Roles of Ion Channels in Carotid Body
Chemotransmission of Acute Hypoxia

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Summary: In this review, we have highlighted the roles of ion channels in carotid body chemotransmission of acute hypoxia. With the application of new technologies, significant breakthroughs have been made in the last decade. The discovery of oxygen-sensitive K⁺ channels in rabbit glomus cells has generated the membrane model of hypoxic chemotransmission: the inhibition of oxygen-sensitive K⁺ channels by hypoxia initiates the depolarization of glomus cells and increases the firing frequency of glomus cells. The depolarization of glomus cells activates voltage-gated Ca²⁺ channels, elevating intracellular Ca²⁺ which triggers the release of neurotransmitters. The correlation of these events in rabbit glomus cells has been shown. However, a large corpus of data indicates that various mechanisms may be involved in different species. In rats, Ca²⁺-activated K⁺ channels are inhibited by hypoxia. The role of this inhibition on rat glomus cell function is controversial, and the contribution of leak-type K⁺ channels to rat glomus cell depolarization has recently been proposed. On the other hand, in cats, nicotinic ACh receptors (ligand-gated cation channels) may play a key role in initiating the depolarization of glomus cells and increasing the cytosolic Ca²⁺ of glomus cells in response to hypoxia. Hypoxic inhibition of oxygen-sensitive K⁺ channels would participate to further depolarize cat glomus cells. Additionally, the activity of Cl⁻ channels and the modulation of ion channels by neurotransmitters may influence the excitability of glomus cells. For generating action potentials in chemoreceptor afferent nerves, nicotinic ACh receptors appear to be involved in cats and rats. [Japanese Journal of Physiology, 49, 213–228]

Key words: Cl⁻ channel, hypoxia, intracellular calcium, K⁺ channel, neuronal nicotinic acetylcholine receptor.

The studies of Heymans and others in the 1920s and 1930s revealed the role of the peripheral arterial chemoreceptors in stimulating the cardiovascular and respiratory systems to supply oxygen to the organism [1]. The major arterial chemoreceptors are the bilateral carotid bodies and the aortic bodies, but the carotid bodies are the main structures notifying the brain of a fall in oxygen tension in the arterial blood. Stimulating the carotid body produces an impressive array of reflex responses in the cardiovascular, pulmonary, endocrine and renal systems, and this aspect of carotid body physiology has been recently reviewed [1–4]. Presently, the main thrust in carotid body investigation is focused on the question of how this arterial chemoreceptor converts the hypoxic signal into increased neural activity, which subsequently induces the systemic reflex responses. With the application of new technologies such as patch clamp, microfluorometric, amperometric and molecular techniques, significant breakthroughs have been made in the last decade. Several excellent reviews have summarized these studies and presented testable working models of hypoxic chemotransmission [5–10]. In this review, we will highlight the roles of ion channels in carotid
body chemotransmission of acute hypoxia. First, we shall describe a generalized model for hypoxic chemotransmission and a critical role for intracellular calcium in the hypoxic response of the carotid body. Second, we shall discuss oxygen-sensitive K⁺ channels and the membrane model for hypoxic chemotransduction of glomus cells. Third, we shall introduce new studies showing oxygen sensitivity in voltage-independent K⁺ channels and nicotinic acetylcholine (ACh) receptors. Further, other factors which modulate the excitability of glomus cells and the nerve endings will be discussed. In general, we shall focus more on the controversial and less attended issues of hypoxic chemotransmission in the carotid body, hoping that by revisiting the controversy we will gain a deeper insight into this tiny but unique organ.

I. A Generalized Model of Hypoxic Neurotransmission and a Role of Intracellular Calcium

The carotid body contains several cellular components including glomus cells (type I cells, chief cells), sheath cells (type II cells, sustentacular cells) and afferent nerve endings. Historically, these three components have been proposed as the chemosensor [11, 12]. However, recent studies indicate that glomus cells are sensitive to oxygen tension and are able to secrete neurotransmitters in response to hypoxia. Hence, most investigators model the basic chemotransductive unit as a glomus cell with an opposing chemoreceptor afferent nerve fiber, a branch of the glossopharyngeal nerve (Fig. 1).

In this model, neurotransmitters released from glomus cells play an active role in generating action potentials in chemoreceptor afferent nerve fibers, although the precise role of each neurotransmitter is still being debated. Dopamine, epinephrine, norepinephrine, serotonin, ACh, substance P and other neuropeptides have been demonstrated in the carotid body of several species [5, 11–13]. Further, nitric oxide and carbon monoxide appear to be produced in the carotid body [14–18]. Substantial data have shown that the carotid bodies of rabbits [19–22], cats [23] and rats [24, 25] release catecholamines at rest and that their release increases during hypoxia. Recently, a similar release pattern of ACh from the cat carotid body has been reported [26]. The release of other neurotransmitters is suspected, but has not yet been measured.

As with other neural tissues, the release of neurotransmitters from the carotid body is expected to be controlled by intracellular calcium ([Ca²⁺]). Indeed, dopamine release from the rabbit or rat carotid body was inhibited by L-type voltage-gated Ca²⁺ channel blockers [22, 25] or by the removal of extracellular calcium [19, 24, 25], indicating an influx of Ca²⁺ via L-type Ca²⁺ channels is critical for regulating dopamine release. Further, a close correlation of the [Ca²⁺] level with catecholamine release was recently presented in cultured adult rabbit glomus cells [20, 21]. Consistent with these data, the carotid body neural response to hypoxia in cats was also significantly attenuated by removal of extracellular calcium or by voltage-gated calcium channel blockers [27], showing a significant role of Ca²⁺ in carotid body neurotransmission.

However, presently available data regarding the changes in [Ca²⁺], are not necessarily consistent (Table 1). For example, during hypoxia, the [Ca²⁺] of glomus cells increased in some reports [20, 21, 28–31], was variable in others [32–35] and even decreased in one study [36]. The reason for this inconsistency is not apparent, but one possibility is that glomus cells do not respond uniformly to hypoxia. For example, Sterni et al. found that [Ca²⁺] increased in 34.5% of the 87 adult rabbit glomus cells tested and in 44% of the 91 newborn glomus cells [33]. Bright et al. [34], examining 182 rat glomus cells, reported that 20% of isolated cells increased [Ca²⁺] in response to hypoxia with various response patterns, but glomus cells in clusters did not respond to hypoxia. Chou et al. observed that hypoxia elevated [Ca²⁺] in only 5% of clusters of cat carotid body cells [35]. Another possibility is that increases in cytosolic Ca²⁺ are localized in the area near the synaptic vesicles containing neurotransmitters, and that the mean [Ca²⁺] may not always increase. The techniques which have been used

Table 1. Changes in \([\text{Ca}^{2+}]_i\) of glomus cells in response to hypoxia.

<table>
<thead>
<tr>
<th>[Ca(^{2+})_i]</th>
<th>Species</th>
<th>Age</th>
<th>Culture duration</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase</td>
<td>Rabbit</td>
<td>Adult</td>
<td>Fresh</td>
<td>[28]</td>
</tr>
<tr>
<td>Increase</td>
<td>Rabbit</td>
<td>Adult</td>
<td>12 h–5 d</td>
<td>[20, 21]</td>
</tr>
<tr>
<td>Increase (2/14;14%)</td>
<td>Rabbit</td>
<td>Adult</td>
<td>Fresh</td>
<td>[32]</td>
</tr>
<tr>
<td>No change (12/14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase (+40%)</td>
<td>Rabbit</td>
<td>Adult and newborn</td>
<td>Fresh</td>
<td>[33]</td>
</tr>
<tr>
<td>Increase</td>
<td>Rabbit</td>
<td>Newborn</td>
<td>2–10 d</td>
<td>[29]</td>
</tr>
<tr>
<td>Decrease</td>
<td>Rat</td>
<td>Adult</td>
<td>Fresh</td>
<td>[36]</td>
</tr>
<tr>
<td>Increase (20%)</td>
<td>Rat</td>
<td>Adult</td>
<td>Overnight</td>
<td>[34]</td>
</tr>
<tr>
<td>No change (cluster)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase</td>
<td>Rat</td>
<td>6–14 d</td>
<td>3–36 h</td>
<td>[30]</td>
</tr>
<tr>
<td>Increase</td>
<td>Rat</td>
<td>Fetus–21 d</td>
<td>Fresh</td>
<td>[31]</td>
</tr>
<tr>
<td>Increase (5%)</td>
<td>Cat</td>
<td>Adult</td>
<td>1–14 d</td>
<td>[35]</td>
</tr>
</tbody>
</table>

To measure the [Ca\(^{2+}\)_i] of glomus cells are not sensitive enough to detect local changes in Ca\(^{2+}\).

Although the [Ca\(^{2+}\)_i] response of each glomus cell may be heterogeneous, the increase in [Ca\(^{2+}\)_i] of glomus cells appears to derive from an influx of Ca\(^{2+}\) through voltage-gated Ca\(^{2+}\) channels. The application of Ca\(^{2+}\)_i channel blockers (dihydropyridines, Cd\(^{2+}\), or Ni\(^{2+}\)) or removal of extracellular Ca\(^{2+}\) blocks the increase in [Ca\(^{2+}\)_i]. [21, 28–30]. As mentioned above, L-type voltage-gated Ca\(^{2+}\) channels appear to be responsible for the hypoxia-driven [Ca\(^{2+}\)_i] increase. However, other types of Ca\(^{2+}\)_i channels are present in glomus cells [37–39], and further studies are required to understand the role of other Ca\(^{2+}\)_i channels in the [Ca\(^{2+}\)_i] response to hypoxia. Patch clamp techniques have shown that voltage-gated whole Ca\(^{2+}\) current in glomus cells is not augmented during hypoxia [40–42]. Hence, the activation of voltage-gated Ca\(^{2+}\) channels during hypoxia may be secondary to glomus cell depolarization. In addition to the influx of Ca\(^{2+}\) via voltage-gated Ca\(^{2+}\) channels, other mechanisms may also be involved in the regulation of [Ca\(^{2+}\)_i] during hypoxia. Bisno and Duchen first postulated the release of Ca\(^{2+}\) from intracellular storage sites in glomus cells during hypoxia [28, 43]. Later, Buckler and Vaughan-Jones demonstrated that the increase in [Ca\(^{2+}\)_i] of glomus cells in response to anoxia was mostly, but not completely, inhibited by voltage-gated Ca\(^{2+}\) channel blockers [30]. When the glomus cells were voltage-clamped close to their resting membrane potential (therefore, voltage-gated Ca\(^{2+}\) channels were not activated), the hypoxic [Ca\(^{2+}\)_i] response was reduced, but not eliminated. These data support the concept that calcium influx via voltage-gated Ca\(^{2+}\) channels may not be the only source for the increase in [Ca\(^{2+}\)_i] of glomus cells during hypoxia. We shall revisit this issue (III-2).

II. The Membrane Model of Hypoxic Chemotransduction in the Carotid Body

1. Discovery of oxygen-sensitive K\(^{+}\) channels

The effect of oxygen tension on ion channel activities was first shown by Lopez-Barneo et al. [40]. They applied patch clamp techniques to dissociated adult rabbit glomus cells and found that the voltage-gated outward K\(^{+}\) current, but not Na\(^{+}\) or Ca\(^{2+}\) currents, was sensitive to oxygen tension. A series of their studies in both whole-cell and excised patch preparations revealed several characteristics of O\(_2\)-sensitive K\(^{+}\) channels in adult rabbit glomus cells. Although the whole K\(^{+}\) current of rabbit glomus cells has at least three components (large conductance Ca\(^{2+}\)-activated current, small conductance current, and fast inactivating current), only the inactivating K\(^{+}\) current is inhibited by hypoxia [44, 45]. The inhibition of the inactivating K\(^{+}\) channels (K\(_{oi}\) channels) by hypoxia appears to be due to a decrease in open probability [44, 46]. Further, the inhibitory effect of hypoxia on K\(_{oi}\) channel activity is maintained during hypoxia [44, 47], and the effect is reversible [40, 41, 44–47]. The inhibition of K\(_{oi}\) channels by hypoxia is observed in excised membrane patch [44, 46, 47], and the inhibitory effect is not altered by the internal application of ATP or the activation of G-proteins [44, 45]. This suggests that hypoxia exerts its effect directly on K\(_{oi}\) channels, or oxygen interacts with molecules in the plasma membrane closely associated with the K\(_{oi}\) channels and affects the channel activity [8]. The O\(_2\)-sensitive component of K\(^{+}\) channels may change during development. For example, in rabbit fetal glomus cells, at least two
types of K⁺ channels are sensitive to O₂ tension: voltage-gated outward K⁺ channels and inwardly rectifying K⁺ channels [42, 48]. The O₂-sensitive component of voltage-gated K⁺ channels in fetal rabbit glomus cells has not been well characterized, and therefore, it is not clear whether these O₂-sensitive K⁺ channels resemble the Kₒ channels in adult rabbit glomus cells.

2. Heterogeneous characteristics of O₂-sensitive K⁺ channels

O₂ sensitivity of K⁺ current was also found in rat and cat glomus cells. In contrast to adult rabbits, the O₂-sensitive component of the K⁺ current in neonatal rat glomus cells is calcium dependent [49–51]. Ca²⁺ channel blockers or charybdotoxin (CBX), a specific blocker for maxi-K⁺ channels (large conductance Ca²⁺-activated K⁺ channels), inhibited outward current. Under the influence of these agents, outward K⁺ current was not further inhibited by hypoxia. In addition, single-channel recordings showed that these O₂-sensitive K⁺ channels were sensitive to [Ca²⁺]ᵢ and had a conductance of 190 pS; these are characteristics of maxi-K⁺ channels. These features of O₂-sensitive K⁺ channels of rat glomus cells differ greatly from those of rabbit glomus cells. Interestingly, the O₂-sensitive K⁺ current in cats was not inhibited by charybdotoxin. The current was not fast inactivating, and was more resistant to tetroethylammonium (TEA) as compared to the O₂-sensitive K⁺ currents of rabbit or rat glomus cells [52]. These differences are depicted in Fig. 2 and summarized in Table 2.

The heterogeneous characteristics of O₂-sensitive K⁺ current may be attributable to differences in species, age or methodology. Lopez-Lopez et al. studied the characteristics of O₂-sensitive K⁺ current in adult rat glomus cells with the same techniques they had been using for adult rabbit glomus cells [53]. They found that the O₂-sensitive K⁺ current of adult rat glomus cells have characteristics similar to those of neonatal rat glomus cells. Their results indicate that differences of O₂-sensitive K⁺ channels between rabbits and rats are attributable to species differences rather than developmental differences or methodological differences between investigators. On the other hand, differences in methodology might be responsible for some controversial results. Particularly, differentiating glomus cells from sheath cells solely on the basis of morphology can be risky. We attempted to differentiate these cell types by applying immunocytochemical techniques, and we correlated the occurrence of O₂ sensitivity of K⁺ current with the cell types (glomerus cells or sheath cells) [54]. In cultured cat carotid body cells, two types of cells were electrophysiologically identified: one whose K⁺ current was inhibited by hypoxia and the other whose K⁺ current

Fig. 2. Comparison of the voltage-gated, O₂-sensitive K⁺ current from adult rabbit, neonatal rat, and adult cat. Current traces and I-V curves were adapted from Lopez-Lopez et al. [41], Peers [50], and Chou and Shirahata [52] with permission. C, control; H, hypoxia; R, recovery; HP, holding potential; TP, test potential.
**Table 2. Characteristics of oxygen-sensitive K⁺ channels.**

<table>
<thead>
<tr>
<th></th>
<th>Rabbit</th>
<th>Rat</th>
<th>Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Response to voltage</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outward rectification</td>
<td>1. Outward rectification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient</td>
<td>2. Inward rectification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activation threshold</td>
<td>−50 to −20 mV</td>
<td>1. −20 to −30 mV</td>
<td>−40 mV</td>
</tr>
<tr>
<td>Activation by calcium</td>
<td>No</td>
<td>Not clear</td>
<td>Yes</td>
</tr>
<tr>
<td>Sensitivity to CBX</td>
<td>No</td>
<td>Not tested</td>
<td>Yes</td>
</tr>
<tr>
<td>Sensitivity to TEA</td>
<td>Yes, IC₅₀ &lt; 5 mM</td>
<td>1. Yes, IC₅₀ = 5 mM</td>
<td>Yes, IC₅₀ &lt; 5 mM</td>
</tr>
<tr>
<td>Sensitivity to 4-AP</td>
<td>Yes, IC₅₀ 0.2 mM</td>
<td>Not tested</td>
<td>No at 1 mM</td>
</tr>
</tbody>
</table>

**References**

[40, 41, 44-47] [42, 48] [53] [49-51, 63, 64] [52, 54]

CSX, charybdotoxin, a selective blocker of maxi-type calcium-activated K⁺ channels; TEA, tetraethylammonium; 4-AP, 4-aminopyridine. All pharmacological agents were externally applied.

was insensitive to hypoxia. However, these two types of cells were morphologically indistinguishable, and the K⁺ currents had similar kinetic and pharmacological properties [52]. With the combination of immunocytochemical and patch clamp techniques, we found that the K⁺ current of glomus cells, but not of sheath cells, is sensitive to hypoxia. Moreover, the response of glomus cells was heterogeneous. One out of 11 glomus cells, identified by the presence of tyrosine hydroxylase, did not have O₂-sensitive K⁺ channels, suggesting that a small population of glomus cells may not respond to hypoxia. Our results may, at least partly, explain why some investigators were unable to find the inhibitory effect of hypoxia on K⁺ currents of “glomerulosa cells” [28, 55]. Since cell types were identified by simple morphology in most of the patch clamp studies, some cells may have been unresponsive glomus cells or sheath cells.

Heterogeneous responses of glomus cells have been shown in several studies. Donnelly [56] and Cheng and Donnelly [57] have suggested the possibility that undissociated glomus cells may respond differently from dissociated glomus cells. They used a whole rat carotid body to record voltage-gated K⁺ current, and found K⁺ current was not always reduced by hypoxia in their preparation. Their observation is intriguing when we compare their results with others. Bright *et al.* found that the [Ca²⁺]i of adult rat glomus cells increased in response to hypoxia when the cells were isolated, but glomus cells in clusters did not respond to hypoxia [34]. The difference in the responses to hypoxia between isolated and non-isolated glomus cells was demonstrated some years ago by Eyzaguirre's group [58, 59]. They also reported electrical coupling between glomus cells [60, 61]. These studies suggest that the communication between glomus cells could generate different responses to hypoxia among individual glomus cells, and that special attention to the state of glomus cells (isolated or in a cluster) may be necessary for interpreting data.

**3. The membrane model of hypoxic chemotransduction**

In spite of some variable responses associated with species and methodological differences, it is reasonable to state, in general, that the K⁺ current of cultured glomus cells is inhibited by hypoxia. Accepting this, the critical question becomes how does this inhibition fit into the picture of hypoxic chemotransduction in the carotid body. The sequence of events most frequently postulated to describe the chemotransductive process is: (1) Hypoxia depolarizes glomus cells due to a decrease in K⁺ outflow through O₂-sensitive K⁺ channels; (2) depolarization activates voltage-gated calcium channels; (3) extracellular Ca²⁺ enters the glomus cell raising [Ca²⁺]; (4) the increase in [Ca²⁺], triggers the release of excitatory and inhibitory neurotransmitters; (5) these neurotransmitters bind to postsynaptic receptors on the chemoreceptor afferent fiber and autoreceptors on glomus cells; and (6) this binding generates and/or modulates the action potential recordable in the chemoreceptor afferent nerves, as well as the further release of neurotransmitters from glomus cells (Fig. 3) [5–8, 10, 62].
CBX depolarized rat glomus cells [49]. Since both studies used similar dissociation/culture procedures and patch clamp conditions, the reason of this discrepancy is not apparent.

Rabbit glomus cells produce spontaneous action potentials [41, 65]. Since $O_2$-sensitive $K^+$ channels are voltage dependent, they can be activated periodically. Thus, the increases in the slope of pacemaker potential and in the firing frequency during hypoxia in rabbit glomus cells [41] may be due to the inhibition of these channels. However, pharmacological experiments have not yet proved this scenario. The application of 4-AP, which inhibits $O_2$-sensitive $K^+$ current in rabbit glomus cells, decreased the speed of the repolarization phase of an electrically induced action potential. This resulted in the widening of the electrically induced action potential [45]. These changes are apparently different from those caused by hypoxia as mentioned above. The effect of 4-AP on spontaneous action potentials in rabbit glomus cells has never been tested. In cat glomus cells, a close correlation between the presence of $O_2$-sensitive $K^+$ channels and hypoxia-induced depolarization of glomus cells has been suggested [54], but the effect of $K^+$ channel blockers on the membrane potential of cat glomus cells is not known.

Another issue with the membrane model is the relationship between the inhibition of $O_2$-sensitive $K^+$ channels and $O_2$ tension. Inhibition of the $K^+$ channel activity in rabbit glomus cells occurs at $P_{O_2} \leq 150$ mmHg with the maximum inhibition observed at $P_{O_2} = 80$ mmHg in both whole-cell (Fig. 4A) and single-channel recordings [41, 47]. However, carotid body neural output is minimal at such $P_{O_2}$ values. Neural output begins to increase at $P_{O_2}$ values in the 70 mmHg range [11, 12]. On the other hand, a recent work by Montoro et al. [20] presented a linear relation between $O_2$ tension and inhibition of $K^+$ current in rabbit glomus cells using conventional whole-cell recordings (Fig. 4B). They explained that $O_2$ tension in the recording chamber was not monitored in previous reports, resulting in an "anomalous" relationship between $O_2$ tension and $K^+$ current inhibition. However, in those previous reports they clearly stated that $O_2$ tension in the chamber was measured [41, 47]. Perhaps the difference is attributable to the methods for expressing the inhibition of $K^+$ current. In the report of 1989 [41], the inhibition of $K^+$ current was expressed as a ratio of $K^+$ current at low $O_2$ to control $K^+$ current (Fig. 4A). Since the degree of $K^+$ current inhibition appears variable, and each data point expressed a measurement from a different cell in the report of 1989 (Fig. 4A), the "anomalous" relationship
Fig. 4. The relationship between the oxygen tension and inhibition of K⁺ current. A: Relative peak K⁺ current obtained during a +40 mV test pulse. Each data point is from a different experiment. Adult rabbit glomus cells were isolated and cultured, and a whole-cell patch clamp was performed. Reproduced from Lopez-Lopez et al. [41] by copyright permission of Rockefeller Univ Press. B: The inhibition of K⁺ current generated during +20 mV test potential was normalized as % of maximal inhibition (mean±SD, n=8). The preparation of the cells was similar to that in A. Reproduced from Montoro et al. [20] by copyright permission of Rockefeller Univ Press. C: K⁺ current inhibition in a cat glomus cell. A whole-cell K⁺ current was generated by a +40 mV test pulse. All three regression lines were drawn by eye, and no statistical evaluation was made in any of these studies.

may simply represent variable hypoxic responses in different cells. In the report of 1996 [20], inhibition was expressed as a percent of maximal change (Fig. 4B) and each data point was a mean of values from 8 cells. In cat glomus cells, O₂-sensitive K⁺ current was linearly inhibited by hypoxia when the inhibition was expressed as a percent of maximal change (Fig. 4C). In these recent studies, EC₅₀ of O₂ tension for K⁺ current inhibition is approximately 80 mmHg (Fig. 4B, C). Again, this relationship is different from that between PO₂ values and cataroid body neural output. From these results, one could speculate that the inhibition of O₂-sensitive K⁺ channels may not initiate hypoxic chemotransduction of the cataroid body. Alternatively, Gonzalez et al. suggested that second messengers may play a role for the full expression of hypoxic inhibition of O₂-sensitive K⁺ channels [10]. For example, hypoxia increases cAMP in glomus cells [66–68] and this effect is maximal at 40–45 mmHg of PO₂. Since cAMP inhibits O₂-sensitive K⁺ channels [45], reduction in O₂ may exert its effect on K⁺ current via both membrane-associated mechanisms and cytosolic mechanisms in intact glomus cells. The latter effect (e.g., the inhibitory effect of cAMP) may be absent in conventional whole-cell current recordings or single-channel recordings.

III. Modification of the Membrane Model

1. Voltage-insensitive K⁺ channels

Recently, Donnelly [56] and Cheng and Donnelly [57] questioned the role of voltage-gated O₂-sensitive K⁺ channels for cataroid body neurotransmission. They simultaneously recorded whole-cell K⁺ current and carotid sinus nerve discharge from the adult rat carotid body. Blockers for O₂-sensitive K⁺ channels in rat glomus cells, TEA, 4-AP or CBX, inhibited voltage-gated K⁺ current as expected, but did not increase neural activity. Further, under the influence of TEA, hypoxia still increased chemoreceptor neural activity (Fig. 5), suggesting a dissociation of the inhibition of K⁺ channel activity from the excitation of carotid chemoreceptor activity. These data are consistent with Buckler's report, which showed no effects of TEA, 4-AP or CBX on [Ca²⁺]₀ and/or membrane potential of neonatal rat glomus cells [64]. Thus, the inhibition of O₂-sensitive, Ca²⁺-activated K⁺ channels may not be sufficient to initiate hypoxic neurotransmission of the rat carotid body. Buckler has further shown that a different type of K⁺ channel may be involved in the hypoxic excitation of rat glomus cells. These channels appear voltage-insensitive, open at resting membrane potentials, and insensitive to TEA and 4-AP. Their resting K⁺ conductance was reduced by graded decreases in O₂ tension. Therefore, the inhibition of this type of K⁺ channel could play a significant role in depolarizing rat glomus cells. However, the contribution of CBX- and O₂-sensitive K⁺ current on the membrane potential of glomus cells cannot be dismissed at this moment due to the controversial results discussed above (i.e., Wyatt and Peers found CBX depolarized rat glomus cells [49]). The presence of an O₂-sensitive, resting K⁺ current is not known in other species, and the absence of specific blockers of this type of K⁺ channel is presently a major obstacle to further investigation of this current.
ACh receptors of glomus cells is stimulatory in cats, rabbits and rats, but the effect of muscarinic receptor activation is species-dependent.

It is well documented that nicotinic ACh receptors in muscles and neurons are ligand-gated cation channels, and those on glomus cells most likely belong to the neuronal type. Neuronal nicotinic receptors are distinct from muscle-type nicotinic receptors in their subunit compositions, sensitivity to agonists/antagonists, ion permeability, and activation/inactivation kinetics. Recent studies on other neural tissues have greatly advanced our understanding of neuronal nicotinic ACh receptors [79–84]. To date, eight neuronal α (α2–9) and three neuronal β (β 2–4) subunits of nicotinic ACh receptors have been encoded in rodent and chick. The receptors are permeable to Ca\(^{2+}\) and Na\(^{+}\), and the permeability ratio of Ca\(^{2+}\) to Na\(^{+}\) ranges from 1 to 20 depending on the types of subunits composing the receptors. It appears that a heteromERIC combination of neuronal α and neuronal β subunits is necessary to form ACh-gated channels, but α7, α8, and α9 subunits can form homomERIC ACh-gated channels. A nanomolar concentration of α-bungarotoxin can block the channels composed of α7–9 subunits, but not the other types.

Wyatt and Peers were the first to demonstrate inward current induced by the activation of nicotinic ACh receptors in glomus cells using neonatal rats [85]. The current showed a strong inward rectification at negative membrane potentials, and it seemed to be carried by both Na\(^{+}\) and Ca\(^{2+}\). Nicotinic receptor activation also depolarized rat glomus cells. Since α-bungarotoxin binding sites have been localized on rat glomus cells using electron microscopy [86], the activation of α-bungarotoxin–sensitive receptors may be responsible for some of the responses to nicotine. However, the sensitivity of the nicotine-induced current to mecamylamine [85] suggests that other types of nicotinic receptors may also be involved, because α-bungarotoxin–sensitive receptors are relatively resistant to mecamylamine [79]. Presently, no information is available on the specific receptor subtypes in rat glomus cells. On the other hand, α4 subunits of neuronal nicotinic ACh receptors in cat glomus cells have been demonstrated using immunocytochemical techniques (Fig. 6) [76]. The electrophysiological characteristics of these receptor channels in cat glomus cells are not yet well studied, but ACh-induced inward current exhibits some characteristics of the current via α4 ACh receptors (unpublished observation by authors).

In the past, ACh receptors on glomus cells have been presumed to play only a modulatory role in the release of catecholamines. However, as discussed
above, the activation of nicotinic ACh receptors depolarizes glomus cells [85], which means that ACh has a strong influence on the excitability of glomus cells. Our recent studies have revealed that the carotid body releases ACh even under normoxic normocapnic conditions [26, 87], indicating that glomus cells are exposed to ACh even at rest. The depolarizing effect of ACh on glomus cells may be significant at rest, because the ACh-induced current shows strong inward rectification at negative membrane potentials [80, 85, 88, 89] (i.e., the more negative the membrane potential, the larger the cation influx). In addition to the depolarizing effect of ACh on glomus cells, nicotinic ACh receptor activation can cause Ca\(^{2+}\) influx. Ca\(^{2+}\) influx via nicotinic ACh receptors can also occur at resting membrane potentials for the reasons described above. Furthermore, our preliminary data have shown that the ACh-induced inward current in cat glomus cells was enhanced by mild hypoxia, suggesting that nicotinic ACh receptors on glomus cells may be sensitive to oxygen tension (Fig. 7). Thus, nicotinic ACh receptors on glomus cells may play a major role in increasing [Ca\(^{2+}\)] and controlling the release of neurotransmitters during hypoxia. Our hypothesis describing the role of nicotinic ACh receptors is presented later (VI).

**IV. Factors Modulating the Excitability of Glomus Cells**

**1. Large-conductance Cl\(^-\) channels**

The Cl\(^-\) current in glomus cells may play a role in regulating intracellular pH and membrane potential, although its contribution has been overlooked. Stea and Nurse have demonstrated large-conductance (296 pS), voltage-independent Cl\(^-\) channels in neonatal rat glomus cells [90]. Anions move through the Cl\(^-\) channel depending on the electrochemical gradient. The activity of the channels appears insensitive to intracellular Ca\(^{2+}\), nucleotides, or pH, and is inhibited by anthracene-9-carboxylic acid. An important feature of the channels is their high permeability to HCO\(_3^-\) (permeability ratio \(P_{\text{HCO}_3^-}/P_{\text{Cl}^-}=0.7\)). In a subsequent study [91], they used perforated-patch recordings in rat glomus cells and showed that a HCO\(_3^-\)-containing perfusate correlated with a much lower input resistance (453.6±65.7 M\(\Omega\)) compared to that with a HCO\(_3^-\)-free perfusate (2.6±0.3 G\(\Omega\)). The leak current of glomus cells increased during HCO\(_3^-\)-containing perfusion. The changes were inhibited by anthracene-9-carboxylic acid. Further, they observed the opening of putative single channels, which was superimposed on whole-cell leakage current during HCO\(_3^-\)-containing perfusion. The conductance of the putative single channel was similar to that of the Cl\(^-\) channel in inside-out patches. Hence, the presence or absence of HCO\(_3^-\) in the perfusate may generate changes in input resistance and leak current via Cl\(^-\) channels.

Under physiological conditions, both Cl\(^-\) and HCO\(_3^-\) can move through Cl\(^-\) channels. Based on the Goldman-Hodgkin-Katz equation, the reversal poten-
tial of the Cl\textsuperscript{−} channel-mediated current (\(E_i\)) would be:

\[ E_i = -\frac{RT/F}{n} \ln \left( \frac{[\text{Cl}^\text{−}]_o + (P_{\text{HCO}}/P_{\text{Cl}}) \times [\text{HCO}_3^\text{−}]_o}{[\text{Cl}^\text{−}]_o + (P_{\text{HCO}}/P_{\text{Cl}}) \times [\text{HCO}_3^\text{−}]_o} \right) \]

\[ = -26.7 \times \ln(105 + 0.7 \times 24)/(27 + 0.7 \times 15) \]

\[ = -32 \text{ (mV)} \]

where [Cl\textsuperscript{−}]_o and [HCO\textsubscript{3}^−]_o were assumed to be 105 and 24 mM, respectively, [Cl\textsuperscript{−}] was estimated from studies of Oyama et al. [92] and Pang and Eyzaguirre [93], and [HCO\textsubscript{3}^−] was estimated from the Henderson-Hasselbalch equation at pH\textsubscript{o} = 7.4 and pH\textsubscript{i} = 7.2. Since \(E_i\) is less negative than the resting membrane potential, Cl\textsuperscript{−} and HCO\textsubscript{3}^− would move out from the glomus cell at rest. The outward movement of these ions may greatly influence glomus cell function (i.e., acidification and depolarization of the glomus cell). Acidification of glomus cells would inhibit the O\textsubscript{2} sensitive K\textsuperscript{+} current [94].

Many patch clamp studies using rabbit and rat glomus cells have been performed in an HCO\textsubscript{3}^−-free environment, and therefore the precise effects of HCO\textsubscript{3}^− movement on glomus cell functions are largely unknown. However, our experiments measuring carotid chemoreceptor neural activity suggested that the activity of Cl\textsuperscript{−} channels is important for hypoxic chemotransmission in the carotid body [95]. We selectively perfused the carotid body with Krebs solution in vivo. When anthracene-9-carboxylic acid was added in the hypoxic perfusate, the neural response to hypoxia was significantly reduced (Fig. 8). Similar results were obtained in the rat carotid body [96]. No studies have yet shown the effect of hypoxia on Cl\textsuperscript{−} channel activities. Clearly, the role of HCO\textsubscript{3}^−-permeable Cl\textsuperscript{−} channels in hypoxic chemotransmission needs further attention.

2. Modulation of ion channel activities by neurotransmitters

As mentioned before, the carotid body contains many kinds of neurotransmitters. They may well influence the ion channel activities of glomus cells because various neurotransmitters modulate several channel activities in other neural systems. For example, the stimulation of D2 receptors enhances delayed rectified and A-type K\textsuperscript{+} currents [97-99] and inhibits L-type and N-type Ca\textsuperscript{2+} channels [100, 101]. Substance P has been shown to modulate many channel functions [102-105]. However, little is known regarding the effects of these neurochemicals on the channel activities of glomus cells. Benot and Lopez-Barneo have shown that dopamine inhibited Ca\textsuperscript{2+} current but did not affect Na\textsuperscript{+} and K\textsuperscript{+} currents in rabbit glomus cells [106].

![Figure 8. Carotid chemoreceptor neural responses to perfusions of hypoxic Krebs with or without 4 mM anthracene-9-carboxylic acid (9-AC). Carotid sinus nerve (CSN) activity was recorded from the whole carotid sinus nerve after denervating baroreceptors. The carotid body was selectively and intermittently perfused with various Krebs solutions. Detailed methods were described by Shirahata and Fitzgerald [27]. The inhibition of chemoreceptor neural response to hypoxia by 9-AC suggests that Cl\textsuperscript{−} channels are involved in hypoxic neurotransmission.](image)

Very recently, Overholt and Prabhakar have reported that norepinephrine also attenuated the Ca\textsuperscript{2+} current in rabbit glomus cells via a pathway involving G-protein βγ [107]. On the other hand, nitric oxide seemed to inhibit L-type Ca\textsuperscript{2+} channels via pathways independent of G-protein [108]. Future investigations should reveal the modulatory effects and mechanisms of other neurotransmitters on the ion channel activities of glomus cells.

V. Ion Channels in Chemoreceptor Afferent Neurons

Although some investigators have proposed that chemoreceptor afferent nerve endings are chemosensors [11, 12, 109], most investigators postulate that chemoreceptor afferent endings play a subordinate role in carotid body chemotransmission. Namely, neurotransmitters which are released from glomus cells evoke receptor potential in chemoreceptor afferent endings and eventually trigger action potentials in chemoreceptor afferent nerves (Figs. 1, 3). Several studies have investigated voltage-gated and ligand-gated ion channels in chemoreceptor afferent neurons and their roles in the generation of action potentials. Gallego [110] and Belmonte and Gallego [111] have characterized the electrophysiological properties of chemoreceptor and baroreceptor neurons in the cat petrosal ganglion (the sensory ganglion of the glosopharyngeal nerve). They showed that the action potentials of cat chemoreceptor neurons were produced.
by Na\sup+ current (a part of which was tetrodotoxin-resistant) and Ca\sup+ current. A prominent, long after-hyperpolarization was due to Ca\sup+-dependent K\sup+ current. Stea and Nurse described two major subpopulations of neurons in cultured rat petrosal ganglia with patch clamp techniques [112]. Na\sup+ current in one type of neurons was tetrodotoxin-sensitive, but the other was not. Their study did not correlate the electrophysiological characteristics of neurons with the identity of sensory neuron types. Hayashida et al. [113] recorded afferent discharge from cat carotid sinus nerve endings in the carotid body. Action potentials were dependent on extracellular Na\sup+ and blocked by tetrodotoxin. The application of ACh evoked receptor potential (slowly developed depolarization) and increased discharge, suggesting that ACh stimulated excitatory ACh receptors in the chemoreceptor afferent endings, or that ACh released excitatory neurotransmitter(s) from glomus cells and these neurotransmitters evoked receptor potential.

Little is known regarding ligand-gated ion channels in chemoreceptor afferent neurons except for nicotinic ACh receptors. Early studies suggested that α-bungarotoxin binding sites (possibly α7 subunits of neuronal nicotinic ACh receptors) were unlikely to be present on chemoreceptor afferents in rabbits, rats, and cats [69, 75, 86]. However, the negative results may be attributable to the low density of the receptors at the nerve ending. In fact, when the carotid sinus nerve was ligated for 10 h, α-bungarotoxin binding sites did accumulate on both sides of the ligature, due possibly to the axonal transport of the receptors [114]. Very recently, we have shown that α7 subunits of neuronal nicotinic ACh receptors are localized in the carotid sinus nerve endings which innervate glomus cells (Fig. 9) [115]. Although the function of α7 subunit-containing ACh receptors needs to be clarified, in our preliminary studies, ACh evoked an inward current in cultured cat petrosal ganglion neurons and depolarized these cells. The current was partially inhibited by methylecanitine, a blocker for α7 ACh receptors [116, 117]. Hence, it is possible that α7-subunit containing nicotinic receptors are involved in neurotransmission in the cat carotid body. Further, Nurse and his colleagues have shown that ACh evoked action potentials in the cultured petrosal ganglion neurons of neonatal rats [118]. When petrosal ganglion neurons were co-cultured with glomus cells, these neurons produced spontaneous action potentials. FIRING frequency increased during hypoxia, and this hypoxic response was blocked by a nicotinic ACh receptor blocker, hexamethonium [119]. These data suggest a critical role of nicotinic ACh receptors in evoking action potentials in chemoreceptor afferent neurons in rats as well.

VI. Concluding Remarks

In the last decade we have obtained a significant amount of knowledge regarding the electrophysiological properties of glomus cells. A big cornerstone was the discovery of oxygen-sensitive K\sup+ channels in glomus cells followed by a proposal of the membrane model (Fig. 3). Subsequently, the oxygen sensitivity of K\sup+ channels was demonstrated in several other tissues such as pulmonary arterial smooth muscle cells [120–122], cells from pulmonary neuroepithelial bodies [123], adrenal chromaffin cells [124, 125], neurons from the central nervous system [126], and PC12 cells (pheochromocytoma-derived cells) [127]. Further, it has been shown that Na\sup+ channels in some neurons [128] and Ca\sup+ channels in vascular smooth muscle cells [129] were inhibited by hypoxia. In adult rabbit glomus cells, Ca\sup+ channels were initially reported to be insensitive to hypoxia [40, 41]. However, a later study showed them to be inhibited by hypoxia [20]. These data suggest that the O\textsubscript{2} sensitivity of ion channels does not seem to be limited to the carotid body, but to be a more generalized phenomenon [130]. However, what is unique to the carotid body is that decreases in arterial O\textsubscript{2} tension are transduced into increased neural activity in a very orderly fashion. This
activity induces reflex responses in many organs, adjusting the oxygen delivery and metabolic need of the organs.

Whether the inhibition of O₂-sensitive K⁺ channels is the initial step in hypoxic chemotransmission of the carotid body is not yet clear. The inhibition of Kᵦ channels may play a significant role in the excitation of rabbit glomus cells. On the other hand, in rats, the hypoxic inhibition of leak-type K⁺ channels may depolarize glomus cells, and the inhibition of Ca²⁺-activated K⁺ channels by hypoxia may have a secondary role in the excitation of glomus cells. Thus, different species may use different mechanisms to depolarize glomus cells.

We have summarized a working model of hypoxic chemotransmission of the cat carotid body in Fig. 10. Under normoxic conditions, a small amount of ACH is continuously released [26, 87]. ACH binds to α4 subunit-containing nicotinic ACh receptors, leading to the influx of Na⁺ and Ca²⁺. The influx of cations tends to depolarize the cell, but the depolarization is not large enough to activate voltage-gated K⁺ or Ca²⁺ channels. With a small decrease in O₂ tension, hypoxia may augment the activity of α4 subunit-containing nicotinic ACh receptors and/or enhance the sensitivity of nicotinic ACh receptors for ACh. Na⁺ and Ca²⁺ rush into the cell via the nicotinic receptors. The glomus cell begins to depolarize. The elevation of [Ca²⁺] via these α4 subunit-containing nicotinic receptors increases the release of ACh and other neurotransmitters (NT). ACh further activates nicotinic ACh receptors, and more Na⁺ and Ca²⁺ enter via these receptors. Membrane depolarization activates voltage-gated K⁺ and Ca²⁺ channels. However, hypoxia is inhibiting the K⁺ channels. The inhibition of the outward movement of K⁺ ions prevents repolarization of the glomus cell. Depolarization of the glomus cell continues and even advances. Ca²⁺ enters the glomus cell via voltage-gated Ca²⁺ channels, and ACh and other neurotransmitters are continually released. The activity of Cl⁻ channels and the effects of neurotransmitters on ion channel activities seem to influence the excitability of glomus cells as well. ACh activates α7 subunit-containing ACh receptors in chemoreceptor afferent endings. The resultant influx of Na⁺ and Ca²⁺ via these receptor channels generates receptor potential followed by the activation of Na⁺ channels and the generation of action potentials in chemoreceptor afferent endings. Other neurotransmitters may participate in the induction of discharge. This model has not been fully validated, but it can be experimentally tested. Although we have focused on the role of ion channels in hypoxic chemotransmission in this review, current carotid body research is also searching for answers to major questions such as the identification of oxygen-sensitive molecules, other mechanisms involved in glomus cell excitation, and the identification of excitatory and inhibitory neurotransmitters.

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