# Review
# Acute oxygen sensing: diverse but convergent mechanisms in airway and arterial chemoreceptors
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## Abstract

Airway neuroepithelial bodies sense changes in inspired $O_2$, whereas arterial $O_2$ levels are monitored primarily by the carotid body. Both respond to hypoxia by initiating corrective cardiorespiratory reflexes, thereby optimising gas exchange in the face of a potentially deleterious $O_2$ supply. One unifying theme underpinning chemotransduction in these tissues is $K^+$ channel inhibition. However, the transduction components, from $O_2$ sensor to $K^+$ channel, display considerable tissue specificity yet result in analogous end points. Here we highlight how emerging data are contributing to a more complete understanding of $O_2$ chemosensing at the molecular level.

**Keywords:** carotid body, chemoreceptor, hypoxia, neuroepithelial body, $O_2$ sensing

## Introduction

Aerobic metabolism requires an adequate supply of $O_2$, and rapid adaptation to changes in the partial pressures of inspired atmospheric gases is crucial to survival. During episodes of compromised $O_2$ availability, numerous chemosensory systems, acting in concert, rapidly modulate pulmonary ventilation and perfusion to optimise the supply of $O_2$ from alveolus to metabolising tissues. This review focuses on two key systems involved in this homeostatic response: the carotid bodies (CBs) and neuroepithelial bodies (NEBs), representative chemoreceptors of the arterial circulation and the airway, respectively [1,2]. So far, CBs and NEBs, together with pulmonary smooth muscle (which will not be examined in great depth here), have been the most extensively studied of $O_2$-sensitive tissues, and recent investigations have provided major new insights into the expression and interactions of molecular components that link a decreased partial pressure of oxygen ($p_{O_2}$) to appropriate cellular responses in the circulation and respiratory systems.

CBs are highly vascularised organs, located at the bifurcations of the common carotid arteries, that rapidly initiate increased activity in afferent chemosensory fibres of the carotid sinus nerve in response to systemic hypoxaemia. There is widespread agreement that the sensory elements of the CB are the type I (glomus) cells, which contain numerous transmitters and lie in synaptic contact with afferent sensory neurones [1,3]. Type I cells release catecholamines, acetylcholine and ATP in response to hypoxia to initiate afferent discharge [4]. Commonly located at airway bifurcations are NEBs, tight clusters of neurone-derived, transmitter-containing cells that synapse with branches of both afferent and efferent neurones. They evoke appropriate responses to airway hypoxia (as opposed to hypoxaemia) by initiating afferent information to the respiratory centres [5] and releasing peptides and amine modulators [particularly 5-hydroxytryptamine (serotonin)] [6] into the local pulmonary circulation [2]. The prominence of NEBs in neonatal lungs and the association of pathological conditions, such as apnoea of prematurity and sudden infant

CB = carotid body; HPV = hypoxic pulmonary vasoconstriction; $K_{2P}$ channel = tandem P-domain $K^+$ channel; NEB = neuroepithelial body; $p_{O_2}$ = partial pressure of oxygen; PKC = protein kinase C; ROS = reactive oxygen species; TASK = TWIK-related, acid-sensitive $K_{2P}$ channel; TWIK = tandem of P-domains, weakly inward rectifying $K_{2P}$ channel.

death syndrome, with NEB cell hyperplasia strongly suggest that NEBs are involved in both the initiation of breathing at birth and cardiorespiratory control postnatally [7].

Although the specific details of the signal transduction mechanisms that link a decreased $p_{O_2}$ to transmitter release in CBs and NEBs exhibit significant differences, the unifying response elements in both are $p_{O_2}$-sensitive $K^+$ channels [8]. Thus, decreasing $p_{O_2}$ causes, sequentially, $K^+$ channel inhibition [9,10], membrane depolarization [11,12], activation of voltage-gated $Ca^{2+}$ channels and $Ca^{2+}$-dependent transmitter release [13]. This is not generally agreed to be so in pulmonary arterioles; there is still controversy about the relative roles of capacitative/voltage-independent $Ca^{2+}$ entry [14] and $O_2$-sensitive $K^+$ channels in hypoxic pulmonary vasoconstriction (HPV) [15,16].

Investigations into the nature of $O_2$ sensing in CBs and NEBs, from sensor to effector, have had surprisingly similar aetiologies. As more detailed dissection of the signal transduction pathways was required, the use of isolated, cultured and cellular models of CBs and NEBs emerged. Thus, the precise mechanistic perspectives that are now available have been derived from the whole gamut of techniques ranging from human studies through intact CB/sinus nerve and lung slice preparations to cellular and molecular studies in PC12 cells (a rat phaeochromocytoma cell line, a model for CBs), H146 cells (a human small cell carcinoma of the lung cell line, a model for NEBs) and, most recently, knockout and recombinant experiments.

## $O_2$ sensor and signal transduction
It has been clear for some time that putative $O_2$ sensors would be drawn from a pool of proteins that naturally underwent oxido-reductive transitions. Candidates included plasma membrane bound enzymes, cytosolic enzymes and mitochondrial complexes that contained, as key elements in the proposed redox mechanism, one or more transition metals. Thus, iron-containing haem proteins, including cytochromes and NADPH oxidases, were proposed some time ago as potential $O_2$ sensors in a variety of cellular systems. In NEBs, a number of lines of evidence point towards a significant, if not exclusive, involvement of NADPH oxidase in airway $O_2$ sensing [17–19]. The NADPH oxidase model for $O_2$ chemoreception suggests that, under normoxic conditions, the oxidase tonically generates superoxide ($O_2^{-•}$) from $O_2$ which is rapidly converted to $H_2O_2$ by several enzymes including superoxide dismutase and catalase. This $H_2O_2$ is believed to promote channel activity. Thus, native, isolated and cultured NEB cells express a number of important proteins that together constitute the multimeric functional NADPH oxidase enzyme complex, including gp91[phox] and p22[phox] [17]. Hypoxia caused decreased fluorescence of rhodamine 123 (indicative of decreased free radical formation) and $K^+$ channel inhibition, effects that were suppressed by the relatively

non-selective NADPH oxidase inhibitor, diphenylene iodonium ('DPI') [17]. Furthermore, $H_2O_2$ (a product of the oxidase activity) was able to stimulate $K^+$ channels [17].

The suggestion that NADPH oxidase acted as a $O_2$ sensor and transduced the signal via changes in the intracellular redox potential was tested in the human NEB model, H146 cells [12], by exploiting the fact that NADPH oxidase activity can be regulated by the protein kinase C (PKC)-dependent phosphorylation of two components of the complex, p67[phox] and p47[phox] [20]. H146 cells express these proteins, hypoxia suppresses $H_2O_2$ levels, $H_2O_2$ activates 4-aminopyridine-insensitive $K^+$ currents, and hypoxic $K^+$ channel inhibition is suppressed by PKC activation [19]. These results provide direct functional evidence to support a role for NADPH oxidase in this important process and also suggest that PKC might modulate chemoreception by altering the affinity of the oxidase for $O_2$. Recently, the involvement of this oxidase has received further reinforcement by the observation that NEB cell $K^+$ currents recorded from gp91[phox] knockout mouse lung slices were acutely insensitive to acute hypoxia [18].
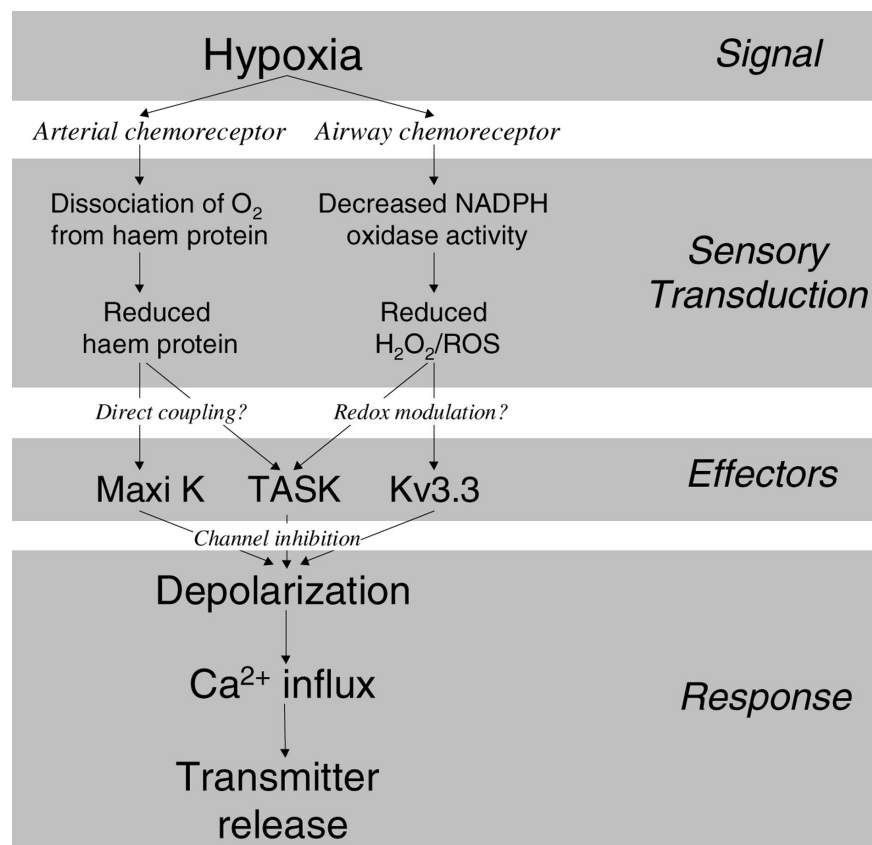
In contrast, the idea that NADPH oxidase provides the upstream signal for $K^+$ channel inhibition has been thoroughly investigated and largely discounted by most investigators in the CB field; the haem hypothesis has gained greater credence since the observation that hypoxic inhibition of $K^+$ channels can be completely reversed upon the application of carbon monoxide [21]. Similarly, the involvement of NADPH oxidase as an $O_2$ sensor in the pulmonary circulation has essentially been discounted by the recent report that HPV is maintained in pulmonary arterioles isolated from gp91[phox] knockout mice [22].

The generation of reactive oxygen species (ROS) from mitochondria, as demonstrated in a number of cell types, has been suggested as one mechanism by which hypoxia can induce a cellular response [23]. However, results from most of these studies are inconsistent with mitochondrial ROS production being the major mechanism for rapid $O_2$ sensing, such as that seen in CBs and NEBs, because ROS are not significantly elevated during the first 10 min of the hypoxic challenge and do not become maximal for up to 2 h [24]. Mitochondrial ROS production is therefore more likely to underlie responses to chronic hypoxia, which exerts effects at the level of the gene. This does not in itself discount mitochondrial involvement in rapid $O_2$ sensing, because specific inhibitors of mitochondrial complexes mimic the actions of hypoxia in isolated type I CB cells [25], suggesting a potential interaction of different ROS-generating systems acting on different timescales.

## Identity of the $O_2$-sensing $K^+$ channels
An interesting parallel has arisen in CB and NEB studies relating to the specific identity of the $K^+$ channels involved

**Figure 1**



Schematic flow diagram illustrating the diverse but converging transduction pathways linking hypoxia to transmitter release from arterial (carotid body) and airway (neuroepithelial body) chemoreceptors. Kv3.3 channel, voltage-activated *shaw* K$^+$ channel (KCNC3); Maxi K, high-conductance, Ca$^{2+}$-activated K$^+$ channel (KCMA1); ROS, reactive oxygen species; TASK, TWIK-related, acid-sensitive K$_{2P}$ channel; TWIK, tandem of P-domains, weakly inward rectifying K$_{2P}$ channel.

in the hypoxic response downstream of the sensor. In both tissues, voltage-dependent and voltage-independent channels have been implicated, and controversy still exists about the physiological contribution of each in the overall cellular response to hypoxia. Studies on CB have been further complicated by genuine species variation [26] (a factor that has not yet been thoroughly investigated for NEBs). In the rat CB, iberiotoxin-sensitive, high-conductance, Ca$^{2+}$-activated K$^+$ (maxi-K) channels were first proposed as being the O$_2$-sensitive channel [27], but several years later this was brought into question with the identification of a low-conductance, acid-sensitive background K$^+$ channel that was proposed to be TWIK-related, acid-sensitive K$_{2P}$ channel-1 (TASK1; TWIK refers to 'tandem of P-domains, weakly inward rectifying K$_{2P}$ channel') – a member of the newly emerging gene family of voltage-insensitive tandem P-domain K$^+$ (K$_{2P}$) channels [28].

The importance of maxi-K in transducing hypoxic stimuli into CB transmitter release had been contested until the recent observation that iberiotoxin (the selective maxi-K

channel inhibitor) could, like acute hypoxia, evoke catecholamine secretion from type I cells in a novel thin slice preparation of CBs [29]. However, the contribution of TASK1 to the overall hypoxic response cannot be discounted, and awaits clarification in a preparation *in situ*. Similarly, a number of K$^+$ channels have been implicated in HPV but recent recombinant studies point toward a voltage-activated *shaw* K$^+$ channel (KCNC1), Kv3.1b, as the primary pulmonary arteriolar effector [16].

In NEBs, a similar controversy has arisen, in part owing to the vexed nature of consistently isolating native NEB cells from airway. At present, hypoxic inhibition of both Ca$^{2+}$-sensitive and Ca$^{2+}$-insensitive K$^+$ currents has been demonstrated in NEBs, both isolated [10] and *in situ* [30], but there has been a paucity of further information on the channels that underlie these currents, because of the unsuitability of primary cultured cells and lung slices for detailed molecular characterisation. A recent approach to this problem has been to establish the H146 cell as an appropriate model in which to study O$_2$ sensing in human

NEB-derived cells [12,19,31]. Employing this model, it has been possible to verify that $O_2$-sensitive channels are insensitive to $Ca^{2+}$ [12], but the contribution of $Ca^{2+}$-activated channels still remains to be investigated robustly in native human cells and lung slices. Notwithstanding that H146 cells and native cells show some differences, it is clear that the $Ca^{2+}$-insensitive components in the two species are almost certainly identical because their pharmacologies and biophysical natures are essentially indistinguishable. On the basis of these observations, debate still rages about the molecular identification of the $Ca^{2+}$-insensitive $K^+$ channel: a voltage-activated *shaw* $K^+$ channel (KCNC3), Kv3.3, is proposed in native NEBs [17] and a TASK-like conductance is suggested in H146 cells [31].

Screening, by reverse-transcriptase-mediated polymerase chain reaction, for all the known human homologues of the $K_{2P}$ gene family has indicated that only TWIK1 and TWIK-related, arachidonic acid-sensitive $K_{2P}$ channel ('TRAAK') are not expressed in H146 cells [32]. Importantly, however, *in situ* hybridisation and immunohistochemical studies have now exclusively localised TASK to mouse NEB cells in lung, and recent antisense knock-down experiments in the H146 cell model have shown a high correlation between quantitative TASK expression and functional hypoxic sensitivity [33]. This antisense approach could not distinguish between TASK1 and TASK3 because they share such high identity in their open reading frame sequences; of considerable import, however, is the recent demonstration that recombinant TASK1 and TASK3 are exquisitely sensitive to decreased $p_{O_2}$ when expressed in HEK 293 cells [34]. Further pharmacological dissection (using $Zn^{2+}$ as a discriminating blocker) has now lent support to the notion that the $O_2$-sensitive channel is TASK3, although heterodimerism in H146 cells cannot at present be excluded (PJ Kemp, GJ Searle and C Peers, unpublished data).

## Conclusion

$O_2$ sensing in NEBs and CBs therefore exhibits diverse yet convergent mechanistic features; these are summarised in Figure 1. Upstream, the main $O_2$ sensors in the two tissues are clearly different, although a contribution by mitochondrial ROS generation might be shared. Transduction of the hypoxic signal almost certainly converges, as a unifying theme, on a $K_{2P}$ channel, but how different $K^+$ channels interact to evoke transmitter release and a full physiological response to hypoxia in CBs and NEBs is still debated fiercely and integrative approaches might again be crucial in resolving this important issue.

## Acknowledgements

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