proliferative vascular diseases referred in stroke. Generally speaking, cell proliferative regulation is a complex network. When ERK1/2 and MMP-9 induce proliferation of VSMCs, they are likely to regulate cyclin D1 and cyclin E. To date, the molecular mechanisms for *CLC-3* regulation of VSMC proliferation is not completely clear and further studies are needed.

The process of cerebrovascular remodeling during hypertension prior to stroke involves not only the increased proliferation but also the decreased apoptosis of VSMCs. In fact, the balance between proliferation and apoptosis is broken. A study showed that overexpression of *CLC-3* significantly decreased the apoptotic rate of H_2O_2-treated BASMCs and increased the cell viability, whereas silencing of *CLC-3* produced opposite effects and increased the apoptotic rate[19]. *CLC-3* overexpression decreased cytochrome *c* release and caspase-3 activation, and increased both the stability of mitochondrial membrane potential and the ratio of Bcl-2/Bax[19]. It also inhibited the degradation of cell skeleton protein Lamin induced by H_2O_2. This report is consistent with previous study in PC12 cells which also demonstrated *CLC-3* mediated apoptotic process induced by thapsigargin through inhibitory mechanism[66]. But another study in human prostate cancer epithelial cells reported opposite link between *CLC-3* and Bcl-2 which demonstrated Bcl-2 up-regulated *CLC-3* expression and increased $I_{Cl,\ vol}$ to inhibit apoptosis[67]. Moreover, endothelial progenitor cell and endothelial cell dysfunction are also important for hypertension development. *CLC-3* could mediate extracellular $O_2^{\cdot-}$ to enter endothelial cell to stimulate the production of ROS which damages endothelial cell to increase endothelin-1 release and decrease NO release contributing to hypertension development[68]. Until now very little is known about the roles of VRCCs and *CLC-3* in endothelial progenitor cells[69].

Atherosclerosis is closely associated with vascular remodeling. Atherosclerosis involves the phenotypic changes of several types of cells, especially monocyte-derived macrophages and VSMCs, and the formation of foam cells[70]. Atherosclerotic plaque rupture and cholesterol release can block vessels in brain and cause stroke. One study suggested that volume-regulated Cl^- movement was augmented during macrophage-derived foam cell formation, and its increment positively correlated with atherosclerotic plaque area in the development of atherosclerosis[71]. Cell swelling occurred in macrophages due to uptake of modified LDL during the process of foam cell formation and the alteration of Cl^- transmembrane movement via VRCCs was involved in the development of atherosclerosis. In contrast, inhibition of VRCCs with Cl^- channel blockers prevented ox-LDL induced foam cell formation. But current pharmacological blockers lack specificity for a particular Cl^- channel and gene targeting study may give a clearer answer about the contribution of VRCCs to atherosclerosis. Furthermore, SMC-derived foam cell formation in atherosclerosis also refers to an increase in cell volume and VRCC activation. So the role of VRCCs and *CLC-3* in SMC-derived foam cell formation warrants further studies in the future.

There is now some evidence that some neurons die as a result of apoptosis after cerebral ischemia. Apoptotic cell volume decrease has also been associated with regulatory volume decrease (RVD) since the decrease of cell volume after apoptotic stimuli is due to activation of the same K^+ and Cl^- channels that are responsible for physiological RVD[72–74]. VRCCs play a predominant role in neuronal apoptosis[72, 75]. Cl^- channel blockers NPPB, SITS and DIDS completely inhibited cell shrinkage, RVD, caspase-3 activation and DNA laddering in neurons, suggesting Cl^- channel blockers had a preventive effect on neuron apoptosis[75, 76]. These blockers abolished structural alterations that occur during cell apoptosis, such as chromatin condensation and leaky nuclear envelopes. But in another study these blockers just mildly attenuated cell death induced by staurosporine, C_2-ceramide, or serum deprivation and had no significant effects on caspase-3 activation and DNA fragmentation at concentration that prevented cell shrinkage[77]. It should be pointed out that, however, all these blockers of Cl^- channels are not specific. They might produce their effects by blocking other ion channels or regulating other signaling pathways.

Nevertheless, regulation function of VRCCs 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 treat-

ment of many vascular diseases, such as hypertension, atherosclerosis and stroke. *CLC-3* may be potential new targets for the prevention of the cerebrovascular remodeling that occurs during the development of hypertension.

CFTR and stroke

CFTR Cl^- channels are expressed in VSMCs and may play an important role in the regulation of vascular tone. Individuals homozygous for the autosomal recessive disorder cystic fibrosis (CF) are known to have low blood pressure[78]. Older female CF carriers had lower systolic and diastolic pressures than matched healthy subjects, with a tendency for blood pressure to increase less with age[79]. A beneficial effect of CF gene mutation may be protection against developing hypertension and significantly reduce stroke and heart disease in the female CF patients[79]. The effect of activation of *CFTR* channels on blood pressure is insufficient to prevent hypertension, though it remains conceivable that the severity might be ameliorated in carriers. How *CFTR* is linked to hypertension and stroke *in vivo* is currently not known. It was proposed that this might be a result of life-long increased sweat Na^+ and Cl^- loss[78, 80]. Activation of *CFTR* by β-adrenergic agonists and vasoactive intestinal peptide led to vasorelaxation *in vitro*[22]. But, only when SMCs were in a depolarizing high potassium solution could *CFTR* be activated to relax SMCs. With SMCs bathed in normal potassium solution, no evidence for active *CFTR* channel activity was observed[22]. Robert *et al* reported that arteries with or without endothelium from $CFTR^{-/-}$ mice became significantly more constricted than that from $CFTR^{+/+}$ mice in response to vasoactive agents and *CFTR* activation contributed to endothelium-independent vasorelaxation[23, 24]. Recent studies indicate a potential role of *CFTR* in the high fructose-salt-induced hypertension[79, 81, 82].

Cl^- current mediated by GABA receptor and stroke

Stroke therapy has focused on reducing risk factors and minimizing secondary brain damage by restoring and maintaining perfusion. However, a third approach, neuroprotection, is now being investigated. GABA is the primary inhibitory neurotransmitter in mammalian brain and increasing GABA function via activation of the $GABA_A$ receptor resulted in increased Cl^- influx and promoted hyperpolarization of neuron membrane. So, during acute ischemic damage in brain increased Cl^- current mediated by GABA receptor probably plays a beneficial role in stroke. There is good evidence that GABA exerted an inhibitory tone on glutamate-mediated neuronal activity[83]. Meldrum proposed that the excitotoxic process of neuron probably depends on a balance between excitatory and inhibitory mechanisms[84]. Agonists of GABA receptors are likely to be new treatment approach for stroke.

Perspectives

Cl^- channels are emerging as novel and important mechanisms for stroke and may become novel therapeutic targets for the treatment and prevention of stroke although at present many questions about these channels in stroke are still not clear. Future studies will focus on the clear identification of functional role of each type of Cl^- channels (*CFTR*, *CLC-3*, and TMEM16A, *etc*) in VSMCs, endothelial cells, and endothelial progenitor cells, in cerebrovascular remodeling and VSMC-derived foam cell formation, and neuron apoptosis and necrosis. The findings from these studies may generate new directions for the development of new therapeutic strategies for the treatment of stroke and may reduce the impact of this enormous economic and social burden.

Acknowledgements

Dayue Darrel DUAN is supported by the NIH grant #HL106256 and AHA Western State Affiliate Grant-in-Aid #11GRNT7610161. Ya-ping ZHANG is supported by the National High Technology Research and Development Program of China #2009AA022703 and National Science and Technology Major Projects for "Major New Drugs Innovation and Development" #2009ZX09501-032.

References

1 Albers GW, Clark WM, Madden KP, Hamilton SA. ATLANTIS trial: results for patients treated within 3 hours of stroke onset. Alteplase thrombolysis for acute noninterventional therapy in ischemic stroke. Stroke 2002; 33: 493–5.
2 Sturm JW, Dewey HM, Donnan GA, Macdonell RA, McNeil JJ, Thrift AG. Handicap after stroke: how does it relate to disability, perception of recovery, and stroke subtype?: the north North East Melbourne Stroke Incidence Study (NEMESIS). Stroke 2002; 33: 762–8.
3 Clarke P, Marshall V, Black SE, Colantonio A. Well-being after stroke in Canadian seniors: findings from the Canadian Study of Health and Aging. Stroke 2002; 33: 1016–21.
4 Michael KM, Shaughnessy M. Stroke prevention and management in older adults. J Cardiovasc Nurs 2006; 21: S21–S26.
5 Tanahashi N. Hypertension associated with stroke. Nihon Rinsho 2011; 69: 2001–6.
6 Pasterkamp G, Galis ZS, de Kleijn DP. Expansive arterial remodeling: location, location, location. Arterioscler Thromb Vasc Biol 2004; 24: 650–7.
7 Heistad DD, Baumbach GL. Cerebral vascular changes during chronic hypertension: good guys and bad guys. J Hypertens Suppl 1992; 10: S71–S75.
8 Johansson BB. Hypertension mechanisms causing stroke. Clin Exp Pharmacol Physiol 1999; 26: 563–5.
9 Sierra C, Coca A, Schiffrin EL. Vascular mechanisms in the pathogenesis of stroke. Curr Hypertens Rep 2011; 13: 200–7.
10 Ungvari Z, Kaley G, de CR, Sonntag WE, Csiszar A. Mechanisms of vascular aging: new perspectives. J Gerontol A Biol Sci Med Sci 2010; 65: 1028–41.
11 Roquer J, Segura T, Serena J, Castillo J. Endothelial dysfunction, vascular disease and stroke: the ARTICO study. Cerebrovasc Dis 2009; 27 Suppl 1: 25–37.
12 Davis AJ, Forrest AS, Jepps TA, Valencik ML, Wiwchar M, Singer CA, *et al*. Expression profile and protein translation of TMEM16A in murine smooth muscle. Am J Physiol Cell Physiol 2010; 299: C948–C959.
13 Thomas-Gatewood C, Neeb ZP, Bulley S, Adebiyi A, Bannister JP, Leo MD, *et al*. TMEM16A channels generate Ca(2)-activated Cl currents in cerebral artery smooth muscle cells. Am J Physiol Heart Circ Physiol 2011; 301: H1819–H1827.
14 Wang M, Yang H, Zheng LY, Zhang Z, Tang YB, Wang GL, *et al*.

Downregulation of TMEM16A calcium-activated chloride channel contributes to cerebrovascular remodeling during hypertension by promoting basilar smooth muscle cell proliferation. Circulation 2012; 125: 697–707.

15 Chu X, Filali M, Stanic B, Takapoo M, Sheehan A, Bhalla R, *et al*. A critical role for chloride channel-3 (ClC-3) in smooth muscle cell activation and neointima formation. Arterioscler Thromb Vasc Biol 2011; 31: 345–51.

16 Duan D, Zhong J, Hermoso M, Satterwhite CM, Rossow CF, Hatton WJ, *et al*. Functional inhibition of native volume-sensitive outwardly rectifying anion channels in muscle cells and *Xenopus* oocytes by anti-ClC-3 antibody. J Physiol 2001; 531: 437–44.

17 Guan YY, Wang GL, Zhou JG. The ClC-3 Cl^- channel in cell volume regulation, proliferation and apoptosis in vascular smooth muscle cells. Trends Pharmacol Sci 2006; 27: 290–6.

18 Hume JR, Wang GX, Yamazaki J, Ng LC, Duan D. CLC-3 chloride channels in the pulmonary vasculature. Adv Exp Med Biol 2010; 661: 237–47.

19 Qian Y, Du YH, Tang YB, Lv XF, Liu J, Zhou JG, *et al*. ClC-3 chloride channel prevents apoptosis induced by hydrogen peroxide in basilar artery smooth muscle cells through mitochondria dependent pathway. Apoptosis 2011; 16: 468–77.

20 Tang YB, Liu YJ, Zhou JG, Wang GL, Qiu QY, Guan YY. Silence of ClC-3 chloride channel inhibits cell proliferation and the cell cycle via G/S phase arrest in rat basilar arterial smooth muscle cells. Cell Prolif 2008; 41: 775–85.

21 Tang YB, Zhou JG, Guan YY. Volume-regulated chloride channels and cerebral vascular remodelling. Clin Exp Pharmacol Physiol 2010; 37: 238–42.

22 Robert R, Thoreau V, Norez C, Cantereau A, Kitzis A, Mettey Y, *et al*. Regulation of the cystic fibrosis transmembrane conductance regulator channel by beta-adrenergic agonists and vasoactive intestinal peptide in rat smooth muscle cells and its role in vaso-relaxation. J Biol Chem 2004; 279: 21160–8.

23 Robert R, Norez C, Becq F. Disruption of CFTR chloride channel alters mechanical properties and cAMP-dependent Cl^- transport of mouse aortic smooth muscle cells. J Physiol 2005; 568: 483–95.

24 Robert R, Savineau JP, Norez C, Becq F, Guibert C. Expression and function of cystic fibrosis transmembrane conductance regulator in rat intrapulmonary arteries. Eur Respir J 2007; 30: 857–64.

25 Nelson MT, Conway MA, Knot HJ, Brayden JE. Chloride channel blockers inhibit myogenic tone in rat cerebral arteries. J Physiol 1997; 502: 259–64.

26 Duan DD. The ClC–3 chloride channels in cardiovascular disease. Acta Pharmacol Sin 2011; 32: 675–84.

27 Shi XL, Wang GL, Zhang Z, Liu YJ, Chen JH, Zhou JG, *et al*. Alteration of volume-regulated chloride movement in rat cerebrovascular smooth muscle cells during hypertension. Hypertension 2007; 49: 1371–7.

28 Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999; 138: S419–S420.

29 Mandegar M, Fung YC, Huang W, Remillard CV, Rubin LJ, Yuan JX. Cellular and molecular mechanisms of pulmonary vascular remodeling: role in the development of pulmonary hypertension. Microvasc Res 2004; 68: 75–103.

30 Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. Hypertension 2001; 38: 581–7.

31 Miledi R. A calcium-dependent transient outward current in *Xenopus* laevis oocytes. Proc R Soc Lond B Biol Sci 1982; 215: 491–7.

32 Barish ME. A transient calcium-dependent chloride current in the immature *Xenopus* oocyte. J Physiol 1983; 342: 309–25.

33 Large WA, Wang Q. Characteristics and physiological role of the Ca^{2+}-activated Cl^- conductance in smooth muscle. Am J Physiol 1996; 271: C435–C454.

34 Nilius B, Prenen J, Szucs G, Wei L, Tanzi F, Voets T, *et al*. Calcium-activated chloride channels in bovine pulmonary artery endothelial cells. J Physiol 1997; 498: 381–96.

35 Hartzell C, Putzier I, Arreola J. Calcium-activated chloride channels. Annu Rev Physiol 2005; 67: 719–58.

36 Large WA, Wang Q. Characteristics and physiological role of the Ca^{2+}-activated Cl^- conductance in smooth muscle. Am J Physiol 1996; 271: C435–C454.

37 Nilius B, Droogmans G. Amazing chloride channels: an overview. Acta Physiol Scand 2003; 177: 119–47.

38 Eggermont J. Calcium-activated chloride channels: (un)known, (un) loved? Proc Am Thorac Soc 2004; 1: 22–7.

39 Duran C, Thompson CH, Xiao Q, Hartzell HC. Chloride channels: often enigmatic, rarely predictable. Annu Rev Physiol 2010; 72: 95–121.

40 Sun H, Tsunenari T, Yau KW, Nathans J. The vitelliform macular dystrophy protein defines a new family of chloride channels. Proc Natl Acad Sci U S A 2002; 99: 4008–13.

41 Barro SR, Spitzner M, Schreiber R, Kunzelmann K. Bestrophin-1 enables Ca^{2+}-activated Cl^- conductance in epithelia. J Biol Chem 2009; 284: 29405–12.

42 Qu Z, Wei RW, Mann W, Hartzell HC. Two bestrophins cloned from Xenopus laevis oocytes express Ca^{2+}-activated Cl^- currents. J Biol Chem 2003; 278: 49563–72.

43 Matchkov VV. Mechanisms of cellular synchronization in the vascular wall. Mechanisms of vasomotion. Dan Med Bull 2010; 57: B4191

44 Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, *et al*. TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity. Science 2008; 322: 590–4.

45 Schroeder BC, Cheng T, Jan YN, Jan LY. Expression cloning of TMEM16A as a calcium-activated chloride channel subunit. Cell 2008; 134: 1019–29.

46 Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim WS, *et al*. TMEM16A confers receptor-activated calcium-dependent chloride conductance. Nature 2008; 455: 1210–5.

47 Stephan AB, Shum EY, Hirsh S, Cygnar KD, Reisert J, Zhao H. ANO2 is the cilial calcium-activated chloride channel that may mediate olfactory amplification. Proc Natl Acad Sci U S A 2009; 106: 11776–81.

48 Stohr H, Heisig JB, Benz PM, Schoberl S, Milenkovic VM, Strauss O, *et al*. TMEM16B, a novel protein with calcium-dependent chloride channel activity, associates with a presynaptic protein complex in photoreceptor terminals. J Neurosci 2009; 29: 6809–18.

49 Manoury B, Tamuleviciute A, Tammaro P. TMEM16A/anoctamin 1 protein mediates calcium-activated chloride currents in pulmonary arterial smooth muscle cells. J Physiol 2010; 588: 2305–14.

50 Klausen TK, Bergdahl A, Hougaard C, Christophersen P, Pedersen SF, Hoffmann EK. Cell cycle-dependent activity of the volume- and Ca^{2+}-activated anion currents in Ehrlich lettre ascites cells. J Cell Physiol 2007; 210: 831–42.

51 Duan D, Winter C, Cowley S, Hume JR, Horowitz B. Molecular identification of a volume-regulated chloride channel. Nature 1997; 390: 417–21.

52 Duan D, Cowley S, Horowitz B, Hume JR. A serine residue in ClC-3 links phosphorylation-dephosphorylation to chloride channel regulation by cell volume. J Gen Physiol 1999; 113: 57–70.

53 Hermoso M, Satterwhite CM, Andrade YN, Hidalgo J, Wilson SM, Horowitz B, *et al*. ClC-3 is a fundamental molecular component of volume-sensitive outwardly rectifying Cl^- channels and volume

regulation in HeLa cells and Xenopus laevis oocytes. J Biol Chem 2002; 277: 40066–74.
54 Zhou JG, Ren JL, Qiu QY, He H, Guan YY. Regulation of intracellular Cl^- concentration through volume-regulated ClC-3 chloride channels in A10 vascular smooth muscle cells. J Biol Chem 2005; 280: 7301–8.
55 Roman RM, Smith RL, Feranchak AP, Clayton GH, Doctor RB, Fitz JG. ClC-2 chloride channels contribute to HTC cell volume homeostasis. Am J Physiol Gastrointest Liver Physiol 2001; 280: G344–G353.
56 Jentsch TJ, Gunther W. Chloride channels: an emerging molecular picture. Bioessays 1997; 19: 117–26.
57 Liu YJ, Wang XG, Tang YB, Chen JH, Lv XF, Zhou JG, *et al*. Simvastatin ameliorates rat cerebrovascular remodeling during hypertension via inhibition of volume-regulated chloride channel. Hypertension 2010; 56: 445–52.
58 Qian JS, Pang RP, Zhu KS, Liu DY, Li ZR, Deng CY, *et al*. Static pressure promotes rat aortic smooth muscle cell proliferation via upregulation of volume-regulated chloride channel. Cell Physiol Biochem 2009; 24: 461–70.
59 Wang GL, Wang XR, Lin MJ, He H, Lan XJ, Guan YY. Deficiency in ClC-3 chloride channels prevents rat aortic smooth muscle cell proliferation. Circ Res 2002; 91: E28–E32.
60 Miller FJ Jr, Filali M, Huss GJ, Stanic B, Chamseddine A, Barna TJ, *et al*. Cytokine activation of nuclear factor kappa B in vascular smooth muscle cells requires signaling endosomes containing Nox1 and ClC-3. Circ Res 2007; 101: 663–71.
61 Matsuda JJ, Filali MS, Volk KA, Collins MM, Moreland JG, Lamb FS. Overexpression of CLC-3 in HEK293T cells yields novel currents that are pH dependent. Am J Physiol Cell Physiol 2008; 294: C251–C262.
62 Lamb FS, Moreland JG, Miller FJ Jr. Electrophysiology of reactive oxygen production in signaling endosomes. Antioxid Redox Signal 2009; 11: 1335–47.
63 Fisher AB. Redox signaling across cell membranes. Antioxid Redox Signal 2009; 11: 1349–56.
64 Mehdi MZ, Azar ZM, Srivastava AK. Role of receptor and nonreceptor protein tyrosine kinases in H_2O_2-induced PKB and ERK1/2 signaling. Cell Biochem Biophys 2007; 47: 1–10.
65 Raines EW, Garton KJ, Ferri N. Beyond the endothelium: NF-kappaB regulation of smooth muscle function. Circ Res 2004; 94: 706–8.
66 Zhang HN, Zhou JG, Qiu QY, Ren JL, Guan YY. ClC-3 chloride channel prevents apoptosis induced by thapsigargin in PC12 cells. Apoptosis 2006; 11: 327–36.
67 Lemonnier L, Shuba Y, Crepin A, Roudbaraki M, Slomianny C, Mauroy B, *et al*. Bcl-2-dependent modulation of swelling-activated Cl^- current and CIC-3 expression in human prostate cancer epithelial cells. Cancer Res 2004; 64: 4841–8.
68 Hawkins BJ, Madesh M, Kirkpatrick CJ, Fisher AB. Superoxide flux in endothelial cells via the chloride channel-3 mediates intracellular signaling. Mol Biol Cell 2007; 18: 2002–12.
69 Xu X, Xia J, Yang X, Huang X, Gao D, Zhou J, *et al*. Intermediate-conductance Ca^{2+}-activated potassium and volume-sensitive chloride channels in endothelial progenitor cells from rat bone marrow mononuclear cells. Acta Physiol (Oxf) 2011; 205: 302–13.
70 Webb NR, Moore KJ. Macrophage-derived foam cells in atherosclerosis: lessons from murine models and implications for therapy. Curr Drug Targets 2007; 8: 1249–63.
71 Hong L, Xie ZZ, Du YH, Tang YB, Tao J, Lv XF, *et al*. Alteration of volume-regulated chloride channel during macrophage–derived foam cell formation in atherosclerosis. Atherosclerosis 2011; 216: 59–66.
72 Maeno E, Ishizaki Y, Kanaseki T, Hazama A, Okada Y. Normotonic cell shrinkage because of disordered volume regulation is an early prerequisite to apoptosis. Proc Natl Acad Sci U S A 2000; 97: 9487–92.
73 Okada Y, Maeno E. Apoptosis, cell volume regulation and volume-regulatory chloride channels. Comp Biochem Physiol A Mol Integr Physiol 2001; 130: 377–83.
74 Elliott JI, Higgins CF. IKCa1 activity is required for cell shrinkage, phosphatidylserine translocation and death in T lymphocyte apoptosis. EMBO Rep 2003; 4: 189–94.
75 Small DL, Tauskela J, Xia Z. Role for chloride but not potassium channels in apoptosis in primary rat cortical cultures. Neurosci Lett 2002; 334: 95–8.
76 Inoue H, Ohtaki H, Nakamachi T, Shioda S, Okada Y. Anion channel blockers attenuate delayed neuronal cell death induced by transient forebrain ischemia. J Neurosci Res 2007; 85: 1427–35.
77 Wei L, Xiao AY, Jin C, Yang A, Lu ZY, Yu SP. Effects of chloride and potassium channel blockers on apoptotic cell shrinkage and apoptosis in cortical neurons. Pflugers Arch 2004; 448: 325–34.
78 Lieberman J, Rodbard S. Low blood pressure in young adults with cystic fibrosis: an effect of chronic salt loss in sweat? Ann Intern Med 1975; 82: 806–8.
79 Super M, Irtiza-Ali A, Roberts SA, Schwarz M, Young M, Smith A, *et al*. Blood pressure and the cystic fibrosis gene: evidence for lower pressure rises with age in female carriers. Hypertension 2004; 44: 878–83.
80 Nowaczynski W, Nakielana EM, Murakami T, Shurmans J. The relationship of plasma aldosterone-binding globulin to blood pressure regulation in young adults with cystic fibrosis. Clin Physiol Biochem 1987; 5: 276–86.
81 Farfel Z, Mayan H, Yaacov Y, Mouallem M, Shaharabany M, Pauzner R, *et al*. WNK4 regulates airway Na^+ transport: study of familial hyperkalaemia and hypertension. Eur J Clin Invest 2005; 35: 410–5.
82 Zhang YP, Ye L, Duan DD. Novel function of CFTR in high salt- and fructose-induced hypertension. Circulation 2012; in press.
83 Kanter ED, Kapur A, Haberly LB. A dendritic $GABA_A$-mediated IPSP regulates facilitation of NMDA-mediated responses to burst stimulation of afferent fibers in piriform cortex. J Neurosci 1996; 16: 307–12.
84 Meldrum B. Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission. Cerebrovasc Brain Metab Rev 1990; 2: 27–57.
85 Dutzler R, Campbell EB, Cadene M, Chait BT, MacKinnon R. X-ray structure of a ClC chloride channel at 3.0 A reveals the molecular basis of anion selectivity. Nature 2002; 415: 287–94.
86 Miller FJ Jr, Filali M, Huss GJ, Stanic B, Chamseddine A, Barna TJ, *et al*. Cytokine activation of nuclear factor kappa B in vascular smooth muscle cells requires signaling endosomes containing Nox1 and ClC-3. Circ Res 2007; 101: 663–71.