

Tails of the L-Type Ca²⁺ Channel To Sense Oxygen or Not

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Voltage-gated L-type Ca²⁺ channels are multisubunit membrane-spanning proteins that play a prominent role in a variety of Ca²⁺ dependent processes in cells including excitation-contraction coupling in muscle cells, excitation-secretion coupling in endocrine and neuronal cells, and gene regulation. The L-type Ca²⁺ channel is composed of a central pore-forming, voltage-sensing α_1 subunit and auxiliary subunits including β , α_2/δ , and γ .¹ Multiple genes are known to encode a variety of isoforms for each subunit. Given their prominent role in the regulation of cellular processes, it is not surprising that these channels are subject to extensive regulation. A myriad of neurohumoral factors can modulate Ca²⁺ channel function via a variety of transmembrane receptors and signaling cascades.¹ The best-studied example is β -adrenergic receptor-mediated stimulation of cardiac L-type Ca²⁺ channels by the cAMP/cAMP-dependent protein kinase pathway. Most of these regulatory pathways are thought to act by altering the phosphorylation status of the channel, although the molecular details of these putative phosphorylation events have not been fully resolved. But the story does not end with these channels responding only to traditional neurohormones. Recent studies have also revealed that the L-type Ca²⁺ channel can be modulated by hypoxia both in native vascular smooth muscle cells,² carotid body chemoreceptor cells,³ and in recombinant systems.⁴

How can acute hypoxia regulate channel activity? Changes in cellular metabolism resulting from hypoxia or ischemia can modulate channel function by changing the phosphorylation status of the channel. However, there are many other manners in which channels may respond more directly and rapidly to changes in O₂ levels.⁵ For example, a channel could contain an O₂ sensing moiety such as a heme group or be closely associated with a protein that contains such an O₂ sensor module. Alternatively, a metabolite of O₂ may be sensed, such as changes in reactive oxygen species or redox state. In the case of voltage-gated K⁺ channels in pulmonary smooth muscle cells, investigators have provided evidence that changes in the local redox environment may be respon-

sible for hypoxic inhibition of these channels.⁶ The idea is that changes in the ratio of reduced/oxidized redox couples such as glutathione (GSH/GSSG) can reduce or oxidize the channels or associated proteins altering their function.

What is known about redox modulation of L-type Ca²⁺ channels? In 1995, Chiamvimonvat et al⁷ demonstrated that Ca²⁺ current (I_{Ca}) expressed by recombinant α_1 subunit of L-type Ca²⁺ channel from rabbit lung was inhibited by 2,2'-dithiodipyridine (DTDP, a specific lipophilic oxidizer of sulfhydryl groups) and that the effect was readily reversed by 1,4-dithiothreitol (DTT, an agent that reduces disulfide bonds). Similar results were obtained by using the hydrophilic sulfhydryl-oxidizing agent, thimerosal. DTT alone had no effect on I_{Ca} . The effects were Ca²⁺ channel-specific: DTDP induced no changes in expressed human cardiac Na⁺ currents. This was the first study that demonstrated that the pore-forming α_1 subunit of the L-type Ca²⁺ channel contains functionally important "free" sulfhydryl groups that may be sensitive to the oxidation state of the cell. Redox modulation of L-type Ca²⁺ channels by oxidizing and reducing agents acting at the thiol group in the channel has also been demonstrated in native channels in ferret ventricular myocytes,⁸ although the details of modulation differ.

Are hypoxic regulation and thiol-specific redox modulation of L-type channels related? Recent studies by Fearon et al⁹ on human α_{1C} recombinant L-type Ca²⁺ channels expressed in HEK cells confirmed the previous findings in that the oxidizing agents thimerosal and *p*-chloromercuribenzenesulfonic acid (PCMB) caused inhibition of Ca²⁺ channel currents, and the reducing agent DTT reversed the inhibitory actions of thimerosal and PCMB. The Ca²⁺ channel currents were also inhibited by pretreatment with the positively charged methanethiosulphonate compound (MTSEA), which can oxidize available cysteines. The effects of the two sulfhydryl-modifying agents PCMB and MTSEA were additive, suggesting that the distinct thiol groups were modulated by these two agents. This study then demonstrated that hypoxic inhibition of Ca²⁺ channel currents was unaffected by pretreatment of cells with MTSEA but was fully prevented by treatment with PCMB, suggesting that distinct cysteine residues on the α_{1C} subunit are sensitive to PCMB treatment (but not those sensitive to MTSEA treatment) are involved in hypoxic inhibition of the channel.

In this issue of *Circulation Research*, Fearon et al¹⁰ take the next step to link O₂-sensing with redox modulation of channel activity by identifying the structural region involved in hypoxia-mediated Ca²⁺ channel regulation. In this study, the authors examined the effects of hypoxia on three naturally occurring splice variants of the human α_{1C} subunit (hHT, rHT, and fHT) of the L-type Ca²⁺ channel that differ only in

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the COOH-tail region.¹¹ Although the initial characterization of these three splice variants showed no clear differences in the properties of the expressed currents,¹¹ the study by Fearon et al¹⁰ remarkably demonstrates that hypoxia inhibits I_{Ca} in cells expressing the hHT splice variant and not the rHT or the fHT splice variants. This result suggests that a 71-amino acid insert present in hHT in the COOH-tail region of the channel confers oxygen sensitivity. Using mutagenesis, this interpretation is substantiated by data demonstrating both loss of function and gain of function. The results further identify a 39-amino acid region in the COOH terminus that is essential for oxygen sensing. This work represents the first structural clue to help unravel the mechanisms of hypoxic regulation of ion channels.

This finding opens the door to a wealth of experiments to further dissect out the molecular details of hypoxia regulation of the channels. First, we still do not know if it is the α_{1C} subunit itself that is directly modified or whether a closely associated protein acts as the O_2 sensor and has its sulfhydryl group(s) modified. An obvious step toward addressing this question would be to test the effect of site-directed mutagenesis of the three cysteine residues present in the identified 39-amino acid essential segment. If this property could be localized to a particular amino acid residue, it would strongly support the hypothesis that the α_{1C} subunit itself is the direct target. In addition, we do not know what redox couple may be responsible during hypoxia for potentially modifying the channel. It may be possible that this represents a site for redox reaction on thiol groups by NO.^{8,12} The results also add to the mystique of the COOH-tail of the α_{1C} channel, which has been associated with Ca^{2+} /calmodulin-dependent inactivation/facilitation,¹³ binding of sorcin,¹⁴ and protein kinase A regulation of the channel.¹⁵ To add to the mystery, the portion of the COOH-tail that contains the hHT splice variation may be truncated in neuronal and cardiac tissues, although recently this truncated fragment of the C-tail has been suggested to remain associated with the channel complex.¹⁶ How the COOH-terminus of the channel is specifically targeted by hypoxia and how this alters channel behavior represent important questions for future research.

The distinct functional properties of these splice variants of the α_{1C} subunit have likely been put to good use by nature. For example, the differential oxygen sensitivity of L-type Ca^{2+} channels in conduit compared with resistance pulmonary artery smooth muscle cells could easily be explained by alternative splice variants being expressed in these cells.¹⁷ In general, vascular smooth muscle cells that respond to hypoxia by relaxing may express the hHT splice variant and display associated Ca^{2+} channel inhibition, whereas those smooth muscle cells that constrict in response to hypoxia, ie, pulmonary resistance vessels, may not express hHT. Additionally, differences in splice variants could be important in a variety of cardiovascular, neurological, and endocrine diseases. For example, reducing Ca^{2+} influx in the setting of myocardial

ischemia could be protective, suggesting that these α_{1C} splice variants could be important in ischemic heart disease. Will this mechanism of channel regulation be able to be put to use in new therapies? Clearly, further research is needed to understand the role of these α_{1C} splice variants and hypoxia in normal physiology and disease, and the results presented by Fearon et al,¹⁰ open the door to exciting new advances.

References

- McDonald TF, Pelzer S, Trautwein W, Pelzer DJ. Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiol Rev*. 1994;74:365–507.
- Franco-Obregon A, Urena J, Lopez-Barneo J. Oxygen-sensitive calcium channels in vascular smooth muscle and their possible role in hypoxic arterial relaxation. *Proc Natl Acad Sci U S A*. 1995;92:4715–4719.
- Montoro RJ, Urena J, Fernandez-Chacon R, De Toldeo GA, Lopez-Barneo J. Oxygen sensing by ion channels and chemotransduction in single glomus cells. *J Gen Physiol*. 1996;107:133–143.
- Fearon IM, Palmer ACV, Balmforth AJ, Ball SG, Mikala G, Schwartz A, Peers C. Hypoxia inhibits the recombinant α_{1C} subunit of the human cardiac L-type Ca^{2+} channel. *J Physiol (Lond)*. 1997;500:551–556.
- Semenza GL. Perspectives on oxygen sensing. *Cell*. 1999;98:281–284.
- Archer SL, Weir EK, Reeve HL, Michelakis E. Molecular identification of O_2 sensory and O_2 -sensitive potassium channels in the pulmonary circulation. *Adv Exp Med Biol*. 2000;475:219–240.
- Chiamvimonvat N, O'Rourke B, Kamp TJ, Kallen RG, Hofmann F, Flockerzi V, Marban E. Functional consequences of sulfhydryl modification in the pore-forming subunits of cardiovascular Ca^{2+} and Na^{+} channels. *Circ Res*. 1994;76:325–334.
- Campbell DL, Stamler JS, Strauss HC. Redox modulation of L-type calcium channels in ferret ventricular myocytes: dual mechanism regulation by nitric oxide and S-nitrosothiols. *J Gen Physiol*. 1996;108:277–293.
- Fearon IM, Palmer AC, Balmforth AJ, Ball SG, Varadi G, Peers C. Modulation of recombinant human cardiac L-type α_{1C} subunits by redox agents and hypoxia. *J Physiol (Lond)*. 1999;514:629–637.
- Fearon IM, Isaacsohn I, Koch S, Varadi G, Ball SG, Peers C. Splice variants reveal the region involved in oxygen sensing by recombinant human cardiac L-type Ca^{2+} channels. *Circ Res*. 2000;87:537–539.
- Klockner U, Mikala G, Eisfeld J, Iles DE, Strobeck M, Mershon JL, Schwartz A, Varadi G. Properties of three COOH-terminal splice variants of a human cardiac L-type Ca^{2+} -channel α_1 -subunit. *Am J Physiol*. 1997;272(3 pt 2):H1372–1381.
- Hu H, Chiamvimonvat N, Yamagishi T, Marban E. Direct inhibition of expressed cardiac L-type Ca^{2+} channels by S-nitrosothiol nitric oxide donors. *Circ Res*. 1997;81:742–752.
- Zuhlke RD, Pitt GS, Deisseroth K, Tsien RW, Reuter H. Calmodulin supports both inactivation and facilitation of L-type calcium channels. *Nature*. 1999;399:159–162.
- Meyers MB, Puri TS, Chien AJ, Gao T, Hsu PH, Hosey MM, Fishman GI. Sorcin associates with the pore-forming subunit of voltage-dependent L-type Ca^{2+} channels. *J Biol Chem*. 1998;273:18930–18935.
- De Jongh KS, Murphy BJ, Colvin AA, Hell JW, Takahashi M, Catterall WA. Specific phosphorylation of a site in the full length form of the α_1 subunit of the cardiac L-type calcium channel by adenosine 3',5'-cyclic monophosphate-dependent protein kinase. *Biochemistry*. 1996;35:10392–10402.
- Gerhardstein BL, Gao T, Bünemann M, Puri TS, Adair A, Ma H, Hosey MD. Proteolytic processing of the C terminus of the α_{1C} subunit of L-type calcium channels and the role of a proline-rich domain in membrane tethering of proteolytic fragments. *J Biol Chem*. 2000;275:8556–8563.
- Franco-Obregon A, Lopez-Barneo J. Differential oxygen sensitivity of calcium channels in rabbit smooth muscle cells of conduit and resistance pulmonary arteries. *J Physiol (Lond)*. 1996;491:511–518.

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