Are Kv Channels the Essence of O_2 Sensing?

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With the exception of anaerobes, organisms depend on oxygen for the production of energy and for biosynthetic reactions. Therefore, cells, tissues, and organisms must be able to sense and respond to changes in the oxygen concentration of their environment. The response of mammalian cells to hypoxia is crucial for their survival, allowing cells to cope with a low-oxygen environment. There are, however, specialized cells located in chemosensory organs (carotid bodies, pulmonary neuroepithelial bodies, and pulmonary artery smooth muscle among others) whose responses to hypoxia are unique, differing both quantitatively and qualitatively from the general self-defense reaction to hypoxia observed in other cell types. The specific hypoxic response of these chemosensitive cells is fast (seconds to minutes), has a lower threshold, requires an increase in their metabolic activity, and leads to changes in their excitability, contractility, and/or secretory activity. All of these features are specializations designed to prevent hypoxia in the entire organism and, thus, to maintain homeostasis.

The physiological role of these specialized responses is well characterized, but the molecular mechanisms of O_2 sensing and their transduction into an adaptive response in chemoreceptor cells is poorly understood. Over the past decade, it has been well established that modulation of ion channel activity by changes in oxygen levels contributes to the chemoreceptor cell response to low P_{O_2}. Since the pioneer description of a low P_{O_2}-modulated K^+ current in rabbit carotid body chemoreceptor cells[1] many other O_2-sensitive K^+ channels have been identified in chemosensory preparations.[2] The high degree of kinetic and pharmacological diversity among O_2-sensitive K^+ channels has focused our interest toward determining the structural requirements for O_2 sensing. Several K^+ channel genes expressed in some of the cell types sensitive to hypoxia have been identified, and for some of them, low P_{O_2} modulation has been studied in heterologous expression systems. However, there are conflicting reports with respect to which of these channels contributes to the native O_2-sensitive K^+ currents. Different genes can produce K^+ channels with similar electrophysiological properties. In addition, K^+ channels can form heterotetrameric complexes and/or associate with auxiliary modulatory subunits that could confer O_2 sensitivity to the resulting channel. Determination of the molecular constituents of the O_2-sensitive K^+ currents in native tissues is a relevant issue, not only to provide a physiological meaning to the reported O_2 modulation of cloned channels expressed in heterologous systems[3–6] but also to understand the molecular mechanisms of O_2 detection in hypoxia-sensitive tissues.

The study presented by Osipenko et al[7] in this issue of *Circulation Research* is an important step in this direction. These authors investigate the effects of hypoxia on several recombinant Kv1 channels previously described in pulmonary artery smooth muscle (PASM) as well as Kv3.1, with kinetics and pharmacological properties similar to the PASM native O_2-sensitive currents. They report that (1) recombinant Kv3.1 is modulated by hypoxia through a membrane-delimited mechanism, (2) Kv3.1 is expressed in PASM cells, and (3) Kv3.1 appears to contribute to the hypoxic inhibition of delayed rectifier K^+ currents in these cells.

From our perspective, this report represents a relevant contribution to the study of oxygen sensing for two main reasons. First, it shows an approach adequate for identification of a putative O_2-sensitive K^+ channel, by moving from the modulation by hypoxia of a recombinant channel back to its physiological context, the role of this channel in the response to low P_{O_2} of the native cells. Second, it demonstrates that hypoxic inhibition of the recombinant Kv3.1 channels is retained in excised membrane patches, pointing to a membrane-delimited mechanism as the origin of hypoxic responses. The importance of this latter finding deserves additional comment.

Kv3.1 represents a new O_2-sensitive K^+ channel described in PASM cells. However, other Kv channels present in these cells have also been implicated in their response to low P_{O_2}, including the Kv2.1/Kv9.3 heteromultimer, the homomeric Kv1.2, or the heteromeric Kv1.2/Kv1.5 (see Reference 7 and references therein). If we add to this picture the fact that both the expression and the functional contribution to the hypoxic response of different Kv channels in PASM seem to exhibit species-related differences, we are far from understanding the complex molecular basis of O_2 sensing in PASM cells. We can add more entropy to this description by pointing out that the same situation (several O_2-sensitive K^+ channels with species-related differences in their molecular identity) has also been found in chemoreceptor cells of the carotid body.[2] Finally, there are also non-K^+ channels that respond to hypoxia. In this context, it is worth speculating about the real essence of O_2 sensing by ionic channels, looking for a possible common link between hypoxia and the distinct O_2-sensitive K^+ channels in different chemosensory tissues. Indeed, the mechanism by which hypoxia modulates channel activity has been a point of discussion during the last few

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years. Two questions are of paramount interest: (1) Does the effect of hypoxia on O_2-sensitive K^+ channels require intracellular mediators? and (2) If low P_{O_2} can modulate O_2-sensitive K^+ channels in the absence of cytoplasmic factors, is the O_2 sensor the channel itself or a closely associated molecule? As mentioned, the present study[7] provides a clear answer to the first question, demonstrating that the hypoxic inhibition of Kv3.1 channels is maintained in cell-free patches in the absence of potential intracellular mediators. This has also been observed for other recombinant O_2-sensitive K^+ channels,[6] as well as for native K^+ currents in chemosensitive tissues.[8,9] In contrast (or perhaps in addition) to this clearly membrane-delimited mechanism, several cytosolic factors have been implicated in the hypoxic modulation of K^+ channels (see Reference 10 and references therein). However, although this redox modulation may well be important, a conclusive link between hypoxia and the redox state of the cell has not been demonstrated.

Regarding the second question raised, ie, the identity of the O_2 sensor, there are two obvious possibilities. First, the K^+ channels themselves function as O_2-sensing devices, and second, there is an O_2-sensing molecule closely associated with the membrane that is capable of interacting with only certain kinds of K^+ channels, modifying their activity in the presence of low levels of O_2. This latter hypothesis certainly provides a unifying explanation of the apparent diversity. As mentioned above, the biochemical adaptations to hypoxia constitute a universal response. So, it is plausible to think of the existence of a universal O_2 sensor that can couple to different effectors in the different cell types thereby providing cell-specific responses. From this point of view, Kv channels are clearly not O_2-sensing devices. They are just the first effectors in the chemotransduction cascade. In any case, with the present report,[7] the spectrum of Kv channels modulated by low O_2 widens a bit more. The literature does not agree yet about which Kv channels are O_2 sensitive and, among them, which are functionally relevant. Nevertheless, it is clear that there is not a single "O_2-sensitive" channel, and that O_2 sensitivity is not a common feature of all Kv channels. Why channels so similar in their molecular structure behave so differently when exposed to a low-oxygen environment is a mystery we do not yet understand. The hypothesis of a general sensor interacting with different channels, directly or through auxiliary subunits, is appealing. It could explain why recombinant Kv1 channels seem to be O_2 sensitive in some expression systems but not in others (see Reference 7 and references therein) or why the molecular identity of the O_2-sensitive K^+ current is so different among different species. The existence of a putative sensor is also supported by the observation that hypoxic inhibition of both native and recombinant O_2-sensitive K^+ channels can be reverted by carbon monoxide.[6,11] Because the only known targets of carbon monoxide in biological systems are metalloproteins, particularly hemoproteins, the observation that carbon monoxide is able to interact with this putative O_2 sensor, replacing O_2 and preventing the inhibition of K^+ currents, strongly suggests that the intrinsic O_2 sensor could be a hemoprotein.

Speculation is a necessary first step in the pursuit of knowledge, but final answers always require well-designed experimental settings. O_2 sensing and Kv channels are still puzzling partners, despite the considerable amount of work performed in the last few years. The approach by Osipenko et al,[7] combining the study of physiological responses in native tissues with the study of expression of recombinant channels in heterologous systems, will certainly bring valuable new information to bear in solving this mystery.

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