Tails of the L-Type Ca\textsuperscript{2+} Channel
To Sense Oxygen or Not

Atsuko Yatani, Timothy J. Kamp

Voltage-gated L-type Ca\textsuperscript{2+} channels are multisubunit membrane-spanning proteins that play a prominent role in a variety of Ca\textsuperscript{2+} 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 Ca\textsuperscript{2+} channel is composed of a central pore-forming, voltage-sensing \( \alpha \) subunit and auxiliary subunits including \( \beta \), \( \alpha_2/\delta \), and \( \gamma \).\textsuperscript{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 Ca\textsuperscript{2+} channel function via a variety of transmembrane receptors and signaling cascades.\textsuperscript{1} The best-studied example is \( \beta \)-adrenergic receptor–mediated stimulation of cardiac L-type Ca\textsuperscript{2+} 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 Ca\textsuperscript{2+} channel can be modulated by hypoxia both in native vascular smooth muscle cells,\textsuperscript{2} carotid body chemoreceptor cells,\textsuperscript{3} and in recombinant systems.\textsuperscript{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 O\textsubscript{2} levels.\textsuperscript{5} For example, a channel could contain an O\textsubscript{2} sensing moiety such as a heme group or be closely associated with a protein that contains such an O\textsubscript{2} sensor module. Alternatively, a metabolite of O\textsubscript{2} may be sensed, such as changes in reactive oxygen species or redox state. In the case of voltage-gated K\textsuperscript{+} channels in pulmonary smooth muscle cells, investigators have provided evidence that changes in the local redox environment may be responsible for hypoxic inhibition of these channels.\textsuperscript{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 Ca\textsuperscript{2+} channels? In 1995, Chiamvimonvat et al\textsuperscript{7} demonstrated that Ca\textsuperscript{2+} current (\( I_{Ca} \)) expressed by recombinant \( \alpha \) subunit of L-type Ca\textsuperscript{2+} channel from rabbit lung was inhibited by 2,2\textsuperscript{'}-dithiodipyridine (DTDP, a specific lipophilic oxidizer of sulphydryl 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 sulphydryl–oxidizing agent, thimerosal. DTT alone had no effect on \( I_{Ca} \). The effects were Ca\textsuperscript{2+} channel-specific: DTDP induced no changes in expressed human cardiac Na\textsuperscript{+} currents. This was the first study that demonstrated that the pore-forming \( \alpha \) subunit of the L-type Ca\textsuperscript{2+} channel contains functionally important “free” sulphydryl groups that may be sensitive to the oxidation state of the cell. Redox modulation of L-type Ca\textsuperscript{2+} 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,\textsuperscript{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 al\textsuperscript{9} on human \( \alpha_{1C} \) recombinant L-type Ca\textsuperscript{2+} channels expressed in HEK cells confirmed the previous findings in that the oxidizing agents thimerosal and \( \rho \)-chloromercuribensulfonic acid (PCMB) caused inhibition of Ca\textsuperscript{2+} channel currents, and the reducing agent DTT reversed the inhibitory actions of thimerosal and PCMB. The Ca\textsuperscript{2+} channel currents were also inhibited by pretreatment with the positively charged methanethiosulphonate compound (MTSEA), which can oxidize available cysteines. The effects of the two sulphydryl-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 Ca\textsuperscript{2+} 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 \( \alpha_{1C} \) 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 al\textsuperscript{10} take the next step to link O\textsubscript{2}-sensing with redox modulation of channel activity by identifying the structural region involved in hypoxia-mediated Ca\textsuperscript{2+} channel regulation. In this study, the authors examined the effects of hypoxia on three naturally occurring splice variants of the human \( \alpha_{1C} \) subunit (hHT, rHT, and fHT) of the L-type Ca\textsuperscript{2+} channel that differ only in
the COOH-tail region. Although the initial characterization of these three splice variants showed no clear differences in the properties of the expressed currents, the study by Fearon et al remarkably demonstrates that hypoxia inhibits $I_{\text{Ca}}$ 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 $\alpha_{1C}$ subunit itself that is directly modified or whether a closely associated protein acts as the $O_2$ sensor and has its sulphhydril 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 $\alpha_{1C}$ 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$.^{10,12}$ The results also add to the mystery of the COOH-tail of the $\alpha_{1C}$ channel, which has been associated with Ca$^{2+}$/calmodulin-dependent inactivation/facilitation, binding of sorcin, and protein kinase $A$ regulation of the channel.$^{13}$ 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 $\alpha_{1C}$ 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 constric 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 $\alpha_{1C}$ 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 $\alpha_{1C}$ 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**


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