invited review

Oxygen sensing by the carotid body chemoreceptors

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Prabhakar, Nanduri R. Oxygen sensing by the carotid body chemoreceptors. J Appl Physiol 88: 2287–2295, 2000.—Carotid bodies are sensory organs that detect changes in arterial blood oxygen, and the ensuing reflexes are critical for maintaining homeostasis during hypoxemia. During the past decade, tremendous progress has been made toward understanding the cellular mechanisms underlying oxygen sensing at the carotid body. The purpose of this minireview is to highlight some recent concepts on sensory transduction and transmission at the carotid body. A bulk of evidence suggests that glomus (type I) cells are the initial site of transduction and that they release transmitters in response to hypoxia, which causes depolarization of nearby afferent nerve endings, leading to an increase in sensory discharge. There are two main hypotheses to explain the transduction process that triggers transmitter release. One hypothesis assumes that a biochemical event associated with a heme protein triggers the transduction cascade. The other hypothesis suggests that a K⁺ channel protein is the oxygen sensor and that inhibition of this channel by hypoxia leading to depolarization is a seminal event in transduction. Although there is body of evidence supporting and questioning each of these, this review will try to point out that the truth lies somewhere in an interrelation between the two. Several transmitters have been identified in glomus cells, and they are released in response to hypoxia. However, their precise roles in sensory transmission remain uncertain. It is hoped that future studies involving transgenic animals with targeted disruption of genes encoding transmitters and their receptors may resolve some of the key issues surrounding the sensory transmission at the carotid body. Further studies are necessary to identify whether a single sensor or multiple oxygen sensors are needed for the transduction process.

heme proteins; potassium channels; sensory transduction; transgenic animals; hypoxia

AN ADEQUATE SUPPLY OF OXYGEN is essential for the survival of mammalian cells. A decrease in oxygen availability (i.e., hypoxia) within seconds increases breathing, which is essential for maintaining oxygenation. These compensatory ventilatory adjustments during hypoxia critically depend on the oxygen-sensing ability of the peripheral chemoreceptors, especially the carotid bodies. Carotid bodies are sensory receptors that detect changes in the oxygen level in the arterial blood, and the sensory information is relayed to brain stem neurons that regulate breathing. It is appreciated that oxygen sensing by the carotid body is crucial for life in mammals and, in fact, may play a life-or-death role in situations involving acute hypoxia. However, the transduction and transmission mechanisms at the carotid body chemoreceptors are not well understood. During the past decade, much progress has been made toward understanding the oxygen-sensing mechanisms at the carotid body. This is mainly due to the application of newly developed techniques such as patch clamp, microfluorometry, and microvoltammetry to the carotid body cells to unravel the cellular mechanisms of sensory transduction. The progress in this area is quite evident from recent proceedings of an international symposium devoted to oxygen-sensing mechanisms in general and to the carotid body in particular (19). The purpose of this minireview is to highlight some of the

First in a series of invited mini-reviews on “Hypoxia Influence on Gene Expression.”
recent concepts regarding transduction and transmission of the hypoxic stimulus at the carotid body and to discuss briefly some of the existing controversies. Elsewhere in this issue, Dr. Donnelly (11a) will discuss the developmental aspects of the carotid body and its role in pathophysiological situations such as sudden infant death syndrome.

SENSORY TRANSDUCTION AT THE CAROTID BODY: EVIDENCE FOR GLOMUS CELLS AS THE PRIMARY SITE OF SENSORY TRANSDUCTION

The chemoreceptor tissue is primarily composed of two cell types: type I and type II. Type I cells (also called glomus cells) are of neural crest origin (and thus are neuronal phenotype) and possess dark, as well as clear, cored vesicles, suggesting that they are secretory cells. The type II cells (also called sustentacular cells) resemble glial cells of the nervous system. The sensory complex is formed by an afferent nerve fiber (a branch of the glosopharyngeal nerve) that branches extensively to innervate clusters of glomus cells. Type II cells envelop this complex. Electron microscopic analysis reveals synaptic contacts between glomus cells and the nerve endings as well as reciprocal synapses (24).

Several hypotheses have been formulated that have advocated each of these elements (i.e., glomus cell, type II, and the nerve ending) as the primary site of sensory transduction. It has been suggested that neurons in the petrosal ganglion (the sensory ganglion that innervates the carotid body) are the putative site of the transduction process (25). Recently, Alcayaga et al. (2) examined the effects of hypoxia and cyanide on the activity of petrosal neurons in vitro. These authors found that cyanide augments but hypoxia has no excitatory effect on the discharge frequency of petrosal neurons. Although it is known that the cell body of petrosal neurons may not respond to hypoxia, the question of whether the sensory activity of dendrites arising from these neurons is affected by low oxygen has not been critically examined. Thus the role of afferent nerve endings and/or petrosal neurons as the site of sensory transduction remains uncertain. Virtually no information exists on the role of type II cells in the transduction process.

The bulk of the evidence, however, suggests that glomus cells are the initial sites of sensory transduction. First, sensory discharge no longer increases in response to hypoxia after cryoablation of glomus cells (42). Second, sinus nerve neuromas do not respond to hypoxia in the absence of the carotid body (34). Third, isolated glomus cells respond to hypoxia by releasing neurotransmitters (26). Fourth, when neurons from the petrosal ganglion are cocultured with glomus cells, only those neurons that developed functional contact with glomus cells respond to hypoxia with an increased neural activity (45). These studies support the notion originally proposed by De Castro (9) that glomus cells are the primary oxygen-sensing cells.

Although it seems certain that glomus cells are crucial for the sensory transduction, it is uncertain whether all glomus cells respond to hypoxia and whether they are a homogeneous population of cells. For instance, it is clear from morphological studies that only a certain population of glomus cells receive afferent innervation. Furthermore, there is marked variation in transmitter composition within glomus cells (43). In addition, cellular responses to hypoxia vary among glomus cells. Pang and Eyzaguirre (31) reported that some glomus cells depolarize and others hyperpolarize in response to a given hypoxic challenge. Similar variations were also noted in the responses of glomus cell to hypoxia in terms of changes in intracellular pH (32) and Ca²⁺ concentration (6). The functional consequence of these variations among glomus cells, however, has not been critically explored. It is possible that the transduction process is initiated only at certain glomus cells (perhaps those cells that receive afferent innervation or vice versa). However, another population of glomus cells, by virtue of intercellular connections (gap junctions, see Electrical Transmission), may regulate the activity of transducing glomus cells.

MECHANISMS OF SENSORY TRANSDUCTION

On the basis of the characteristics of glomus cells, it has been proposed that hypoxia releases an excitatory neurotransmitter(s) that acts on nearby afferent nerve endings leading to an increase in sensory discharge. There are several hypotheses to explain how hypoxia triggers transmitter release from glomus cells (i.e., the transduction process). Figure 1 illustrates the current models of oxygen sensing by the glomus cells. Essentially, the theories proposed can be categorized under two major hypotheses. One hypothesis assumes that a heme and/or a redox-sensitive enzyme is the oxygen sensor and that a biochemical event associated with the heme protein triggers the transduction cascade. The other hypothesis suggests that a K⁺ channel protein is the primary oxygen sensor and that inhibition of this channel by hypoxia is the seminal event in transduction. There is a body of experimental evidence supporting and questioning each of these hypotheses. Both theories are, in fact, interrelated and not mutually exclusive, and perhaps the truth lies somewhere between both.

Heme-Containing Enzymes as Oxygen Sensors

Mitochondrial cytochromes. Molecular oxygen is an essential substrate for many biochemical reactions, including those involved in energy production. Oxygen binds to heme with high affinity, and several biologically important enzymes, especially those associated with mitochondrial respiration (such as cytochromes), contain heme. According to this idea, the oxygen-sensing process involves mitochondria in glomus cells and a cytochrome with an unusually low affinity for oxygen serves as the oxygen sensor. In support of the mitochondrial hypothesis, Bisceo et al. (3) reported that hypoxia causes mitochondrial depolarization in glomus cells. More importantly, hypoxia-induced mitochondrial depolarization in glomus cells occurred at much higher PO₂ values compared with depolarization of
mitochondria in neurons of the dorsal root ganglion (3). These observations indicate that mitochondria in glomus cells are sensitive to modest changes in oxygen compared with other neuronal cells. It has been repeatedly demonstrated that substances such as cyanide, which inhibit mitochondrial respiration, mimic the effects of hypoxia on sensory discharge. Specific inhibitors of mitochondrial respiration (e.g., antimycin A) also augment the basal sensory activity and prevent the sensory response to hypoxia but not to CO2 (27). More recent studies have shown that high concentrations of CO augment sensory discharge in a reversible manner similar to hypoxia (20, 44). The stimulatory effect of CO seems to coincide with inhibition of cytochrome(s) presumably of mitochondrial origin, as evidenced by spectral analysis. Although these studies seem to support the role of mitochondria in transduction, there are still some unanswered issues regarding this hypothesis. First, and perhaps most importantly, how a change in the oxidation state of a mitochondrial cytochrome(s) is linked to an increase in afferent nerve activity remains uncertain. Second, the identity of a cytochrome with low affinity for oxygen remains elusive.

High concentrations of CO also inhibit the activity of other heme-containing enzymes, most notably that of nitric oxide synthases (NOS). Recent studies have shown that NOS-1 is expressed to mitochondria in certain cells and that inhibition of NOS stimulates carotid body activity (36). Therefore, it would be of considerable interest in future studies to explore whether NOS is expressed in mitochondria of glomus cells and, if so, to assess its significance in transduction. Thus further studies are definitely needed to
identify the nature and significance of mitochondrial cytochrome(s) and/or other heme-containing enzymes in glomus cells in the transduction process.

Nonmitochondrial heme-containing enzymes. There is increasing evidence that several nonmitochondrial enzymes contain heme and that their enzymatic activity critically depends on the oxygen availability. The potential role(s) of the nonmitochondrial heme-related enzymes in the sensory transduction is discussed below.

NADPH oxidases and reactive oxygen species. It has been postulated that NADPH oxidase, a heme-containing nonmitochondrial enzyme, is involved in oxygen sensing (1). According to this model, NADPH oxidase is expressed in glomus cells and produces reactive oxygen species (ROS) such as \( \text{H}_2\text{O}_2 \), which in turn regulate membrane potential via \( K^+ \) channels. Oxygen supply determines the amount of \( \text{H}_2\text{O}_2 \) formed by the oxidase, and hypoxia decreases \( \text{H}_2\text{O}_2 \) formation, leading to depolarization and transmitter release. This hypothesis is supported by evidence showing that \( \text{H}_2\text{O}_2 \) or organic peroxides inhibit sensory discharge of the carotid bodies (1, 8). Furthermore, various protein subunits of the NADPH oxidase complex have been identified in type I cells of human carotid bodies (18). Diphenyllethiodonium (DPI), a purported inhibitor of NADPH oxidase, augmented the basal sensory activity and blocked further augmentation by hypoxia (8). However, recent studies questioned the role of NADPH oxidase in oxygen-sensing mechanisms. First, DPI is not a specific inhibitor of NADPH oxidase, in that it can inhibit a variety of other flavoproteins. Other putative inhibitors of NADPH oxidase, such as neopterin or phenylarsine oxide, do not mimic the effects of hypoxia on catecholamine release from carotid bodies (28). More importantly, the chemosensory response to hypoxia seems to be unaffected in mutant mice deficient in the gp91 phox subunit of the NADPH oxidase (16). Recent studies, however, indicate a lack of correlation between catecholamine release and the sensory response to hypoxia (10). Thus monitoring only the catecholamine release as an index of oxygen sensing may not be adequate for assessing the role of NADPH oxidase in the carotid body response to hypoxia. Furthermore, it is possible that NADPH oxidase may still be generating ROS, albeit at a lower level, in the absence of the gp91 phox subunit. Therefore, it is not surprising that the sensory response of the carotid body is still preserved after the gp91 phox subunit of NADPH oxidase is deleted. In addition to NADPH oxidase, several redox-sensitive enzymes, including NADPH-cytochrome P-450 reductases and xanthine oxidases, also generate ROS. Glomus cells from rat carotid body express NADPH-cytochrome P-450 reductase, and its activity can be inhibited by DPI (unpublished observations, see below). In view of these observations, the potential contribution of other ROS-generating enzymes to oxygen sensing in the carotid body should be considered in future studies. It is essential to determine whether ROS levels are decreased in glomus cells by hypoxia and to determine whether changes in ROS are coupled to alterations in membrane potential and transmitter release. Thus much work needs to be done to further understand the role of NADPH oxidase and/or other related enzymes that generate ROS in the transduction process at the carotid body.

NOS, heme oxygenases, and NADPH cytochrome c reductase. Nitric oxide (NO) is endogenously generated by NOS. The synthesis of NO critically depends on oxygen, and its biological actions depend on binding to heme ligands; both of these facts led to the suggestion that NO is involved in oxygen chemoreception at the carotid body (36). NOS-1 and NOS-3 are constitutively expressed and contain heme, and the COOH-terminal fragment of NOS is homologous to NADPH-cytochrome c reductase, a flavoprotein. Histochemical data showed that NOS-1 and NOS-3 are expressed in afferent nerve fibers and blood vessels of the carotid body, respectively (36). The following lines of evidence support the idea that NO, like oxygen, inhibits carotid body activity. First, inhibitors of NOS mimic the effects of hypoxia. Second, NO donors inhibit the sensory activity (see Ref. 36). It is likely that NO released from nerve endings regulates glomus cell activity by acting as a retrograde messenger, as it does elsewhere in the nervous system. Consistent with this idea, it has been shown that NO donors decrease intracellular Ca\(^{2+}\) concentration and inhibit Ca\(^{2+}\)-currents in glomus cells (36, 39). Interestingly, like oxygen, NO affects preferentially L-type Ca\(^{2+}\) channels in glomus cells (39).

Studies with mutant mice deficient in NOS isoforms. Mice with targeted disruption of NOS-1 and NOS-3 isoforms are available, and they offer excellent models for delineating the role of NO in the carotid body. Recently, the ventilatory responses to hypoxia of mutant mice deficient in NOS-1 isoform were examined (see Ref. 36). These investigators found that NOS-1 mutant mice exhibited enhanced ventilatory responses to hypoxia. Respiratory responses to systemic administration of cyanide and depression of breathing in response to brief hyperoxia (Dejour’s test) were also enhanced in mice deficient in NOS-1. These observations provide indirect evidence for enhanced peripheral chemoreceptor sensitivity in NOS-1 mutant mice and suggest that NO generated by NOS-1 is inhibitory to the carotid body activity.

How might NO contribute to transduction of the hypoxic stimulus? In vitro biochemical studies have shown that hypoxia inhibits NOS activity in the carotid body (36). On the basis of biochemical data, it has been proposed that NO is continuously generated during normoxia and keeps the sensory discharge low; in part by its action on glomus cells. Hypoxia decreases the rate of NO generation, thus relieving the inhibition (disinhibition), causing increased sensory discharge (36). Although this is an interesting idea, the evidence thus far indicates that NOS is not expressed in glomus cells, the putative site of transduction in the carotid body. Rather, it is localized to afferent nerve fibers innervating glomus cells (see Ref. 36). Recently, on the basis of spectral analyses, it has been proposed that a
heme-containing protein expressed in the afferent nerve fibers might be involved in oxygen sensing (20). Because afferent nerve fibers in the carotid body express NOS, which is a heme-containing enzyme, it is likely that NOS might serve as the oxygen sensor.

Endogenously generated CO has many physiological roles parallel to NO. CO is generated by heme oxygenases (HO-1 and HO-2) in concert with NADPH cytochrome c reductase. The similarities between CO and NO prompted our interest in whether the carotid body expresses HO-1 or HO-2 and, if so, whether CO plays a significant role in oxygen sensing. Immunocytochemical data revealed HO-2-like immunoreactivity in glomus cells of both cats and rats (36). Recently, NADPH cytochrome c reductase-like immunoreactivity was found in glomus cells of the rat carotid body (unpublished observations). These observations indicate that glomus cells have enzymatic machinery necessary for the synthesis of CO. Inhibitors of HO-2 augmented sensory discharge of the carotid bodies in vitro and elevated the intracellular Ca$^{2+}$ concentration in glomus cells (36). These studies suggest that endogenously generated CO inhibits carotid body activity.

As suggested previously, NADPH cytochrome c reductase generates low levels of ROS and it is inhibited by DPI (41). It is possible that the enzymes HO-2 and NADPH cytochrome c reductase, which require oxygen for their activities, might keep the sensory discharge low under normoxia by way of CO as well as ROS generation. During hypoxia, generation of these molecules is inhibited or reduced, thus removing the inhibition that leads to augmentation of the sensory discharge. Although this idea remains attractive, further experiments are necessary to establish whether hypoxia reduces CO, ROS, or NO levels in intact carotid bodies and, if so, whether the kinetics of the changes in gas molecules parallel the sensory response to hypoxia.

**Ion Channels as Oxygen Sensors**

On the basis of an original idea by De Castro (9), it has been proposed that depolarization of glomus cells by hypoxia is central to the transduction process (23). According to this scheme, low oxygen directly depolarizes glomus cells, causing an influx of Ca$^{2+}$ through voltage-gated Ca$^{2+}$ channels, leading to release of neurotransmitters (Fig. 1B). Although this is seemingly a simple scheme, until a decade ago very little was known about the cellular basis for the initial depolarization of glomus cells induced by hypoxia. With the advent of patch-clamp technology, considerable advances have been made in the identification of various ionic conductances in glomus cells. It is fairly well established that glomus cells express K$^+$, Ca$^{2+}$, Na$^+$ (perhaps in rabbit only), and Cl$^-$ currents. Potential involvement of ion channels, especially K$^+$ channels, in the sensory transduction at the carotid body is discussed below.

K$^+$ channels in the transduction process. Lopez-Barneo and co-workers (22) were the first to report that hypoxia inhibits a K$^+$ current in glomus cells. Subsequent studies have confirmed these observations (23). Although it is clear that hypoxia inhibits K$^+$ currents in glomus cells, the type of K$^+$ current varies among species. In rabbit glomus cells, hypoxia inhibits transient K$^+$ current, whereas, in rat glomus cells, it inhibits a large-conductance Ca$^{2+}$-activated K$^+$ current. More importantly, the inhibition of K$^+$ current by hypoxia could be seen in isolated membrane patches in rabbit glomus cells but not in the rat, suggesting a direct effect of low oxygen on the channel protein in the former but not in the latter (21). These studies led to the view that K$^+$ channels are the primary sensors for oxygen and that the hypoxia-sensitive K$^+$ channels contribute to the initial depolarization essential for the transduction process. However, the contribution of oxygen-sensitive K$^+$ channels to the current at the resting membrane potential is questionable. First, these channels are not active at the reported resting membrane potential of glomus cells. Second, known blockers of oxygen-sensitive K$^+$ channels had no effect on basal or hypoxia-stimulated sensory activity of the carotid body or on intracellular Ca$^{2+}$ in isolated glomus cells (11). These observations question the central role of oxygen-sensitive K$^+$ channels originally described by Lopez-Barneo et al. in the transduction process. However, more recent studies have identified other K$^+$ conductances in glomus cells that might potentially contribute to regulation of resting membrane potential and therefore to the transduction process.

Oxygen-sensitive background and/or leak K$^+$ currents. Buckler (7) identified another K$^+$ current that is sensitive to hypoxia in neonatal rat type I cells. This K$^+$ current is quite distinct from the oxygen-sensitive K$^+$ or Ca$^{2+}$-dependent K$^+$ channels described previously in that it is not inhibited by tetraethylammonium (TEA) or 4-aminopyridine and it lacks intrinsic voltage sensitivity or time dependence (7). These biophysical characteristics qualify it as a background or leak current that would be active at the resting membrane potential. In addition to hypoxia, the leak current is reportedly inhibited by metabolic inhibitors, such as cyanide, as well as by uncouplers of mitochondrial oxidative phosphorylation (e.g., dinitrophenol and carbonyl cyanide p-trifluoromethoxyphenylhydrazone). The correlation between the effects of mitochondrial inhibitors on carotid body activity and the inhibition of the leak current suggests that the channel protein responsible for conducting the leak current may be the key element in the transduction pathway. However, confirmation of this idea will require further studies to identify the channel proteins and to identify the effects of specific inhibitors of the channel on carotid body activity.

Evidence for HERG-like K$^+$ channel in regulation of the membrane potential of glomus cells. Inwardly rectifying K$^+$ channels contribute to the resting membrane potential in many different cell types that are of neural origin. One such channel is the protein encoded by the human ether-a-gogo-related gene (HERG). The main features of HERG current are a peculiar inward rectification mechanism and a unique sensitivity to methanethiosulfonilide drugs such as dofetilide. Recently, Overholt et al. (29) identified a HERG-like K$^+$ current in type I cells of rabbit carotid bodies. Dofetilide, a specific
blocker of HERG channels, also blocked this current in a concentration-dependent manner, as did high concentrations of Ba\(^{2+}\), but not TEA. The steady-state activation properties of the HERG-like current suggest that it is active at resting membrane potential, and dofetilide, but not TEA, caused a significant depolarizing shift in the resting membrane potential. Furthermore, dofetilide elevated the cytosolic Ca\(^{2+}\) concentration in glomus cells and augmented the sensory discharge of the carotid bodies in vitro. In addition, RT-PCR analysis of rat carotid bodies revealed mRNAs encoding ether-a-go-go-related gene (ERG)-1 and ERG-3 (unpublished observations). These observations suggest that glomus cells express a HERG-like current that is active at resting membrane potential and responsible for controlling the resting membrane potential in rabbit glomus cells. However, the oxygen sensitivity of HERG-like channels has not yet been examined. In this context, it is interesting to note that the HERG channel protein contains a PAS domain that is highly conserved among other proteins that are sensitive to oxygen (33). Furthermore, ROS have been shown to modulate the activity of HERG channels expressed in Xenopus oocytes (40). Therefore, it is possible that hypoxia modulates HERG-like channels either directly by affecting the channel protein or indirectly via ROS or related gas molecules. These possibilities, however, remain to be investigated.

In summary, the patch-clamp technique has been instrumental in elucidating the role of ion channels in glomus cells and their possible roles in the transduction process. However, many questions remain unanswered with respect to the identification of ion channels that regulate membrane potential and the mechanisms by which hypoxia regulates ion channel activity.

Second Messenger Systems

It is fairly well established that hypoxia increases the intracellular Ca\(^{2+}\) concentration in glomus cells and much of the Ca\(^{2+}\) flux is mediated by voltage-activated Ca\(^{2+}\) channels. Recent studies have shown that Ca\(^{2+}\) currents in glomus cells are conducted by at least four types of voltage-gated Ca\(^{2+}\) channels, including L, P, N, and a resistant current (30). There is no evidence for the presence of low-voltage activated (T-type) Ca\(^{2+}\) channels in glomus cells. Initial studies reported that hypoxia either has no effect or inhibits Ca\(^{2+}\) currents in glomus cells (23). However, Summers et al. (38) recently reexamined the effects of hypoxia on Ca\(^{2+}\) currents in glomus cells from rabbit carotid bodies. In their experiments, hypoxia augmented Ca\(^{2+}\) currents in glomus cells. This effect was seen in physiologically relevant CO\(_2\)/HCO\(_3\)-buffered medium but not in medium containing HEPES buffer. Furthermore, they also found that the effects of hypoxia were confined primarily to L-type Ca\(^{2+}\) currents and depended on protein kinase C activation. These observations indicate that L-type Ca\(^{2+}\) current, like K\(^{+}\) current in glomus cells, can be modulated by hypoxia. However, further studies are needed to delineate the contribution of individual types of voltage-gated Ca\(^{2+}\) channels to the increases in intracellular Ca\(^{2+}\) concentration caused by hypoxia. In addition to voltage-gated Ca\(^{2+}\) flux, there is some evidence for mobilization of intracellular Ca\(^{2+}\) stores by hypoxia (3). However, the source and the underlying mechanisms for mobilization of intracellular Ca\(^{2+}\) have not yet been examined.

It is evident that an increase in intracellular Ca\(^{2+}\) concentration is critical for neurotransmitter release by hypoxia. In addition, Ca\(^{2+}\) also activates several enzymes, including protein kinases that are known to mediate cellular responses to a variety of extracellular stimuli. However, besides changes in Ca\(^{2+}\), little is known about the other second messengers associated with the sensory response to hypoxia.

SENSORY TRANSMISSION AT THE CAROTID BODY

Chemical Transmission

Glomus cells satisfy several criteria expected of a presynaptic element involved in chemical transmission in the nervous system. They synthesize and store a variety of neurotransmitters (Table 1). In fact, it is amazing that the carotid body, despite being a small tissue weighing <1 mg, expresses as many neurotransmitters as brain tissue. There is little doubt that neurotransmitters play essential roles in sensory transmission at the carotid body. The question is which one of them is responsible for sensory excitation in response to hypoxia? The following discussion addresses some of the main issues surrounding the chemical transmission hypothesis.

Evidence that hypoxia releases multiple neurotransmitters. Catecholamines, especially dopamine (DA), are the most abundantly expressed neurochemicals in glomus cells. Consequently, much attention has been focused on examining its release in response to hypoxia. Now, it is fairly established that hypoxia releases DA from glomus cells in a Ca\(^{2+}\)-dependent manner (13). However, recent studies (10) indicate that the sensory response to hypoxia is independent of DA release. Fitzgerald et al. (14) have shown that ACh is released from cat carotid bodies in response to hypoxia. Recently, Kumar and co-workers (17) have shown that hypoxia releases substance P (SP)-like peptides from rabbit carotid bodies and that SP-like immunoreactivity is localized to glomus cells and nerve fibers. These investigators have further shown that SP release by hypoxia is Ca\(^{2+}\)-dependent and involves L- and N-type Ca\(^{2+}\) channels. Collectively, these studies demonstrate that hypoxia releases multiple neurotransmitters from the carotid body.

### Table 1. Neurotransmitters in glomus cells

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<th>Biogenic Amines</th>
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<th>Gas Molecules</th>
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<tr>
<td>Acetylcholine</td>
<td>Enkephalins</td>
<td>Heme oxygenase-2</td>
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<tr>
<td>Dopamine</td>
<td>Substance P</td>
<td>NADPH-cytochrome c</td>
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<td>Norepinephrine</td>
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Identity of excitatory neurotransmitter. A large body of evidence indicates that exogenous application of putative neurotransmitter(s) has profound effects on carotid body sensory discharge, ranging from excitation to inhibition (13). A detailed discussion of various transmitters in the sensory transmission at the carotid body is beyond the scope of this review. The present discussion focuses on DA, ACh, and SP because they have received considerable attention as putative excitatory transmitter(s) associated with the sensory response to hypoxia. However, there are several problems surrounding the identity of excitatory neurotransmitter(s). First, the effects of exogenous application of putative transmitters show that there are marked species variations, although hypoxia augments the sensory discharge in all species. For example, DA inhibits the activity of cat but not rabbit carotid bodies, and ACh stimulates cat but inhibits rabbit carotid bodies. Second, it is uncertain whether the effects of endogenously released transmitters on sensory discharge are due to direct action on the afferent nerve ending or indirect action on glomus cells. Third, and perhaps the most serious problem regarding the excitatory neurotransmitter, pharmacological antagonists fail to block the sensory response to hypoxia. For instance, DA receptor antagonists reduce sensory discharge, consistent with the idea that DA functions as an inhibitory transmitter. With respect to ACh, the sensory response to hypoxia could only be partially blocked with combined application of extremely large doses of nicotinic and muscarinic receptor antagonists (see Ref. 14). The neuropeptide SP also stimulates the carotid bodies of various species (35) (except goats) (5), and SP antagonists block the sensory response to hypoxia but not hypercapnia (35). However, with the exception of cats, there is no evidence for the presence of SP in glomus cells or its receptors on afferent nerve endings in other species.

Transgenic animals as potential experimental tools to assess the roles of transmitters in the sensory transmission at the carotid body. In recent years, several transgenic mice with targeted deletion of genes encoding various transmitters and their receptors have been developed. The morphology of the mouse carotid body is similar to that described in other species, and it responds to hypoxia in a manner similar to that described in other species (4). It is hoped that future studies with transgenic mice models combined with in vitro coculture of glomus cells with petrosal neurons may resolve some of the important questions surrounding the sensory transmission at the carotid body.

Interactions between transmitters and their roles in maintenance of the sensory response to hypoxia. The carotid body is a slowly adapting type of sensory receptor in that the sensory excitation continues during the entire period of hypoxia. Therefore, neurotransmitters should initiate as well as maintain the increased sensory discharge during long periods of hypoxia. It is conceivable that excitatory neurotransmitters initiate the sensory response to hypoxia, whereas the inhibitory transmitters prevent overexcitation and thus help to sustain the sensory response. In other words, the excitatory and inhibitory neurotransmitters may work in concert with each other as a push-pull regulatory system. Thus, in future studies, it is worthwhile to focus on the interaction of neurotransmitters in understanding their importance in the maintenance of the sensory response to hypoxia rather than searching for a single excitatory transmitter.

Electrical Transmission

Many cells, including neurons in the central nervous system, are electrically coupled via gap junction proteins. Because of several unresolved issues associated with the chemical transmission hypothesis, it has been proposed that sensory transmission may partly involve electrical coupling (12). According to this model, under resting condition, glomus cells are relatively coupled, allowing flow of molecules and ions between the cells, and release of transmitters is minimal. Hypoxia causes uncoupling and release of neurotransmitters. Furthermore, this hypothesis assumes that electrical coupling plays an important role in the maintenance of sensory discharge during a prolonged hypoxic stimulus. Although this is an interesting hypothesis, additional studies are needed in identifying gap junction proteins in the carotid body and the mechanisms underlying the uncoupling.

SUMMARY AND FUTURE DIRECTIONS

In this review, I have attempted to point out that the prevailing theories on sensory transduction are not mutually exclusive and may, in fact, work in concert. Given that oxygen is a physiological substrate for many biochemical reactions, it is more than probable that the initial event in the transduction involves a chemical event. Consistent with this idea are recent studies suggesting that the β-subunit of the K+ channel resembles the enzyme NADPH-oxidoreductase (15). As previously stated, this enzyme generates ROS, which requires oxygen (41). Perhaps the most challenging question is whether transduction involves single or multiple oxygen sensors. It is likely that additional sensory mechanisms are recruited as the severity of hypoxia increases. Thus, in future studies, it is crucial to identify the role of each of the purported sensors to the carotid body response at varying levels of oxygen. Furthermore, it is well established that the sensory response of the carotid body to hypoxia critically depends on prevailing CO2. Currently proposed transduction schemes for oxygen sensing have not critically examined the role of CO2. With regard to the second messenger systems, much needs to be done. For instance, the role of Ca2+ channels other than L-type in transmitter release has not been critically examined. It is possible that a specific type of Ca2+ channel may be linked to release of a specific neurotransmitter. Although the advances on transduction process have been quite rapid and substantial, progress toward understanding sensory transmission has been relatively slow, and there is a considerable paucity of...
information. One reason seems to be the existing pharmacological approaches, which are not entirely adequate for providing convincing evidence for sensory transmission in general and for identifying an excitatory transmitter(s) in particular. Future studies with transgenic animal models may aid in resolving some of the key issues surrounding the sensory transmission at the carotid body.

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