British Journal of Pharmacology (2005) 145, 1–2
www.nature.com/bjp

COMMENTARY

ClC-3: more than just a volume-sensitive Cl^- channel

[1]Carmelle V. Remillard & *,[1]Jason X.-J. Yuan

[1]Department of Medicine, School of Medicine, University of California, 9500 Gilman Drive, San Diego, La Jolla, CA 92093-0725, U.S.A.

Pulmonary vascular medial hypertrophy due to enhanced pulmonary artery smooth muscle cell (PASMC) proliferation and/or decreased PASMC apoptosis is a primary cause of increased pulmonary vascular resistance and arterial pressure in patients with pulmonary arterial hypertension. While many factors can contribute to this form of vascular remodeling, it is generally agreed upon that altered transmembrane ion flux *via* ion channels is involved. While much focus has centered on the role of cations and cation channels in controlling PASMC contraction and proliferation, anion efflux *via* Cl^- channels has recently gained interest for its role in SMC proliferation, differentiation, migration, contraction, and angiogenesis. In this issue, Dai *et al*. report that the putative volume-sensitive ClC-3 channel is upregulated in PASMC from monocrotaline-induced pulmonary hypertensive rats and in inflammatory cytokine-treated canine PASMC. They also provide evidence that ClC-3 upregulation may protect against oxidative stress-induced PASMC necrosis, thereby improving PASMC survival and promoting medial hypertrophy.
British Journal of Pharmacology (2005) **145,** 1–2. doi:10.1038/sj.bjp.0706136
Published online 21 February 2005

Keywords: Pulmonary arterial hypertension; inflammation; monocrotaline; ClC-3; proliferation; cell survival; vascular smooth muscle

Abbreviations: ET-1, endothelin-1; H_2O_2, hydrogen peroxide; IL-1β, interleukin-1β; PASMC, pulmonary artery smooth muscle cell; PDGF, platelet-derived growth factor; SMC, smooth muscle cell; TNF-α, tumor necrosis factor-α

Intuitively, one would expect enhanced Cl^- efflux due to Cl^- channel activation to promote cell shrinkage due to osmolarity changes, accelerating apoptotic volume decrease, and prompting apoptosis (Maeno *et al*., 2000). Contrary to this belief, there is mounting evidence that transmembrane diffusion of Cl^- in fact stimulates vascular smooth muscle cell (SMC) proliferation and differentiation (Voets *et al*., 1997; Wang *et al*., 2002). Owing to the sheer number of Cl^- channel subtypes (Jentsch & Günther, 1997) and their distinct functions in different cell types, identifying the ideal candidate underlying vascular proliferation and volume regulation has proven to be a daunting task. Nonetheless, the ClC-3 channel has risen to the forefront in the search for the volume-regulating Cl^- channel, a property that has been attributed in both cardiac (Duan *et al*., 1997) and vascular preparations (Yamazaki *et al*., 1998; Lamb *et al*., 1999). More recently, Nakazawa *et al*. (2001) published evidence that upregulation of a DIDS-sensitive Cl^- channel in pulmonary artery SMC (PASMCs) is involved in monocrotaline-induced pulmonary vasoconstriction. However, their data indicated that the channel was not likely to be either ClC-3 or ClCA. Therefore, the role of ClC-3 channels in regulating vascular tone is still unclear.

The *tunica media* of arteries is composed mainly of SMCs. Therefore, stimulation of SMC proliferation results in arterial medial hypertrophy due to a combination of enhanced SMC proliferation and decreased SMC apoptosis, ultimately leading to narrowing of the vessel lumen and increased vascular resistance and arterial pressure or, in short, hypertension. Obliteration of small pulmonary arteries and vascular wall thickening due to enhanced PASMC proliferation is disastrous in the pulmonary vasculature, causing pulmonary hypertension and eventual right heart failure. Indeed, both experimental and genetic pulmonary hypertension models demonstrate that pulmonary vascular remodeling, in the form of arterial medial hypertrophy, is one the most critical aspects underlying the hemodynamic changes associated with pulmonary arterial hypertension (Stenmark & Mecham, 1997). Although a dearth of information has been unearthed regarding the role of mitogenic and angiogenic compounds, of growth factors, and of transcription factors in pulmonary vascular remodeling, there is a consensus that deregulated transmembrane ion flux in PASMCs influences proliferation, apoptosis, and contraction of PASMCs. Indeed, much of the research over the last 20 years has centered on the role of Ca^{2+} and K^+ channels in mediating these phenomena (Mandegar *et al*., 2002). With the latter channels' roles clearly established, the page has been turned back to re-examine and further the break-through results of Voets *et al*. (1997) *vis-à-vis* the physiological role(s) of Cl^- channels.

In this issue of the *British Journal of Pharmacology*, Dai *et al*. (2005) examine the modulation of ClC-3 channel gene (*ClCn-3*) expression in the pulmonary vascular remodeling process associated with monocrotaline-induced pulmonary hypertension. They show that mRNA and protein expression of *ClCn-3* is upregulated in PASMC from rats with monocrotaline-mediated pulmonary hypertension as well as in canine PASMC treated with inflammatory cytokines and mitogens known to stimulate PASMC proliferation, migration, and differentiation (i.e. ET-1, PDGF, TNF-α, IL-1β). ClC-3 protein levels are also increased in cardiac myocytes from the right (but

*Author for correspondence; E-mail: xiyuan@ucsd.edu
Published online 21 February 2005

not the left) ventricle of monocrotaline-treated rats, further suggesting that ClC-3 upregulation contributes to right heart failure secondary to pulmonary hypertension. Finally, they demonstrate that H_2O_2-induced PASMC necrosis is attenuated in *ClCn-3*-overexpressing PASMCs. These data suggest that enhanced ClC-3 expression may improve PASMC survival during oxidative stress, such as that promoted by monocrotaline and H_2O_2. Enhanced PASMC survival then contributes to the angiogenesis and medial hypertrophy associated with pulmonary hypertension and vascular inflammation. In addition to the inhibitory effect on cell necrosis, upregulated ClC-3 channels or increased Cl^- efflux across the plasma membrane would also mediate (a) membrane depolarization because cytoplasmic Cl^- concentration ($[Cl^-]_{cyt}$) in PASMC is very high (~ 50 mM), and thus the equilibrium potential for Cl^- (E_{Cl}, approximately -25 mV) is much less negative than the equilibrium potential for K^+ (E_K, approximately -85 mV) and (b) elevation of cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) due to Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The membrane depolarization-mediated increase in $[Ca^{2+}]_{cyt}$ in PASMC and sustained vasoconstriction, as a result of increased ClC-3 channel activity, would further contribute to the elevated pulmonary vascular resistance in rats with monocrotaline-induced pulmonary hypertension.

Cell swelling is an important feature in necrosis, whereas cell shrinkage is an early hallmark of apoptosis. Activity of Cl^- channels or Cl^- flux across the plasma membrane appears to be involved in regulating both necrotic cell swelling (Dai *et al*., 2005) and apoptotic cell shrinkage (Maeno *et al*., 2000). Overexpressed ClC-3 channels in different cell types, such as pulmonary vascular endothelial and smooth muscle cells and cardiomyocytes, may play distinct functional roles in the regulation of cell proliferation, apoptosis, and necrosis, as well as cell migration and contraction. The significantly different $[Cl^-]_{cyt}$ among different cell types (e.g. PASMC vs right ventricular myocytes) is probably a critical determinant for the direction of Cl^- flux across the plasma membrane (causing cell swelling or shrinkage). Comparison of the functional role of ClC-3 channels in various cell types would provide important information for developing therapeutic approaches (targeting on specific Cl^- channels) for patients with pulmonary arterial hypertension.

References

DAI, Y.-P., BONGALON, S., HATTON, W.J., HUME, J.R. & YAMBOLIEV, I.A. (2005). ClC-3 chloride channel is upregulated by hypertrophy and inflammation in rat and canine pulmonary artery. *Br. J. Pharmacol.,* **145,** 5–14 (this issue).

DUAN, D., WINTER, C., COWLEY, S., HUME, J.R. & HOROWITZ, B. (1997). Molecular identification of a volume-regulated chloride channel. *Nature,* **390,** 417–421.

JENTSCH, T.J. & GÜNTHER, W. (1997). Chloride channels: an emerging molecular picture. *BioEssays,* **19,** 117–126.

LAMB, F.S., CLAYTON, G.H., LIU, B.X., SMITH, R.L., BARNA, T.J. & SCHUTTE, B.C. (1999). Expression of CLCN voltage-gated chloride channel genes in human blood vessels. *J. Mol. Cell. Cardiol.,* **31,** 657–666.

MAENO, E., ISHIZAKI, Y., KANASEKI, T. & OKADA, Y. (2000). Normotonic cell shrinkage because of disordered volume regulation is an early prerequisite to apoptosis B. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 9487–9492.

MANDEGAR, M., REMILLARD, C.V. & YUAN, J.X.-J. (2002). Ion channels in pulmonary arterial hypertension. *Prog. Cardiovasc. Dis.,* **45,** 81–114.

NAKAZAWA, H., HORI, M., MURATA, T., OZAKI, H. & KARAKI, H. (2001). Contribution of chloride channel activation to the elevated muscular tone of the pulmonary artery in monocrotaline-induced pulmonary hypertensive rats. *Jpn. J. Pharmacol.,* **86,** 310–315.

STENMARK, K.R. & MECHAM, R.P. (1997). Cellular and molecular mechanisms of pulmonary vascular remodeling. *Annu. Rev. Physiol.,* **59,** 89–144.

VOETS, T., WEI, L., DE SMET, P., VAN DRIESSCHE, W., EGGERMONT, J., DROOGMANS, G. & NILIUS, B. (1997). Downregulation of volume-activated Cl^- currents during muscle differentiation. *Am. J. Physiol. Cell Physiol.,* **272,** C667–C674.

WANG, G.-L., WANG, X.-R., LIN, M.-J., HE, H., LAN, X.-J. & GUAN, Y.-Y. (2002). Deficiency in ClC-3 chloride channels prevents rat aortic smooth muscle cell proliferation. *Circ. Res.,* **91,** e28–e32.

YAMAZAKI, J., DUAN, D., JANIAK, R., KUENZLI, K., HOROWITZ, B. & HUME, J.R. (1998). Functional and molecular expression of volume-regulated chloride channels in canine vascular smooth muscle cells. *J. Physiol.,* **507,** 729–736.

(Received November 25, 2004
Accepted November 29, 2004)