Respiratory Physiology & Neurobiology 174 (2010) 317–330

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Review

A revisit to O_2 sensing and transduction in the carotid body chemoreceptors in the context of reactive oxygen species biology☆

C. Gonzalez*, M.T. Agapito, A. Rocher, A. Gomez-Niño, R. Rigual, J. Castañeda, S.V. Conde, A. Obeso

Departamento de Bioquímica y Biología Molecular y Fisiología, Instituto de Biología y Genética Molecular y CIBER de Enfermedades Respiratorias, Universidad de Valladolid, Consejo Superior de Investigaciones Científicas e Instituto Carlos III, Facultad de Medicina, 47005 Valladolid, Spain

ARTICLE INFO

Article history:
Accepted 1 September 2010

Keywords:
Oxygen-sensing
Transduction
Sensor
Transducer

ABSTRACT

Oxygen-sensing and transduction in purposeful responses in cells and organisms is of great physiological and medical interest. All animals, including humans, encounter in their lifespan many situations in which oxygen availability might be insufficient, whether acutely or chronically, physiologically or pathologically. Therefore to trace at the molecular level the sequence of events or steps connecting the oxygen deficit with the cell responses is of interest in itself as an achievement of science. In addition, it is also of great medical interest as such knowledge might facilitate the therapeutical approach to patients and to design strategies to minimize hypoxic damage. In our article we define the concepts of sensors and transducers, the steps of the hypoxic transduction cascade in the carotid body chemoreceptor cells and also discuss current models of oxygen- sensing (bioenergetic, biosynthetic and conformational) with their supportive and unsupportive data from updated literature. We envision oxygen-sensing in carotid body chemoreceptor cells as a process initiated at the level of plasma membrane and performed by a hemoprotein, which might be NOX4 or a hemoprotein not yet chemically identified. Upon oxygen-desaturation, the sensor would experience conformational changes allosterically transmitted to oxygen regulated K^+ channels, the initial effectors in the transduction cascade. A decrease in their opening probability would produce cell depolarization, activation of voltage dependent calcium channels and release of neurotransmitters. Neurotransmitters would activate the nerve endings of the carotid body sensory nerve to convey the information of the hypoxic situation to the central nervous system that would command ventilation to fight hypoxia.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

1.1. Sensors and transducers

The words sensor and transducer are widely used in biology, frequently with identical meanings. Both words have Latin roots: sensor derives from the verb *sentire* which means to perceive or to feel, the suffix *-or* indicates the subject that performs the action of perceiving. Transducer is a compound word of the preposition *trans* (across, over, beyond) and the verb *ducere* (to lead, to guide) with the suffix *-er* meaning the one that performs the actions, the one that *leads across*. Thus, from an etymological point of view both words would have different meanings. A sensor is a device, a molecular entity or even a more organized cellular element capable of perceiving a change in its surroundings and generating a measurable change. *The sensor elements enter in contact with the stimulus, the measured variable of the surrounding milieu*. For example, sensing of light intensity by photoreceptors consists in the absorption of photons energy by 11-*cis*-retinal and the genesis of 11-all-*trans*-retinal; i.e., the measurable change in light sensing is the genesis of 11-all-*trans*-retinal. In carotid body (CB) arterial chemoreceptors we shall propose as a O_2 sensor a hemoprotein (*vide infra*), which having a given molecular spatial structure when saturated with O_2, acquires a new spatial configuration on O_2 desaturation; this conformational change, much alike occurring in hemoglobin on its O_2 saturation–desaturation cycle, would be the measurable signal generated by the O_2-sensor. From there on, transduction starts (Fig. 1). *The transducer element(s) do(es) not enter in contact with the variable measured, it/they cannot directly "sense" the stimulus.* Instead the transducer elements or the transduction process convey the measurable signal generated at the sensor to the effector, in a single or multi step process, transforming it in a new signal intelligible to the system. In sensory physiology the transduction process usually is a multistep process known as a whole as the transduction cascade, and the intelligible signal to the sys-

☆ This paper is part of a special issue entitled "Physiological Redox: Regulation in Respiratory, Vascular, and Neural Cells", guest-edited by Paul T. Schumacker and Jeremy P.T. Ward.

* Corresponding author. Tel.: +34 983 42 30 89; fax: +34 983 42 35 88.
E-mail address: constanc@ibgm.uva.es (C. Gonzalez).

1569-9048/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.resp.2010.09.002

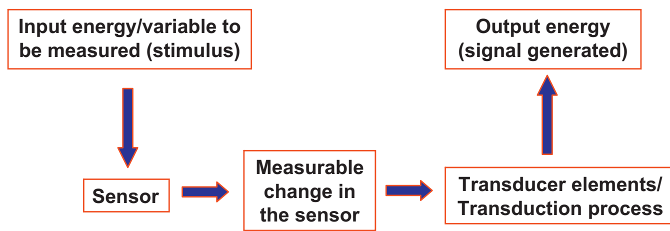


Fig. 1. Flow diagram showing the minimal operational steps in a sensor coupled to a transduction machinery. Note that "sensu stricto" in the basic functioning of the sensor, in the sensing process, there is a transduction. It might be a dilation or contraction as it is the case in a thermometer of mercury or a conformational change of a protein as it is the case in the odorant receptor molecules. The transduction machinery captures the signal generated by the sensor and in a single or multistep process generates an output, a new signal that being intelligible to the system contains a representation of the parameters of the stimulus.

tem is electrogenesis (Grundfest, 1971), i.e., the genesis of action potentials with a frequency and interval distribution specific for the nature and intensity of the sensed signal. Taking another example from sensory physiology we can consider odorant sensing and odorant transduction cascade. Odorant sensing should be defined as the conformational change that occurs in the odorant receptor when an odorant molecule binds to it. The conformational change triggers the transduction cascade which is initiated by the activation of specific G-proteins which in turn promote the activation of an adenylyl cyclase and the increase of cAMP levels; cAMP activates cationic channels which allow the movement of positive charged ionic species into the olfactory cilia causing their depolarization and subsequent triggering of conducted action potentials. These considerations not only apply to sensory physiology, as the action of hormones and growth factors in bodily cells, or antigen recognition by cell of the immune system, are initiated by sensing their presence by receptors (sensors). These receptors, upon binding of the signaling molecule, experience conformational changes which initiate the transduction cascade specific to the signal.

We have mentioned before that in sensory physiology, and the CB is a sensory receptor, the transduction cascade culminates in electrogenesis with specific codes of frequencies and intervals that are specific to the stimulus detected by the sensor. This assert is precise in primary sensory receptors (i.e., sensory receptors in which the sensing device and the transduction and electrogenesis machineries are located in the primary sensory neuron). However, in secondary sensory receptors the sensing device and transduction cascade reside in a cell that communicates with the cell responsible for the electrogenesis via a synapse. Therefore the transduction cascade culminates in a modification (usually an increase) of the rate of the release of neurotransmitters, being the stimulus-induced release of neurotransmitter which conveys the information to the cell responsible for the generation of the conducted electrogenesis (Grundfest, 1971). Although it is not uncommon to include the synaptic transmission within the transduction cascade in secondary sensory receptors, following a reductionist approach, we believe that it is more appropriate to consider the transduction cascade and the synaptic transmission as independent processes, both having specific tools and methodologies of investigation. Sensing and transduction process can even be more complex as it happens for example in photoreceptors, where the conveying of the information contained in the stimulus to cells endowed with the capacity to generate action potentials and to conduct them to central nervous system (the retinal ganglion cells) is achieved through a complex net of cells and synapses.

1.2. The need for oxygen sensing and transduction cascades

Mammalian cells depend on oxygen for survival. In cell mitochondria the equivalents of reduction present in digestion-processed nutrients are transferred in an ordered sequence through the respiratory chain complexes down to molecular O_2, which represents the final acceptor of electrons to generate water. The stepwise transfer of electrons through the respiratory chain is accompanied by H^+ extrusion out the mitochondria. This H^+ extrusion, coupled to a near impermeability of internal mitochondrial membrane, generates a strong electrochemical gradient for H^+ with the mitochondrial interior negative and alkaline in reference to cell cytoplasm. This H^+ gradient directed to the mitochondrial interior is used to force a special ATPase to work in backwards mode and to synthesize ATP from ADP + Pi. In this manner, a significant part of the energy-content of nutrients (another significant part is lost as heat) is transferred to ATP, a compound that due to its chemical structure is capable of releasing such energy to be used by cells. The H^+ entering via mitochondrial ATPase react with reduced molecular O_2 to form water. From this outline it is evident that O_2 availability is nearly equivalent to ATP availability for cells to perform their functions (we are neglecting glycolytic anaerobic pathway which in most cells generates about 10% of ATP). These functions include the maintenance of their homeostasis and integrity, via de novo synthesis of continuously renewed cell constituents, the performance of external work.

The issue on this article is to discuss what happens when cells became hypoxic, i.e., when the PO_2 or O_2 molar concentration in the mitochondrial interior is low enough to be capable of accepting the amounts of electrons required to support an adequate rate of ATP synthesis. Do cells sense the O_2-deficit? Which is (are) the molecular identity(ies) of the O_2-sensor(s)? How do cells transduce the signal generated by the O_2-sensor in teleologically meaningful responses to cell and organism survival?

In this regard, we can distinguish in mammalian organisms two types or categories of cells. The first category is represented by the great majority of cells of the organism. Cells in this category are endowed with capacity to sense O_2 deficit with a high threshold, i.e., they detect intense hypoxias and only react to O_2 deficiency when PO_2 is very low generating responses directed to their own survival. The second category (Fig. 2) is formed by small groups of specialized cells that sense O_2 deficit with a low threshold, i.e., they detect moderate decreases in PO_2 and, through regulatory loops, generate responses aimed to restore or secure an adequate PO_2 for the entire organism. This category of specialized cells includes: chemoreceptor cells of the CB and aortic bodies chemoreceptors, smooth muscle cells of the pulmonary arteries and erythropoietin producing cells of the kidney. We can name these cells as cells endowed with sensitivity to physiological hypoxia (Gonzalez, 1998). Neonatal adrenal medulla chromaffin cells are also endowed with sensitivity to physiological hypoxia, however they lose their intrinsic O_2-sensitivity early in postnatal age when adrenal medulla becomes functionally innervated by splanchnic nerves (Seidler and Slotkin, 1985; Rico et al., 2005). Chemoreceptor cells of the airways neuroepithelial bodies also are oxygen sensitive in neonatal animals (Cutz et al., 2009), but become greatly atrophic in adult mammals (Cutz, 1997).

In the present article we are going to refer mostly to acute oxygen sensing in the CB, but for comparative purposes we will also make occasional considerations to acute oxygen sensing in other cells with physiological sensitivity to hypoxia. Additionally, we will also mention oxygen sensing and transduction when hypoxia is sustained; we will do so first, because it is physiologically relevant as it happens when permanently living or sojourning at high altitude and secondly, because there are many pathological process that course with general systemic (e.g., lung pathology) or locally restricted hypoxia (e.g. or vascular illnesses).

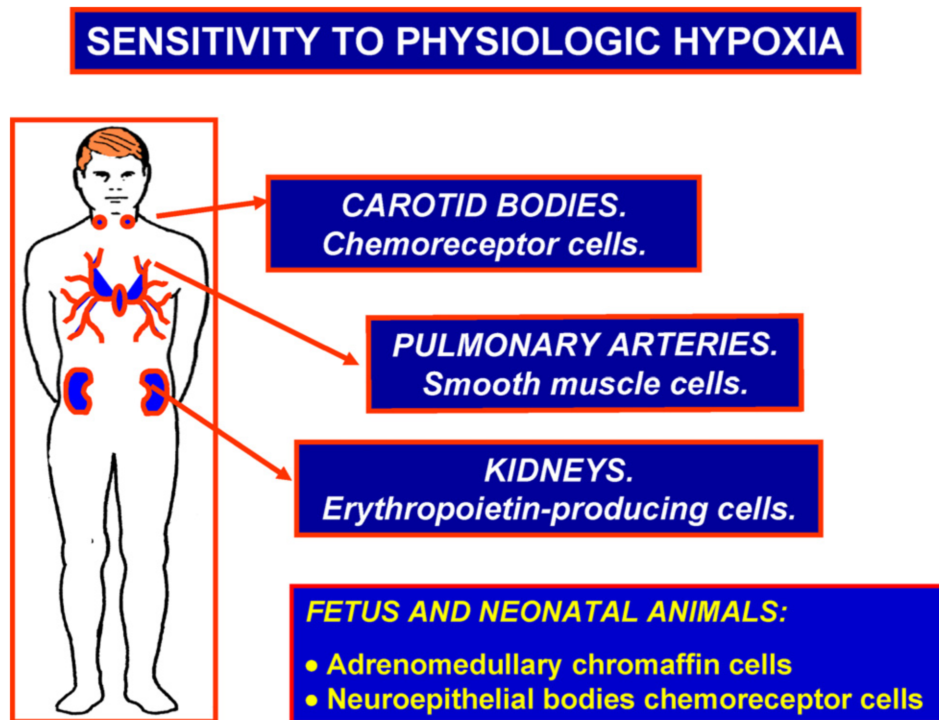


Fig. 2. Cells with low threshold sensitivity to hypoxia. They detect moderate decreases in their surrounding PO_2 and, through regulatory loops, generate responses aimed to restore or secure an adequate PO_2 for the entire organism.

A recent review on oxygen-sensing (Ward, 2008) has a very important section entitled "matching the sensor to the sensed" in which it is nicely discussed the O_2 handling properties of the proposed sensors (K_m or P_{50} for O_2 utilization) with prevailing arterial or ideally cytoplasmic or mitochondrial $[O_2]$ or PO_2 and the functional properties or activity of the O_2 sensing organ. To his knowing ideas on these and others aspects, such as the importance of the concentration of the putative sensor itself, as well as the great importance of the seldom considered concentrations of cofactors or co-substrates in the reactions involved in O_2 sensing, we want to add some extra comment on these aspects pertaining to the CB (see Obeso et al., 1997).

The CB is the organ with the largest blood flow of the entire organism. Whether measured by gravidimetric or radioactive microsphere methods, CB blood flow oscillates between 1.5 and 2 l/100 g/min. Accordingly to this high blood flow, the density of capillaries in the CB represents 25–33% of the surface of histological sections obtained from organs perfused at normal (80–100 mmHg) pressure; just for comparative purposes we should mention that blood flow in rat brain is around 120 ml/100 g/min and the density of capillaries in the cortex is <5% of the surface of the sections. O_2 consumption in the CB measured by several methods (arteriovenous O_2 difference, kinetics of CB tissue PO_2 decay after flow stopping and confinement methods) yields values of around 1.5 ml/100 g/min (i.e., comparatively it is 6–8 times lower than brain cortex). The high blood flow and relatively low O_2-consumption rate implies a very small arteriovenous O_2 difference. Altogether these facts would predict a high tissue PO_2. In fact, models for O_2 diffusion using the above set of values and morphometric data obtained in cat CB showing that the distance from the center of most glomus cells to capillaries is between 10 and 20 μm, have allowed to calculate that less than 4% of CB tissue would have a PO_2 below 40 mmHg, unless a high density of arteriovenous shunts or a high degree of plasma skimming occurs in CB circulation. Since morphological data do not support these particularities for the circulation of the main arterial chemoreceptor organ the conclusion should be that CB tissue PO_2 is high. However, experimental data obtained with O_2 sensitive Clark type microelectrodes *in situ* or in artificially perfused organs with either blood or saline or with phosphorescent dyes have not always generated congruent results (Table 1). From the O_2 consumption rates, the calculations on O_2 diffusion referred above, the data presented in Table 1 and additional data of Whalen and Nair (1976, 1983), we can summarize data of interest to discuss the possible nature of the O_2 sensor in chemoreceptor cells as follows: (a) venous PO_2 in the CB in normoxia would be close to 90 mmHg; (b) mean tissue PO_2 in normoxia would be around 75 mmHg; (c) threshold tissue PO_2 for the activation of the afferent activity in the CSN oscillates between 50 and 65 mmHg; (d) tissue P_{50} for afferent activity in the CSN has been measured to be between 10 and 32 mmHg; (e) maximum activity in the CSN was obtained at tissues PO_2 of 3–5 mmHg. Since as pointed out by Ward (2008) most commonly we refer our findings to arterial blood (or perfusate) PO_2 we could define the general behavior of the CB in the following terms: PO_2 threshold, an arterial PO_2 of 70–75 mmHg; P_{50}, an arterial PO_2 of 40 mmHg; and PO_2 to obtain maximum afferent CSN activity, 10 mmHg (Fidone and González, 1986; Gonzalez et al., 1994). Since even at PO_2 much higher than physiological there is some activity in the CSN, threshold is as the arterial PO_2 on decreasing arterial PO_2 from >100 down to 0 mmHg at which it occurs a change of slope in the activity of the CSN. As expected, in comparing both set of PO_2 values, CB tissue PO_2 and arterial blood PO_2, it is evident a drop in PO_2 from capillaries to tissue of around 15–20 mmHg at arterial blood PO_2 higher than 60 mmHg, corresponding to the flat part of dissociation curve of HbO_2, and smaller drops at arterial blood PO_2 below 60 mmHg, i.e. in the steepest part of the HbO_2 curve.

These characteristics of blood flow, oxygen utilization and tissue PO_2 in the CB conforms with the ideal for the location of an O_2-sensor, as described by Halperin et al. (2006) referring to location of erythropoietin producing cells in the renal cortex. Namely:

Table 1
Reported cat carotid body tissue PO_2.

CB Perfusate	Perfusate pressure	Perfusate PO_2	CB Tissue PO_2	Author
Blood	120	95–113	25	Acker et al. (1971)Acker and Lübbers (1977)
Blood	>70	39	20	Whalen and Nair (1976)
Blood	>70	84	>65	Whalen and Nair (1983)
Saline	*80*	*111 ± 15*	*23 ± 3*	Rumsey et al. (1991)
Saline	*80*	*131 ± 12*	*74 ± 11*	
Saline	*80*	*109 ± 5*	*59 ± 13*	Rumsey et al. (1992)
Blood	*100*	*91*	*49 ± 3*	

All values presented in the table refer to means (±SEM) as reported by the authors. In normal characters data obtained with microelectrodes, in italics data obtained with phosphorescent indicators that measure intravascular PO_2, mostly microvascular PO_2 (Modified from Obeso et al., 1997).

(a) the tissue where the O_2 sensor is located extracts a small proportion of the oxygen that is delivered by unit of blood, making the PO_2 signal easier to recognize; (b) the high blood flow rate improves O_2 diffusion from capillaries to the O_2 sensor, and; (c) there is a constant ratio of the work performed to the CB blood flow rate (i.e., CB consumption of O_2/CB delivery of O_2). The two initial properties are straight forward and easily understood for both renal cortex or CB. The third property makes reference in the case of the kidney to the fact that blood flow and glomerular filtration rate are tightly matched (be aware that glomerular filtration rate dictates the amount of Na^+ to be reabsorbed and, therefore, renal O_2 consumption and PO_2). In the case of the CB it is unknown if the relationship is so tight as it is in the kidney, but it is known than during acute hypoxia (arterial PO_2 close to P_{50}) when the CB activity is enhanced as it is responding to hypoxia, there is an increase in CB blood flow morphometrically verified by several authors, and estimated gravidimetrically in ≈35% increase. Just to mention that this hyperemic response should be the sum of hypoxic (i.e., general metabolic factors involved in hypoxic hyperaemia in any other tissue) and functional, as it is known that some of the neurotransmitters released during hypoxic activation of the CB (e.g., dopamine and nitric oxide) are powerful vasodilators. Even further, it is known that in chronic hypoxia, when the activity of the CB is permanently active, there is a marked enlargement of the CB which results mostly from the increase of CB capillaries (up to 10 times the control capillary volume) and a marked reduction in the mean distance from capillaries to the edge of chemoreceptor cells (see Obeso et al., 1997 for references). A fourth property that Halperin et al. (2006) discuss in relationship with renal cortex blood flow and erythropoietin producing cells is that high renal cortical PCO_2 prevents an additional shift of the hemoglobin dissociation curve by other factors from being a confounding variable. This property would not apply to CB as it has intrinsic sensitivity to variations in arterial PCO_2 which *per se* would probably generate greater changes in CB afferent activity that those indirectly derived from the shifting of the hemoglobin dissociation curve.

2. O_2-sensing and transduction

2.1. The putative nature of oxygen sensors

In the aforementioned article (Ward, 2008; see also Evans and Ward, 2009) distinguished two generic mechanisms of potential O_2 sensors: bioenergetic and biosynthetic. The former type of sensors would signal perturbations of mitochondrial function and energy state, while the second would signal perturbations of O_2-dependent synthesis or degradation of mediators, i.e., they would signal the concentration of a metabolite whose synthesis or degradation requires oxygen; however, as Ward points out, in some places this distinction might become blurred. To these two categories we shall add a third generic mechanism that we will name conformational. In our view conformational O_2 sensors would signal hypoxia as an internal molecular reorganization allosterically transmitted to the next (first) element in the transduction cascade (Lopez-Lopez and Gonzalez, 1992; Gonzalez et al., 1992, 1994, 2007; Riesco-Fagundo et al., 2001; Park et al., 2009).

In Fig. 3 we show, to the left, a flow diagram of the transduction cascade for oxygen chemoreception as our laboratory proposed it in 1992 (Gonzalez et al., 1992). Circled in the flow diagram we include the O_2 sensor and coupling mechanisms and, linked to them by thick arrows, the three putative generic categories of O_2 sensors for acute hypoxia in CB chemoreceptor cells. Each putative category of O_2 sensor is included in an ovoid that contains the principle of its operation and tentative O_2 sensor candidates. The double arrowhead interconnecting the sensor categories intend to indicate that they might be interrelated as for example the main responsible for the bioenergetic status of the cells are mitochondria which in turn are potential sources of ROS, which in turn are the products of NOX4 (and other NOX isoforms). In the transduction cascade we present in *italics* the first known element of the transduction cascade, oxygen sensitive K^+ channels (Almaraz et al., 1986; Lopez-Barneo et al., 1988); the *italics* intend to indicate, aside from the great diversity of O_2 sensitive K^+ channels and the variability of channels amongst species, the discrepancies in the interpretation of the functional significance of some of the O_2-sensitive channels, perhaps best exemplified by maxi K (Wyatt et al., 1995; Buckler, 1997; Donnelly, 1997; Pardal et al., 2000; Gomez-Niño et al., 2009a,b; see Gonzalez et al., 2009). The rest of the steps of the transduction cascade are well accepted and present no major discrepancies except for neurotransmission which, although conflictive, has been recently and excellently reviewed (e.g. Iturriaga and Alcayaga, 2004; Iturriaga et al., 2009; Conde et al., 2009).

2.2. Bioenergetic O_2 sensors

In steady state conditions, the combined functioning of the respiratory (ventilatory) and cardiovascular systems secure and adequate supply of O_2 to the entire population of cells that compose the complex mammalian organisms. In these conditions the amount of O_2 delivered to every cell supports an adequate rate of ATP synthesis for cells to maintain their homeostasis, biosynthetic processes and to perform, in specialized cells, external work. Even in a resting mammalian organism the O_2 (and ATP) requirements vary enormously, existing cells with a high rate of O_2 consumption (e.g., neurons) and cells with much lower energetic demands (e.g. adipocytes). The overall design of the cardiovascular system copes with these resting differences in metabolic requirements providing each tissue-organ with a vascular network of proportional density (Borowsky and Collins, 1989; Obeso et al., 1997). However, as best exemplified by skeletal muscle cells or gut absorptive cells, the energy demand by cells varies according to their degree of activity. Thus, the design must comply with these dynamic variations in energetic requirements.

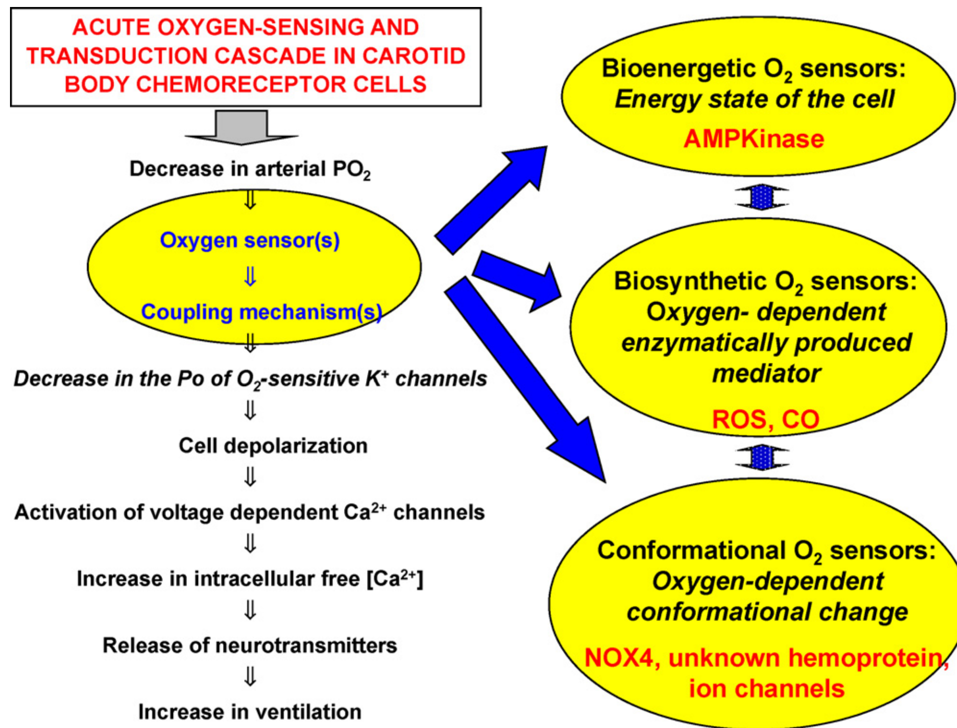


Fig. 3. Oxygen transduction cascade and putative nature of O_2 sensors (see text for explanations).

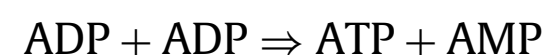
A first and universal mechanism of dynamic adjustment is provided by cells themselves: the products of cell metabolism, which obviously increase in proportion to cell activity, are potent vasodilators making possible a diversion of blood to active tissues. Of course, if the mass of the active tissue is large (e.g. exercise comprising large muscular groups), it might also be required more integrated responses for the respiratory system and heart pumping to deliver enough O_2 to active cells.

A second, and probably also universal, mechanism is represented by the 5′-adenosine-monophosphate-activated protein kinase (AMPK). AMP-activated protein kinases are heterotrimeric complexes consisting of catalytic α-subunits and regulatory β- and γ-subunits. Several isoforms of the three subunits exists in mammalians, and alternative splicing can enlarge the diversity of AMPK in different cells and tissues. The α-subunits have serine/threonine kinase domains at the N-terminus. One of these residues, Thr-172, is conserved in all isoforms and its phosphorylation by upstream kinases (LKB1 serine-threonine kinase; Woods et al., 2003) is absolutely required for AMPK activation and phosphorylation of downstream targets. Binding of AMP to AMPK (in fact to the γ subunit) promotes net phosphorylation of Thr-172 by inhibiting its dephosphorylation and allosterically activating the phosphorylated form of the AMPK. These two effects of AMP cause a multiplicative effect on the activity of AMPK, i.e., a small increase in cellular AMP levels cause a large effect on the kinase (Hardie et al., 1999; Hardie, 2008).

The cells "currency" to perform their normal functions is defined by the energy charge of cells defined by the equation:

$$\text{energy charge} = \frac{[\text{ATP}] + \frac{1}{2}[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

Energy charge of cells can take theoretical values between 1 and 0, all adenosine phosphates are as ATP or all are in the form of AMP, but most cells have steady state normal energy charges between 0.9 and 0.8. On decreasing PO_2 energy charge decreases steadily to a new lower value with a slope defined by the rate of ATP generation at the new PO_2 and the rate of energy expenditure in the tissue or cell type under study; then, the slope of the decrease is higher the lower the PO_2 and final energy charge attained is lower the lower the PO_2. Molar concentrations of free ATP, ADP and AMP vary among cells, with typical values for the three nucleotides, in the range of 5, 0.5 and 0.15 mM/l[1] intracellular water (or about half these values if expressed by kg of fresh tissues). Therefore, typical ADP/ATP and AMP/ATP ratios in normally oxygenated tissues are 0.15–0.1 and 0.06–0.03 (Ridge, 1972; Evans et al., 2005; Roepstorff et al., 2006). Most of ATP is used by cells in enzymatic reactions collectively known as orthophosphate cleavage reactions yielding ADP + Pi. The cleavage of the phosphate bond is energy-yielding and is coupled enzymatically to reactions that utilize the energy to run the cell functions. However, in some very important cellular reactions (e.g., protein elongation) a pyrophosphate cleavage of ATP takes place yielding AMP + P∼P, and no doubt these reactions represent an important source of AMP in cells. Yet probably the highest amount of AMP in cells comes form the action of adenylate kinase, a system of several isoforms of enzymes that catalyze the reaction:



$$\text{ADP} + \text{ADP} \Rightarrow \text{ATP} + \text{AMP}$$

Although this reaction is reversible, in cells in periods of high activity when high amounts of ADP are generated or in situations of hypoxia (or shortage of energetic substrates) when the rate of ATP genesis is slow, the accumulation of ADP displaces the reaction to the right, as a fast strategy to maintain the availability of ATP. Obviously in these circumstances the AMP/ATP ratio increases and this

[1] Correct measurements of AMP and estimation of free AMP are demanding procedures and while ATP and ADP levels, and thereby ADP/ATP ratios are easy to define the AMP/ATP ratio is more variable among different studies. A commonly admitted rule for most cells in steady state conditions is that ATP:ADP:AMP are in proportions 100:10:1, implying that the AMP/ATP ratio will vary as the square of the ADP/ATP ratio, making the former ratio a very sensitive indicator of cellular energy status (Hardie, 2008).

ratio controls the activity of AMPK. Thus in a strict sense AMPK is not an O_2-sensor but rather is a sensor of the energetic status of cells.

In these situations of *metabolic crisis* (Taylor, 2008), generated by the exaggerated ATP consumption during increased activity of cells or by insufficient ATP genesis due to hypoxia or a combination of both, it is needed a fundamental shift in the cellular metabolic strategy to facilitate the creation of an adaptive state which ultimately supports tissue survival. Insufficient ATP genesis due to insufficient substrate delivery to cells is a situation not encountered *in vivo*, because the levels of glucose (the readily energetic substrate for cells) compatible with the life of the animals are high enough to keep the glucose utilizing machinery in cells working efficiently, otherwise animals will enter into hypoglycemia with serious risk to survive. However, in situations of ischemia a chronic decrease in substrate delivery and hypoxia are concurrent. In these situations, the action of AMPK goes beyond the regulation of fast post-translational events aimed to acutely maintain energy balance (see below) and participates along with HIF-1α and 2α and sirtuins (particularly SIRT1) in an orchestrated control of differential expressions of genes in the different cell types in the ischemic tissues directed to secure cell survival and blood vessel growth to solve the ischemic process (Cantó et al., 2009; Guarani and Potente, 2010; Ruderman et al., 2010). These and other aspects (Kahn et al., 2005) of chronic AMPK actions would not be considered further.

The point of interest in our context is which are the effects resulting from AMPK activation as a result of a *metabolic crisis* produced by acute hypoxia and if these effects should be considered as mediators of oxygen-sensing and/or part of the transduction cascade in the chemoreceptor cells of the CB. In every cell studied it has been found that AMPK phosphorylates a great number of enzymes with the end result of promoting catabolic ATP-generating processes and inhibiting ATP-consuming anabolic processes and thereby tending to maintain the cell energy balance (Hardie, 2008; Taylor, 2008; Steinberg and Kemp, 2009). In the case of the CB, in an ample range of hypoxia down to very low arterial PO_2 (25–30 mmHg; Biscoe et al., 1970; Nielsen et al., 1988) it would be, in a sense, inadequate talking of *metabolic crisis* because in spite of the intense hypoxia there was no decline in frequency of action potentials even at these low oxygen tensions, implying a very high rate of ATP synthesis to maintain the enhanced hypoxic activity. In other words, it would not be mitochondrial hypofunction the responsible of the putative decrease in the energy charge of chemoreceptor cells, rather it would be an increase in the ATP consumption result of hypoxic activation (action potential generation, ionic pumping, neurotransmitter release, etc.) which would determine a decrease in the energy charge of the cell and a pull for mitochondrial respiration and increase in ATP synthesis (Erecińska and Wilson, 1982) to match ATP expenditure. Yet, in all probability AMPK plays a critical role in the maintenance of the required energy status by facilitating glucose uptake and oxidation by chemoreceptor cells (Obeso et al., 1989, 1993) as well as transference of fatty acid and their oxidation in mitochondria. In fact in an vitro study on the levels of adenine nucleotides in the CB using normoxic air-equilibrated superfusates *vs.* 10% O_2 as hypoxic solutions, no significant change was observed in ATP or ADP levels at either 4 or 30 min of hypoxia, but after 4 min there was an increase in AMP by a factor of 10 in the hypoxic CBs with no differences observed at 30 min (Verna et al., 1990) and in other study a modest decrease in ATP levels was observed on incubating the CBs with air vs. 100% O_2 equilibrated solutions (Obeso et al., 1985). Therefore, it appears that the requirements for AMPK activation during hypoxic stimulation of the CB are met, as it is the case in pulmonary arteries smooth muscle lysates where is has been shown (Evans et al., 2005) that the AMP/ATP ratio rose from 0.040 under normoxia (155–160 mmHg, 2 h) to 0.083 under hypoxic conditions (16–21 mmHg, 1 h). In keeping with the CB, Wyatt et al. (2007) reported that in neonatal rat AMPK co-localizes at the plasma membrane of chemoreceptor cells with maxiK, that the AMPK activator AICAR inhibited both O_2-sensitive currents expressed in these cells, maxiK and TASK-like channels carried currents, caused chemoreceptor cell depolarization and augmented the afferent activity in the CSN. In a more recent study Dallas et al. (2009) showed using recombinant TASK channels that isoform sensitive to AICAR is TASK-3, a finding which in turn conforms recent findings of Kim et al. (2009) showing that the main O_2 sensitive background current in rat chemoreceptor cells is mostly carried by heteromeric TASK-1/TASK-3 channels and that in mouse TASK-3 is expressed at a higher level than TASK-1 (Ortega-Sáenz et al., 2010). In the study by Wyatt et al. (2007) it was also shown that the AMPK antagonist compound C reversed the just described effects of hypoxia and AICAR on chemoreceptor cells. Although ironically, from a cynic point of view, it might be said that most of the effects seen on AMPK activation and inhibition are epiphenomenal to O_2-sensing, because maxiK inhibition (Gomez-Niño et al., 2009a,b) and genetic elimination of TASK-1 and TASK-3 do not affect hypoxic behaviour of chemoreceptor cells (Ortega-Sáenz et al., 2010). It must be unambiguously stated that the data generated by Wyatt et al. (2007) would indicate that AMPK dependent phosphorylation of K^+ channels in CB chemoreceptor cells mimic hypoxia and appears capable of eliciting integrated CB afferent activity, arguing in the favour that AMPK activation is a key element in the hypoxic transduction cascade. But the question on debate is if AMPK is the primary sensor of low PO_2 in chemoreceptor cells or rather it is an amplifying element in the hypoxic transduction cascade which by phosphorylating channels amplifies their responsiveness to hypoxia. It is the authors opinion that AMPK is not the primary sensor. Data supporting our contention comes from several laboratories that using rabbit and rat chemoreceptor cells to record native O_2 sensitive K currents and channels in isolated membrane patches in the inside out configuration have evidenced that O_2 sensitivity of chemoreceptor cells is a membrane delimited process that does not require any particular metabolic substrate or enzymatic reaction. The observations have also been extended to recombinant channels expressed in heterologous systems (see Section 2.4).

2.3. Biosynthetic O_2 sensors

We are referring to sensors based in the measurement of the concentration of O_2 dependent enzymatically produced mediators such as ROS and CO. In the last three years, since the previous review of our laboratory on the role ROS on oxygen chemoreception in the CB (Gonzalez et al., 2007) there have been substantial changes in the field, both in relation to mitochondrial derived ROS and more significantly on NADPH oxidase structure, subcellular location of potentiality of NADPH oxidase derived ROS for signalling.

2.3.1. Mitochondrial ROS

Literature on acute oxygen sensing and mitochondrial ROS has been dominated during almost the entire decade by opposite views on their significance, mostly centered in hypoxic pulmonary vasoconstriction (HPV) but including oxygen chemoreception in the CB (see Evans and Ward, 2009). In the one side was Schumacker laboratory marshalling the case of hypoxia as trigger of increased ROS production at the complex III of the mitochondrial respiratory chain. Schumacker laboratory proposed that mitochondria functions as *the* O_2 sensors by increasing ROS which would represent the coupling mechanisms with O_2-sensitive K^+ channels (or key players in stabilizing HIF-1α; Chandel et al., 1998, 2000; Waypa et al., 2001; Waypa and Schumacker, 2005, 2006, 2008); in their review of year 2000 they extended their model to O_2 chemoreception in the CB (Chandel and Schumacker, 2000). The opposite view was championed by Archer's laboratory (see Moudgil et al., 2005;

Archer et al., 2008 for representative reviews). In their article of 1995 Weir and Archer concluded: "in pulmonary vascular smooth muscle as in the type I (chemoreceptor) cell of the carotid body, hypoxia causes a reduction in whole-cell potassium current . . .it is likely that the potassium current is controlled by changes in redox status, which could reflect the level of ROS generation by mitochondria or by an oxidase like NAD(P)H oxidase". They also stated that K channels would be closed when reduced (R–SH SH–R) and open when oxidized (R–S–S–R). In 1999 Archer et al. tested the hypothesis using gp91^{phox} (i.e. NOX2) knockouts, and found that animals have preserved HPV and a very low rate of ROS production in comparison to wild type controls. In additional experiments carried out in rat pulmonary arterial rings, they found that diphenyleneiodonium (DPI; an inhibitor of NADPH oxidase) dramatically decreased the normoxic rate of ROS production, indicating that most ROS in the rings in normoxic conditions are originated (within the specificity of DPI; Aldieri et al., 2008) at the NADPH oxidase level; they also observed that hypoxia reduced significantly ROS production to an identical level in control and in DPI treated rings, implying that hypoxia inhibits ROS production both at NADPH oxidase and mitochondria level. Conjugating findings in the knockout mice with the rat data they concluded that NADPH oxidase was not the sensor nor NADPH oxidase derived ROS were involved in O_2-sensing, rather mitochondria would be *the* sensor and the decrease of ROS levels produced by hypoxia the signals causing the reduced closed state of K^+ channels. The same notions were defended in additional articles. For example in Reeve et al. (2001) it was proposed that the increased basal PAP and the attenuation of HPV after CH would be due to a loss of the inhibition of K^+ channel activity by acute hypoxia which in turn would result from the inability of acute hypoxia to further modify the cytosolic redox status beyond the already reduced environment caused by CH itself. In another study (Michelakis et al., 2002) it was shown that while in PASMC acute hypoxia (36–40 mmHg) caused a decrease in ROS production, in renal artery smooth muscle cells (which exhibit a lower rate of normoxic ROS production) hypoxia increased ROS, being proposed that this difference would explain the opposite vasomotor effects of hypoxia in both arterial beds. Yet, Wu et al. (2007) compared PASMC and coronary artery smooth muscle cells and found that acute hypoxia PO_2 = 25–30 mmHg for 5–10 min significantly and rapidly decreased ROS levels in both cell types.

The putative significance of mitochondrial ROS in HPV, and by extension on CB chemoreception, has not concluded with these opposing views. In a very recent study Schumacker group (Waypa et al., 2010) based on data obtained with cell compartment targeted ROS indicators propose that hypoxia (superfusion of PASMC isolated cells with 1.5% O_2-equilibrated solutions) augments in a regulated manner ROS in the intermembrane mitochondrial space and by diffusion into the cell cytoplasm while lowering mitochondrial matrix ROS levels; it would be the regulated increased cytoplasmic ROS level the trigger of HPV. It is the authors opinion that the slow time courses of ROS changes measured in present experiments do not correlate with the fast contraction or Ca^{2+} transients of PASMC in response to hypoxia observed both in vivo and in vitro preparations (see Evans and Ward, 2009); additionally Waypa et al. observed that systemic arteries smooth muscle cells behaved alike PASMC (see above).

Quite recently other authors have made to intervene ROS of both origins, mitochondrial and NADPH oxidases, in the genesis of HPV, albeit in different manners (see Weissmann et al., 2006; Rathore et al., 2006, 2008; Cogolludo et al., 2009 and under consideration; Wang and Zheng, 2010; Daiber, 2010). Although these aspects surely will be covered in greater detail in other articles of this issue, we should mention that Cogolludo et al. (2009) found that neutral sphingomyelinase generated ceramide and PKCζ activation are early and necessary events in the genesis of the HPV. In a recent article of Perez-Vizcaino laboratory (Frazziano et al., submitted) it is reported that PKCζ activates phosphorylation of p47^{phox} and activation of NADPH oxidase with the ROS generated playing a necessary role in controlling the open probability of Kv channels in PASMC; in addition they also present evidence suggestive that increased mitochondrial ROS production could play a critical role in the activation of neutral sphingomyelinase. In our laboratory, we have tested a wide variety of reducing and oxidizing agents (Sanz-Alfayate et al., 2001; Gonzalez et al., 2004a,b; Agapito et al., 2009) and we could not establish a relationship between the general redox status of the cell and the activity of chemoreceptor cells. In a recent study (Gomez-Niño et al., 2009a,b) we aimed to manipulate mitochondrial rate of ROS production and redox status with the use of metabolic poisons and uncouplers in isolation and in the presence of N-acetylcysteine. As shown in Table 2 data indicate that mitochondrial alteration of the general status of cells is not linked to chemoreceptor activation. Thus, our findings would suggest that acute O_2 sensing in CB chemoreceptor cells and PASMC do not follow the same paths. To further explore similarities between acute O_2-sensing in PASMC and CB chemoreceptor cells we have performed the group of experiments shown in Fig. 4. Data indicate that the specific neutral sphingomyelinase inhibitor GW4869 at the same concentration (10 μM) that decreased by about 80% the tension developed by pulmonary artery segments in response to hypoxia did not alter the activity of chemoreceptor cells.

We do not want to conclude this section on mitochondrial ROS without stating some reflections made in previous reviews (Gonzalez et al., 2002, 2004b, 2007) and to add some additional comments. First, methods used to assess ROS concentrations or redox status are not reliable (see in addition Jelic and Le Jemtel, 2008). Second, to rise conclusions on the significance of oxidative status on a given cell function based of antagonism of the observed effects by antioxidants might be marred by secondary effects of the antioxidant used (e.g. Sekiguchi et al., 2003). In this regard we want to make some additional reflections taken from (Armitage et al., 2009); for example antioxidants can in fact promote chain reactions initiated in their oxidized forms, and more importantly, if the

Table 2
Affects of Metabolic Poisons alone or in combination with N-acetylcysteine on the general redox status of cells, chemoreceptor cells catecholamine release and CB ATP levels.

Condition Parameter	Control	Rotenone (1 μM)	3-NP (5 mM)	Antimicyn A (0,2 μg/ml)	Na Azide (5 mM)	DNP (100 μM)
E_{GSH} (mV)	−181/−186^{+}	−175*/−179	−160**/−180	−165***/−178	−163**/−179	−180/−180
^{3}H-CA release (% tissue content)	1.61/1.57	23.19/21.9	1.64/1.88	45.49/45.53	33.88/34.55	9.04/9.19
[ATP] (μmol/g tissue)	1.46/1.46	0.50^{+++}/0.51	1.54/1.54	0.72^{+++}/0.81	0.60^{+++}/0.48	1.46/1.46 0.80^{+++}/073 α

In all instances data are means of 10–12 values (E_{GSH}), 8–10 individual data for ^{3}H-CA release and 6–8 values for ATP levels. Also in all cases the set of two values in each box represent mean values (SEM) were omitted, the first value was obtained in the absence of N-acetylcysteine while the second was obtained after preincubation of tissues for 10 min in the presence of 2 mM N-acetylcysteine. $^{+}$, $^{++}$ and $^{+++}$ Different from untreated control ($p < 0.05$; $p < 0.01$ and $p < 0.001$, respectively). *($p < 0.05$), **($p < 0.01$) and ***($p < 0.001$) different from N-acetylcysteine treated tissues. α these values correspond to CBs incubated with 500 μM DNP.

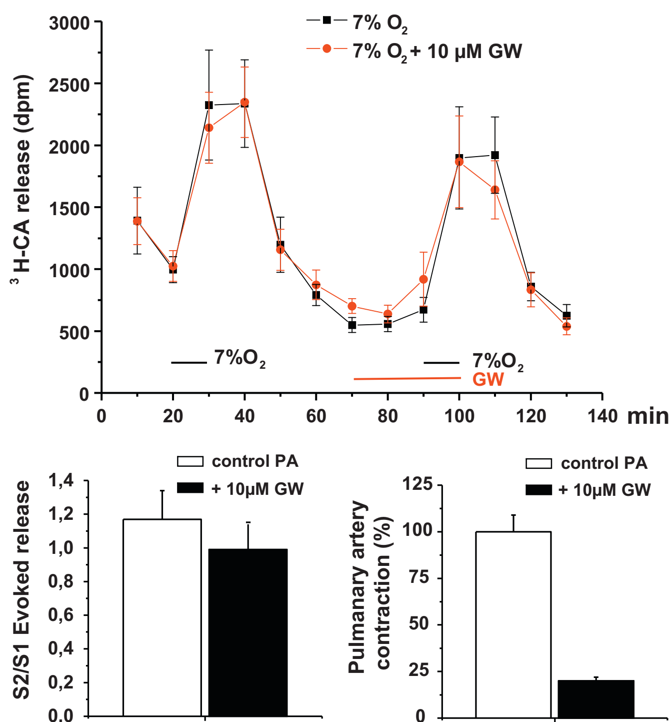


Fig. 4. Effects of the GW4869, a specific inhibitor of neutral sphingomyelinase, on the activity of chemoreceptor cells in normoxia and hypoxia. The activity of chemoreceptor cells was measured as their release of CA. In a group of 10 CBs two hypoxic stimuli (10 min superfusion with a solution equilibrated with 7% O_2 instead of the normoxic 20% O_2) and other group of equally 10 CBs was similarly stimulated, but prior and during the application of the second hypoxic stimulus 10 µM of GW4869 was present. In the upper figure it is shown the mean time course of the release evidencing that the presence of the sphingomyelinase inhibitor did not alter it. In the lower row of the figure at the left are shown the ratios of the evoked release in the second to the first stimulus in control and inhibitor treated organs. For comparative purpose are shown mean effects of the same concentrations of GW4869 in pulmonary artery rings contraction; these last data are taken from Cogolludo et al. (2009).

antioxidant is fully effective the global removal of ROS may result in unwanted effects since ROS at many cell places (and not only at the one we want target) also regulate important physiological functions. Third, the compartimentalization of ROS generation and the likelihood of local signalling (D'Autréaux and Toledano, 2007; Fisher, 2009; Paulsen and Carroll, 2010; Lassègue and Griendling, 2010) would require in future years to develop reliable compartment specific indicators for the different ROS to truly understand the significance of ROS; in this regard the developments in the Ca^{2+} methodology and function understanding (Montero et al., 2000; Alvarez and Montero, 2002; Santodomingo et al., 2008) would be a path to emulate. Fourth, owed to the expression in mitochondria of superoxide dismutase and glutathione peroxidase as well as to their content in lipoic acid and glutathione, forces the teleological conclusion that mitochondria are capable of producing ROS as indeed has been proven under a great variety of conditions. The question as posed by Nohl et al. (2005) is how much ROS mitochondria produce in physiological conditions, being their view that physiological rate of ROS production is minimal (see below) and that the strong anti-ROS armamentarium that mitochondria possess should be considered as a functional reserve to fight ROS in pathophysiological conditions. Fifth, from this conception we can ask how many ROS mitochondria would produce at the levels of hypoxia physiologically detected, both in CB chemoreceptor cells and in PASM which have comparable P_{50} and shape of response curves to hypoxia (Ward, 2008; see above).

Going behind an answer to this question we will refer to several considerations made by Murphy (2009) in his excellent review, as we consider they are germane in our context: (a) how much superoxide anion ($O_2^{\bullet-}$) do mammalian mitochondria produce in vivo? Maximal rate of $O_2^{\bullet-}$ production by isolated mitochondria reaches up to 2% of their O_2 consumption and this occurs in conditions uncertain to occur in living cells, i.e., when mitochondria are not synthesizing ATP, have a high electrochemical H^+ gradient and the coenzyme Q is reduced. In these conditions most of $O_2^{\bullet-}$ produced occurs at the complex I level, by a rotenone sensitive backflow of electrons from coenzyme Q to complex I. This value of 1–2% of respiration going to $O_2^{\bullet-}$ has propagated through the literature, being taken as an estimate of the rate mitochondrial $O_2^{\bullet-}$ production *in vivo*. (b) Murphy (2009) points out al least three reasons why this figure does not relate to the in vivo situation: one, backflow of electrons occurs with saturating concentration of succinate and when physiological concentrations of substrates are used the rate would drop to 0.12–0.15% of O_2 consumed; two, the most common concentration of O_2 used when working with isolated mitochondria is that given by room air (≈200 µM) and in vivo the concentration is between 10 and 50 µM (in chemoreceptor cells and PASMC should be in the upper range), which would decrease the rate of production by a factor of at least four,[2] leaving the rate of production <0.1% of O_2 consumption; and three, *in vivo* mitochondria are making ATP, implying that the electrochemical gradient for H^+ is not maximal and that neither NADH and CoQ pools are fully reduced. The proportion of time that mitochondria are in state 3 (i.e. actively making ATP) *vs.* state 4 (i.e. with low rate of ATP synthesis and high electrochemical gradient) is not known, but for sure the active synthesis of ATP would further reduce the figures previously calculated for isolated mitochondria.

These concepts are extremely important for O_2-sensing in the context of ROS production, because in the three tissues endowed with physiological sensitivity to hypoxia (Fig. 2), hypoxia necessarily means increased activity, in the case of chemoreceptor cells and PASMC, without adaptation (i.e., sustained increased activity as long as hypoxia last) and therefore the rate of ATP synthesis at the mitochondria must be increased to support the increased activity (Obeso et al., 1993, 1997; see above). Then all factors, hypoxia itself, high rate of ATP synthesis and consequently a submaximal H^+ electrochemical gradient and a rather low fraction of reduced components in the respiratory chain would imply a lower than normoxic mitochondrial rate of ROS production in cells with physiological sensitivity to hypoxia. In other words, active mitochondrial ATP synthesis requires transport of ADP and inorganic phosphate and backflow of H^+ at the mitochondrial ATPase which altogether decrease electrochemical gradient and direct electrons to fully reduce molecular O_2 to water. Following this rationale it is not possible to envision an increase in mitochondrial ROS production by hypoxia, unless some additional factor(s) enters into play to alter the parameters of the equation in footnote 2. Nitric oxide ($NO^{\bullet}$) is one candidate to alter the values of the members of equation (Murphy, 2009). $NO^{\bullet}$ can reach mitochondria from cytoplasm NOS, can be synthesized by mitochondrial NOS or can even, in situations of extreme hypoxia, be synthesized by reduction of NO_2^- by cytochrome oxidase (Carreras and Poderoso, 2007). However, even if the $NO^{\bullet}$ concentrations reached at the mitochondrial matrix are adequate to generate the inhibitory effects on respiration by inhibiting cytochrome oxidase and to increase the rate of

[2] The rate of $O_2^{\bullet-}$ in a complex system like mitochondria would vary according to the following equation: $d[O_2^{\bullet-}]/dt = [O_2]\Sigma(keP_R[E])$ where it is evident the direct proportionality between $[O_2]$ and rate of superoxide production. *ke* are the second order rate constants for the electron carrier reactions with O_2 to form $O_2^{\bullet-}$ and P_R are the fractions of the different enzymes (E) that are in their reduced form.

$O_2^{\bullet-}$ production by increasing the level in the reduction of mitochondrial components (Carreras and Poderoso, 2007), it is our view that cells whose function is to be fully active in hypoxia, and with greater activity the greater the intensity of hypoxia, this biological design would be purposeless. Yet, if several laboratories, including ourselves found that mitochondria of PASMC increase their rate of ROS production in hypoxia. Either the ROS measuring tools are faulty or we are missing the factor that without breaking ATP synthesis activates $O_2^{\bullet-}$ production. In this regard it should be noted that according to some authors the potentially exist to have a reduced electron transport chain in an ample range of hypoxic levels well before the K_m of cytochrome oxidase for O_2 is reached and O_2 consumption is restricted (Erecińska and Wilson, 1982; see Guzy and Schumacker, 2006). Thus, according to the so-called near-equilibrium hypothesis of Erecińska and Wilson (1982), hypoxia would intrinsically tend to augment the fractions of the different components of the respiratory chain that are in their reduced form, this factor tending to favour the production of ROS. Note, however, that at the same the PO_2 decrease would tend to diminish ROS production.

2.3.2. NADPH oxidase derived ROS

In recent years have emerged several new aspects of the biology of NADPH oxidases, NOX, that are excellently compiled in recent reviews (Brown and Griendling, 2009; Fisher, 2009; Oakley et al., 2009; Ushio-Fukai, 2009; Lassègue and Griendling, 2010). Here we will summarize the basics necessary to follow recent endeavours on NADPH oxidase researches in the CB chemoreception. (1) Although of some NOX subtype (e.g. NOX 4) there have been described several isoforms, they are recognized seven NOX types (NOX1-5, DUX1 AND DUOX 2). (2) A given cell type may express several NOX isoforms, rising the possibility that different cell processes are regulated by signals that modulate the activity of different NOX. (3) Cellular localization of NOX is variable, being the plasma membrane and the endoplasmic reticulum the most frequent locations; other common and specific locations for NOX expression include the nucleus, lipid-rafts-caveolae, cell matrix adhesions and cell to cell contacts, cell migrating edges and endosomes resulting from early endocytosis mediated by receptor resulting in specialized vesicular compartments, redoxomes, where ROS ($O_2^{\bullet-}$) are generated. (4) The classical concept of NADPH oxidase as an unassembled multimolecular complex that requires stimulus mediated assembling to be activated is still true for NOX1 (ubiquitously expressed, but is highly expressed in endothelial and vascular smooth muscle cells and thereby playing critical roles in vessel pathophysiology and in intestinal epithelium where its function would be mostly defensive), NOX2 (the classical phagocyte isoform but also expressed in many cell types but particularly in all cell types of vessels existing a direct correlation between NOX2 expression and hypertension [a high level of expression in vessel cells would lead to neutralization of the vasodilator action of $NO^\bullet$ as it would react with the NOX derived $O_2^{\bullet-}$ generating $ONOO^-$ which is highly reactive and damage propagating]) and NOX3 (expressed in the ear where it has been related to inner ear and vestibular function and lung where it has been associated to the development of emphysema by controlling the destruction of elastic fibers of the alveolar wall). The NOX4 isoform only requires subunit $p22^{phox}$ to express its full activity. NOX4 was originally described as Renox, being very abundant in kidney and in many cell types from skin to neurons including vessel cells and hepatocytes; it is constitutively active and its main regulation appears to be mediated at transcriptional level, although some regulation via $p22^{phox}$ specific ligands also seems to exist. NOX5 is conformed by the single NOX subunit with its cytosolic N-terminal region being very reach in Ca^{2+} binding to EF-hand domains; it is expressed in lymphatic tissue, vessel cells and prostate, and appears to participate in the control of cell proliferation, but interestingly it is not expressed in rodents. DUOX1 and DUOX2 activation involves Ca^{2+} binding to EF-hand domains in the cytosol generating directly H_2O_2 by two electrons reduction of molecular O_2 without the intermediate step of forming $O_2^{\bullet-}$; they are expressed in thyroid cells where they are critical to metabolism of iodine and thyroid hormone synthesis, being also abundant in the epithelial cells of the respiratory tract where they would play defence-type functions. (5) The above summarized recent findings provide an explanation to the postulate put forward by Saran and Bors (1994; see also Gonzalez et al., 2002): "in order to properly convey a message from one cellular location to another, the messenger must get to the point of message reception in unaltered form or through a series of well-defined reproducible steps is of course trivial with respect to stable molecular messengers like hormones. Regarding radicals, which by their very nature are reactive species, the postulate is less trivial". Aiming to clarify the feasibility of specific signalling by ROS we should describe two situations. When NOX is expressed in a caveolae (invaginations with typical diameter of 80–120 nm and open pores to cell surroundings of 30–50 nm in diameter; Westermann et al., 1999), the $O_2^{\bullet-}$ would be released into a compartment that, in the one hand, is specific for its contents and the abutting proteins and, in the other hand, taking into consideration diffusion coefficient and half life for $O_2^{\bullet-}$ (Saran and Bors, 1994), it could be expected that many of the cell molecules exposed to the caveolar surface can be direct targets of significant fractions of the $O_2^{\bullet-}$ produced. Even in the absence of any special constituent (transition metals, ascorbic acid, etc.) the $O_2^{\bullet-}$ concentration decay path is steep due to the spontaneous dismutation and a focal genesis of H_2O_2. This fact is very important because for $O_2^{\bullet-}$ to signal it certainly must be there, but it is equally necessary something to take the message (i.e., "a receptor" for $O_2^{\bullet-}$). In this regard it should be recalled that ROS chemistry dictates reactivity towards selective atomic targets in proteins, with $O_2^{\bullet-}$ being active towards iron–sulphur clusters and H_2O_2 targets are reactive –SH of cysteine residues; i.e., it is the subcellular colocalization of specific ROS and their targets what dictates signaling specificity (D'Autréaux and Toledano, 2007). At the extracellular surface of caveolae it is unlikely the presence of iron-sulfur clusters, but certainly many proteins likely have cysteine residues with different reactivity with H_2O_2 (Paulsen and Carroll, 2010) providing specificity to signalling. When a redoxome is formed by receptor mediated endocytosis of a plasma membrane patch containing a NOX complex capable of generating $O_2^{\bullet-}$ inside the endosome, superoxide production would stop unless $O_2^{\bullet-}$ processing enzymes (SOD capable of generating freely diffusible H_2O_2) or chloride channels and/or anion exchangers capable of mediating $O_2^{\bullet-}$ export to cell cytoplasm and maintaining membrane gradients required for NOX activity are present in the endosome membrane. It is obvious that receptor mediated redoxome formation is specific, as they are every other step capable of spilling ROS to cell cytoplasm (orientation of anion channels or exchangers, aquaporin molecules focusing the exit of H_2O_2 formed).

Departing from here we can ask how many NOX subtypes are expressed in the CB, and more specifically in chemoreceptor cells where they, via their enzymatic products, contribute to control O_2-sensing and transduction cascade. Initial studies of Kummer and Acker (1995) located $p22^{phox}$, $gp91^{phox}$, $p47^{phox}$, and $p67^{phox}$, i.e. NOX2 in rat chemoreceptor cells, but a few years later Kummer's laboratory (Dvorakova et al., 2000) using specific monoclonal antibodies against $gp91^{phox}$ and additional markers concluded that macrophages located in the rat CB represent the major location of NOX2. This finding in turn would give a full justification to the functional studies of Roy et al. (2000) and He et al. (2002) showing that $gp91^{phox}$ KO mice have preserved normal K^+ currents and magnitude of hypoxic inhibition and normal hypoxic Ca^{2+} transients in chemoreceptor cells as well as normal CSN activity and ventilation

to acute hypoxia. These findings as a whole would certify that NOX2 is not involved in acute O_2-sensing or transduction cascade in mice and probably also in the rat.

However, the very same year, Fidone's laboratory (Sanders et al., 2002) showed that p47^{phox} knockout mice exhibited exaggerated CSN discharges and ventilatory responses to acute hypoxic tests and normal levels of erythropoietin messenger expression after 72 h of hypoxia. In a further study of Fidone's laboratory (He et al., 2005) in a comparative study of chemoreceptor cell responses to hypoxia in chemoreceptor cells of p47^{phox} KO and correspondent wild type controls it was shown: (a) basal normoxic ROS production was comparable in control and KO cells; (b) hypoxia increased superoxide production (dihydroethidine oxidation) in control but not in KO cells; (c) hypoxic ROS production was sensitive to NADPH oxidase inhibitors; (d) sodium azide (a cytochrome oxidase inhibitor) increased $O_2^{\bullet -}$ production equally in control and KO cells; (e) percentage K^+ current inhibition by hypoxia was greater in KO than in control cells, and; (f) Ca^{2+} transients elicited by hypoxia were higher in KO than in control cells. These findings and additional northern blot data (see He et al., 2010) and the use of L-type Ca^{2+} channel blocker (unpublished) allowed us to conclude that NOX expressed in chemoreceptor cells was NOX4 (see Gonzalez et al., 2007), that NOX was not the oxygen sensor, that NOX derived ROS acted as negative modulators of the acute hypoxic transduction cascade, that Ca^{2+} entering through L-type channels was responsible for NOX activation during hypoxia and finally, that mitochondria did not increase their rate of ROS production in hypoxia as the rate of dihydroethidine oxidation was not altered by hypoxia in KO cells. Obviously these findings need to be reinterpreted at the light of new findings. Since we know today that p47^{phox} does not form part of NOX4 it is obvious that chemoreceptor cells must express at least one more NOX isoform requiring this subunit (i.e., NO1, NOX2 or NOX3) to explain the loss of chemoreceptor cells capacity to produce ROS in the p47^{phox} KO animals. Since in pancreatic acinar cells Ca^{2+} may mediate the activation of NOX1 and thus ROS production (Yu et al., 2007) we would suggest that this is the p47^{phox} containing isoform present in chemoreceptor cells and activated as a result of Ca^{2+} entering the cells by acute hypoxic activation. Our suggestion is supported in part by the study of Peng et al. (2009) showing that rat CB expresses NOX1. However, they also showed that the major isoform expressed in the CB, at least in terms of mRNA fold increase during intermittent hypoxia was NOX2 (but see above). They reported that the subcellular location in chemoreceptor cells was different as NOX2 (and probably NOX1) was cytoplasmic and NOX4 was perinuclear; no cellular or subcellular location was provided to NOX3, which is also expressed in the CB, but whose expression was not modified by intermittent hypoxia. Peng et al. (2009) showed additionally that neither apocinyn, AEBFS or DPI inhibited acute response to repetitive trains of hypoxic stimulation, conforming our previous conclusion that NOX were not the O_2 sensor and that NOX-derived ROS are not necessary coupling factors in the transduction cascade; the three NADPH inhibitors prevented long term facilitation of sensory nerve discharge induced by repetitive stimulation that was also absent in NOX1 knockout mice.

2.4. Conformational O_2 sensors

We are referring to molecules that would bind O_2 in concentrations proportional to their surrounding PO_2, and as a result they would experience conformational changes that would be allosterically transmitted to the first effectors of the transduction cascade, i.e., oxygen sensitive K^+ channels. An unknown hemoprotein, ion channels themselves and NOX4 have been proposed as sensors of this category.

Leaving aside ion channels as putative O_2 sensors, there are two concepts contained in our proposal of the conformational model as *the* O_2 sensor for acute hypoxia in chemoreceptor cells. First, that it should be a hemoprotein and second, that it must be located in the plasma membrane or strongly bound to some membrane component. In the remaining of this section we should intend to justify our views on the basis of existing literature.

Why a hemoprotein? First, the notion is not new as old CB literature already contains the idea that the O_2 sensing device can be a haemoglobin-like hemoprotein with a P_{50} close to 40 mmHg and a special Bohr effect (see Fidone and González, 1986 for old references). Second, the idea returned when Lopez-Lopez and Gonzalez (1982) showed that in rabbit chemoreceptor recorded in whole-cell configurations CO prevented or reversed the inhibition of K^+ currents produced by hypoxia. A year later Lahiri et al. (1993) showed that CO at low concentrations inhibited the activity of the CSN elicited by hypoxia while at high concentrations (4–5× prevailing PO_2) stimulated the activity in the CSN, being this last excitatory, but not the inhibitory effect, sensitive to light, an unequivocal indication that excitation was due to cytochrome oxidase inhibition, while the inhibitory effect was independent of CO actions at the mitochondrial level. Third, a survey to general biology books indicated that all proteins handling O_2 are metallo- or hemoproteins, being also known that in biological systems CO is very unreactive, and as O_2, CO is handled by metallo- or hemoproteins proteins (Coburn and Forman, 1987). Fourth, the notion of channel gating implied molecular rearrangements of the channel protein as to open the channel pore and allow the ion to pass through the selectivity filter. Fifth, It was also known that hemoproteins, as exemplified by myoglobin and particularly haemoglobin, on binding and releasing O_2 suffer conformational changes in their tertiary or quaternary structures. Sixth, putting together points fourth and fifth it is evident that a conformational change in a hemoprotein can be allosterically transmitted to the ion channels to cause important modifications in their kinetic properties if the physical relationships between both elements are the appropriated. Finally, as Streit et al. (2010) put it, heme is one of the nature's most versatile catalytic building blocks as heme-binding proteins facilitate a range of redox, atom or electron-transfer, and small-molecule sensing and trafficking processes, with a specificity that is sometimes difficult or impossible to reproduce synthetically. However, it should be added that specific kinetic parameters of hemoprotein functions are dependent on the protein moiety as exemplified by differences in O_2 *vs.* CO affinities binding in hemoglobin (CO affinity nearly 300 times higher than O_2 affinity) *vs.* cytochrome oxidase (CO affinity about 5–10 times higher than O_2 affinity). Another example that we want to bring here is the case of NOX2; in their classical review on NADPH oxidase (NOX2) Cross and Jones (1991) give K_m for O_2 oscillating between 5 and 30 μM, but according to Iizuka et al. (1985) and Parkos et al. (1988) it does not bind CO at all (but see Nakahira et al., 2006; Basuroy et al., 2009).

Why in the plasma membrane? Very early after the description of the O_2 sensitive K^+ channels (Lopez-Barneo et al., 1988), Ganfornina and Lopez-Barneo (1991) showed that isolated rabbit chemoreceptor cell membrane patches recorded in the inside out configuration contained low unitary conductance K^+ channels that were reversibly inhibited by low PO_2, implying that the plasma membrane contained all elements to sense O_2 and to couple the O_2 sensor to the first line effectors in the transduction cascade, the K^+ channels. A few years later, our laboratory showed with inside out isolated patches obtained from HEK cells cotransfected with Kv4.2 + Kvβ1.2 regulatory subunits that the channels were O_2-sensitive, being reversibly inhibited by hypoxia and also were sensitive to CO in such a manner that CO prevented the low PO_2 inhibition (Perez-Garcia et al., 1999). In an ample study working with inside out isolated patches of rat

chemoreceptor cells we demonstrated that low PO_2 diminished the open probability of maxiK channels (Riesco-Fagundo et al., 2001). The inhibition of the channel activity was dependent on Ca^{2+} and membrane potential, being maximal at low Ca^{2+} concentrations and at negative membrane potentials; extrapolation of experimental data would indicate that at voltages near the threshold for most voltage-dependent currents in chemoreceptor cells (around −40 mV) hypoxic inhibition of maxiK would be nearly complete (Riesco-Fagundo et al., 2001). This behaviour of maxiK has been considered of great physiological importance because due to the great density of outward comparatively to inward currents in chemoreceptor cells, and their near identical E_m threshold, a marked inhibition of some component of the outward current must occur to allow cells to depolarize and to generate Ca^{2+} dependent neurotransmitter release (Gomez-Niño et al., 2009a,b). Another finding of great interest in the study of Riesco-Fagundo et al. (2001) was that CO could fully reverse the effects of hypoxia on isolated patches on the diminution of open probability. Although no detailed kinetic analysis of the CO effects was carried out it would appear that the affinity of the O_2 sensor molecule would have comparable affinities for O_2 and CO. As mentioned above, in a whole-cell preparation of rabbit chemoreceptor cells where the main (or unique) O_2 sensitive K^+ current is carried by a channel expressing a transient current, in all likelihood Kv4.3 (Sanchez et al., 2002; Kääb et al., 2005), we also found that CO was able to substitute O_2 at the molecular O_2 sensor with a comparable affinity (Lopez-Lopez and Gonzalez, 1992). In this context, and in spite of the experimental data provided above on HPV, we should mention that in artificially perfused preparation *in situ* of rat pulmonary circulation we observed that CO equally prevented or reversed HPV (Tamayo et al., 1997).

Oxygen-sensitivity of native maxi K^+ channels in inside-out isolated membrane patches of chemoreceptor cells has also been shown by Williams et al. (2004). McCartney et al. (2005) showed that native maxiK in AtT20 cells (derived from a murine adenohypophyseal tumor) identified as the STREX maxiK^+ isoform, as well as the same isoform expressed in HEK cells, have the capacity of being regulated by hypoxia while other isoforms of maxiK were not PO_2 regulated. These findings, plus the additional observation that all isoforms of maxiK were activated by CO, and additional findings in point mutations in the STREX domain led McCartney et al. (2005) to conclude that the O_2-sensitivity was a specific property generated by the cysteine-rich motive of STREX channels while CO ability to activate maxiK is a common property of all channel isoforms. Since Riesco-Fagundo et al. (2001) found that in patches of rat chemoreceptor cells with a single maxiK channel low PO_2 diminished the open probability of the channel and CO restored it, data would allow to provisionally conclude that native maxiK in rat chemoreceptor cells is the STREX isoform. However, as pointed out by Peers and Wyatt (2007) it is difficult to reconcile McCartney data with those of Lewis et al. (2002), Kemp et al. (2003) and Williams et al. (2004, 2008) showing that low PO_2 inhibits and CO activates human recombinant maxiK channels (which are not the STREX isoform) by a membrane delimited mechanism. In the last study quoted, Williams et al. (2008) studied in detail molecular mechanisms of CO sensitivity in recombinant α1-subunit of human *slo* stably and transiently expressed in HEK cells. They mutated histidine residues previously implicated in the binding of heme that putatively conferred the CO sensitivity (H616R) to maxiK, and (H254R), and found that neither mutation in isolation or combined suppressed CO sensitivity. Williams et al. (2008) went further and constructed a chimera substituting the distal S9 and S10 modules of human *slo* α1-subunit by corresponding modules of human α3-subunit, and found that the resulting channel although being activated by maxiK openers has completely lost the ability of being activated by CO, implying that it is the distal part of C terminal of the channel the responsible for CO sensitivity. They conclude that binding of CO to a receptor protein/compound that associates with this distal S9–S10 channel modules of the C-terminal tail of the channel can satisfactorily explain CO ability to regulate maxiK. Pursuing with his studies on the mechanisms of CO activation of maxiK, Kemp's laboratory (Brazier et al., 2009) studied the effects of point mutating four cysteines residues (C820, C911, C995 and C1028) in the C terminal region and found that only C911G substitution in the proximity of the Ca^{2+} bowl (alone or in combination) reduced the sensitivity of maxiK to CO by nearly doubling its EC_{50}. They suggested that CO may bind to this channel subunit in a manner similar to the transition metal-dependent co-ordination which is characteristic of several enzymes, such as CO dehydrogenase found in prokaryotes.

Membrane delimited sensitivity is not restricted to the oxygen sensitive Kv channel expressed in rabbit chemoreceptor cells (probably Kv4.3) or maxiK in rat chemoreceptor cells; it has also been evidenced in HEK cells expressing either Kv4.2 or maxi K. In addition Osipenko et al. (2000) showed that hypoxia inhibited Kv3.1b (natively expressing O_2 sensitivity in pulmonary artery smooth muscle cells) channels expressed in L929 cells, both in the whole-cell preparation and single-channel activity whether recorded in cell attached or in isolated inside out patches, without affecting unitary conductance, leading them to conclude that O_2-sensing was a membrane-delimited mechanism. It is this molecular diversity of O_2 and CO-sensitive channels what makes unlikely that channels themselves are the sensors as common motifs candidate as sensors have not been recognized (Gonzalez et al., 2009). The notion of a unique hemoprotein sensor capable of interacting allosterically with several ion channels in the same manner that kinases interact with diverse substrate proteins is conceptually more conceivable.

In fact, recently Park's laboratory has two publications (Lee et al., 2006; Park et al., 2009) supporting the hemoprotein concept, and indicate additionally that the up to now named "an unknown hemoprotein in chemoreceptor cells plasma membrane" could in fact be NOX4. In the first study they showed recombinant TASK-1 per se expressed in HEK cells cannot sense O_2. However, either endogenously expressed or transfected NOX4, which colocalized at the plasma membrane in HEK cells, conferred O_2 sensitivity to TASK channels. This hypoxic sensitivity was sensitive to DPI (1 h preincubation with 20 μM) and $NADP^+$ (1 h preincubation with 2 mM). Further any hypoxic sensitivity was abolished by NOX4 siRNA. They concluded that a novel function for NOX4 would be to provide TASK-1 with the property of being oxygen regulated. In the second study using the same preparation, they tested CO, and found that it blocked the inhibitory effect of hypoxia on TASK-1 currents, implying that CO interacts with NOX4 (Basuroy et al., 2009). They also showed that addition of H_2O_2 did not alter, and ascorbic acid did not reverse the inhibitory effects of hypoxia on TASK-1. This implies that the effects of hypoxia although NOX4 mediated do not depend on ROS generation, a conclusion that was confirmed by the construction of specific mutants of NOX4. Their final conclusion was that it is the conformational change of NOX4 on its binding or releasing O_2 (or CO) what, with the intermediate participation of $p22^{phox}$, is transmitted to TASK-1 channels to change their open probability in such a manner that on O_2 desaturation the opening probability decreases (see excellent drawing of Figure 8 in Park et al., 2009). Obviously the use of recombinant effectors and heterologous systems have unavoidable handicaps, and the encountered situation with recombinant channels and NOX might differ from the native real thing, but Park's findings connect a set of data of many laboratories to reaffirm that O_2 sensing in chemoreceptor cells is a membrane delimited process that requires the participation of a hemoprotein that might happen to be NOX4, although the location for NOX4 proposed by Peng et al. (2009) would not satisfactorily

meet the notion that both receptor and signal must encounter in the right place to produce the biological effects.

Acknowledgements

We want to thank Elena Olea and Mª de los Llanos Bravo for technical assistance. Supported Grants: BFU2007-61848 (DGICYT), CIBER CB06/06/0050 (FISS-ICiii) and JCyL-GR242.

References

Acker, H., Lübbers, D.W., Purves, M.J., 1971. Local oxygen tension field in the glomus caroticum of the cat and its change at changing arterial PO_2. Pflügers Arch. 329, 136–155.

Acker, H., Lübbers, D.W., 1977. Relationship between local flow, tissue PO2, and total flow of the cat carotid body. In: Acker, H., Fidone, S., Pallot, D., Eyzaguirre, C., Lübbers, D.W., Torrance, R.W. (Eds.), Chemoreception in the Carotid Body. Springer-Verlag, Berlin, pp. 271–276.

Agapito, M.T., Sanz-Alfayate, G., Gomez-Niño, A., Gonzalez, C., Obeso, A., 2009. General redox environment and carotid body chemoreceptor function. Am. J. Physiol. Cell Physiol. 296, C620–C631.

Aldieri, E., Riganti, C., Polimeni, M., Gazzano, E., Lussiana, C., Campia, I., Ghigo, D., 2008. Classical inhibitors of NOX NAD(P)H oxidases are not specific. Curr. Drug Metab. 9, 686–696.

Almaraz, L., Gonzalez, C., Obeso, A., 1986. Effects of high potassium on the release of [^{3}H]dopamine from the cat carotid body in vitro. J. Physiol. 379, 293–307.

Alvarez, J., Montero, M., 2002. Measuring [Ca^{2+}] in the endoplasmic reticulum with aequorin. Cell Calcium 32, 251–260.

Archer, S.L., Reeve, H.L., Michelakis, E., Puttagunta, L., Waite, R., Nelson, D.P., Dinauer, M.C., Weir, E.K., 1999. O_2 sensing is preserved in mice lacking the gp91 phox subunit of NADPH oxidase. Proc. Natl. Acad. Sci. U. S. A. 96, 7944–7949.

Archer, S.L., Gomberg-Maitland, M., Maitland, M.L., Rich, S., Garcia, J.G., Weir, E.K., 2008. Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1alpha-Kv1.5 O_2-sensing pathway at the intersection of pulmonary hypertension and cancer. Am. J. Physiol. Heart Circ. Physiol. 294 (February (2)), H570–H578.

Armitage, M.E., Wingler, K., Schmidt, H.H., 2009. Translating the oxidative stress hypothesis into the clinic: NOX versus NOS. J. Mol. Med. 87, 1071–1076.

Basuroy, S., Bhattacharya, S., Leffler, C.W., Parfenova, H., 2009. Nox4 NADPH oxidase mediates oxidative stress and apoptosis caused by TNF-alpha in cerebral vascular endothelial cells. Am. J. Physiol. Cell Physiol. 296, C422–432.

Biscoe, T.J., Purves, M.J., Sampson, S.R., 1970. The frequency of nerve impulses in single carotid body chemoreceptor afferent fibres recorded in vivo with intact circulation. J. Physiol. 208, 121–131.

Borowsky, I.W., Collins, R.C., 1989. Metabolic anatomy of brain: a comparison of regional capillary density, glucose metabolism, and enzyme activities. J. Comp. Neurol. 15 (288), 401–413.

Brazier, S.P., Telezhkin, V., Mears, R., Müller, C.T., Riccardi, D., Kemp, P.J., 2009. Cysteine residues in the C-terminal tail of the human BK(Ca)alpha subunit are important for channel sensitivity to carbon monoxide. Adv. Exp. Med. Biol. 648, 49–56.

Brown, D.I., Griendling, K.K., 2009. Nox proteins in signal transduction. Free. Radic. Biol. Med. 47, 1239–1253.

Buckler, K.J., 1997. A novel oxygen-sensitive potassium current in rat carotid body type I cells. J. Physiol. 498, 649–662.

Cantó, C., Gerhart-Hines, Z., Feige, J.N., Lagouge, M., Noriega, L., Milne, J.C., Elliott, P.J., Puigserver, P., Auwerx, J., 2009. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature 458, 1056–1060.

Carreras, M.C., Poderoso, J.J., 2007. Mitochondrial nitric oxide in the signaling of cell integrated responses. Am. J. Physiol. Cell Physiol. 292, C1569–1580.

Chandel, N.S., Schumacker, P.T., 2000. Cellular oxygen sensing by mitochondria: old questions, new insight. J. Appl. Physiol. 88, 1880–1889.

Chandel, N.S., McClintock, D.S., Feliciano, C.E., Wood, T.M., Melendez, J.A., Rodriguez, A.M., Schumacker, P.T., 2000. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. J. Biol. Chem. 275, 25130–25138.

Chandel, N.S., Maltepe, E., Goldwasser, E., Mathieu, C.E., Simon, M.C., Schumacker, P.T., 1998. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc. Natl. Acad. Sci. U.S.A. 95, 11715–11720.

Coburn, R.F., Forman, H.J., 1987. Carbon monoxide toxicity. In: Farhi, L.E., Tenney, S.M. (Eds.), Handbook of Physiology, Section 3. Respiratory System, Vol. 4. Gas Exchange. Am. Physiol. Soc., Bethesda, MD, pp. 439–456.

Cogolludo, A., Moreno, L., Frazziano, G., Moral-Sanz, J., Menendez, C., Castañeda, J., González, C., Villamor, E., Perez-Vizcaino, F., 2009. Activation of neutral sphingomyelinase is involved in acute hypoxic pulmonary vasoconstriction. Cardiovasc. Res. 82, 296–302.

Conde, S.V., Monteiro, E.C., Obeso, A., Gonzalez, C., 2009. Adenosine in peripheral chemoreception: new insights into a historically overlooked molecule – invited article. Adv. Exp. Med. Biol. 648, 145–159.

Cross, A.R., Jones, O.T., 1991. Enzymic mechanisms of superoxide production. Biochim. Biophys. Acta 1057, 281–298.

Cutz, E., Pan, J., Yeger, H., 2009. The role of NOX2 and "novel oxidases" in airway chemoreceptor O_2 sensing. Adv. Exp. Med. Biol. 648, 427–438.

Cutz, E. (Ed.), 1997. Cellular and Molecular Biology of Airway Chemoreceptors. Springer, NY, pp. 1–33.

Daiber, A., 2010. Redox signaling (cross-talk) from and to mitochondria involves mitochondrial pores and reactive oxygen species. Biochim. Biophys. Acta [Epub ahead of print].

Dallas, M.L., Scragg, J.L., Wyatt, C.N., Ross, F., Hardie, D.G., Evans, A.M., Peers, C., 2009. Modulation of O(2) sensitive K (+) channels by AMP-activated protein kinase. Adv. Exp. Med. Biol. 648, 57–63.

D'Autréaux, B., Toledano, M.B., 2007. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nat. Rev. Mol. Cell. Biol. 8, 813–824.

Donnelly, D.F., 1997. Are oxygen dependent K^+ channels essential for carotid body chemo-transduction? Respir. Physiol. 110, 211–218.

Dvorakova, M., Hohler, B., Vollerthun, R., Fischbach, T., Kummer, W., 2000. Macrophages: a major source of cytochrome b558 in the rat carotid body. Brain Res. 852, 349–354.

Erecińska, M., Wilson, D.F., 1982. Regulation of cellular energy metabolism. J. Membr. Biol. 70, 1–14.

Evans, A.M., Mustard, K.J., Wyatt, C.N., Peers, C., Dipp, M., Kumar, P., Kinnear, N.P., Hardie, D.G., 2005. Does AMP-activated protein kinase couple inhibition of mitochondrial oxidative phosphorylation by hypoxia to calcium signaling in O2-sensing cells? J. Biol. Chem. 280, 41504–41511.

Evans, M.A., Ward, J.P., 2009. Hypoxic pulmonary vasoconstriction. Adv. Exp. Med. Biol. 648, 351–360.

Fidone, S.J., González, C., 1986. Initiation and control of chemoreceptor activity in the carotid body. In: Fishman, A.P. (Ed.), Handbook of Physiology. The Respiratory System. Amer. Physiol. Soc, Bethesda, MD, pp. 247–312.

Fisher, A.B., 2009. Redox signaling across cell membranes. Antioxid. Redox Signal. 11, 1349–1356.

Frazziano, G., Moreno L., Moral-Sanz J., Menendez C., Escolano L., González C., Villamor E., Cogolludo A., Perez-Vizcaino F., submitted. Neutral sphingomyelinase, NADPH oxidase and reactive oxygen species. Role in acute hypoxic pulmonary vasoconstriction.

Ganfornina, M.D., Lopez-Barneo, J., 1991. Single K^+ channels in membrane patches of arterial chemoreceptor cells are modulated by O_2 tension. Proc. Natl. Acad. Sci. U.S.A. 88, 2927–2930.

Gomez-Niño, A., Obeso, A., Baranda, J.A., Santo-Domingo, J., Lopez-Lopez, J.R., Gonzalez, C., 2009a. MaxiK potassium channels in the function of chemoreceptor cells of the rat carotid body. Am. J. Physiol. Cell Physiol. 297, C715–722.

Gomez-Niño, A., Agapito, M.T., Obeso, A., Gonzalez, C., 2009b. Effects of mitochondrial poisons on glutathione redox potential and carotid body chemoreceptor activity. Respir. Physiol. Neurobiol. 165, 104–111.

Gonzalez, C., Almaraz, L., Obeso, A., Rigual, R., 1992. Oxygen and acid chemoreception in the carotid body chemoreceptors. Trends Neurosci. 15, 146–153.

Gonzalez, C., Sanz-Alfayate, G., Agapito, M.T., Gomez-Nino, A., Rocher, A., Obeso, A., 2002. Significance of ROS in oxygen sensing in cell systems with sensitivity to physiological hypoxia. Respir. Physiol. Neurobiol. 132, 17–41.

Gonzalez, C., Sanz-Alfayate, G., Obeso, A., Agapito, M.T., 2004a. Role of glutathione redox state in oxygen sensing by carotid body chemoreceptor cells. Methods Enzymol. 381, 40–71.

Gonzalez, C., Sanz-Alyayate, G., Agapito, M.T., Obeso, A., 2004b. Effects of reducing agents on glutathione metabolism and the function of carotid body chemoreceptor cells. Biol. Chem. 385, 265–274.

Gonzalez, C., Agapito, M.T., Rocher, A., Gonzalez-Martin, M.C., Vega-Agapito, V., Gomez-Niño, A., Rigual, R., Castañeda, J., Obeso, A., 2007. Chemoreception in the context of the general biology of ROS. Respir. Physiol. Neurobiol. 157, 30–44.

Gonzalez, C., Vaquero, L.M., López-López, J.R., Pérez-García, M.T., 2009. Oxygen-sensitive potassium channels in chemoreceptor cell physiology: making a virtue of necessity. Ann. N. Y. Acad. Sci. 1177, 82–88.

Gonzalez, C., 1998. Sensitivity to physiologic hypoxia. In: Lopez-Barneo, J., Weir, E.K. (Eds.), Oxygen regulation of ion channels and gene expression. Futura Publishing Co. Inc., Armonk, NY, pp. 321–336.

Gonzalez, C., Almaraz, L., Obeso, A., Rigual, R., 1994. Carotid body chemoreceptors: from natural stimuli to sensory discharges. Physiol. Rev. 74, 829–898.

Grundfest, H., 1971. The general electrophysiology of input membrane in the electrogenic excitable cells. In: Loewenstein, W.R. (Ed.), Handbook of Sensory Physiology, Vol. I Principles of Receptor Physiology. Springer Verlag, Berlin, pp. 135–165.

Guarani, V., Potente, M., 2010. SIRT1 - a metabolic sensor that controls blood vessel growth. Curr. Opin. Pharmacol. 10, 139–145.

Guzy, R.D., Schumacker, P.T., 2006. Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. Exp. Physiol. 91, 807–819.

Halperin, M.L., Cheema-Dhadli, S., Lin, S.H., Kamel, K.S., 2006. Properties permitting the renal cortex to be the oxygen sensor for the release of erythropoietin: clinical implications. Clin. J. Am. Soc. Nephrol. 1, 1049–1053.

Hardie, D.G., 2008. AMPK: a key regulator of energy balance in the single cell and the whole organism. Int. J. Obes. (Lond.) 32 (Suppl. 4), S7–12.

Hardie, D.G., Salt, I.P., Hawley, S.A., Davies, S.P., 1999. AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. Biochem. J. 338 (Pt 3), 717–722.

He, L., Chen, J., Dinger, B., Sanders, K., Sundar, K., Hoidal, J., Fidone, S., 2002. Characteristics of carotid body chemosensitivity in NADPH oxidase-deficient mice. Am. J. Physiol. Cell Physiol. 282, C27–33.

He, L., Liu, X., Chen, J., Dinger, B., Stensaas, L., Fidone, S., 2010. Modulation of chronic hypoxia-induced chemoreceptor hypersensitivity by NADPH oxidase subunits in rat carotid body. J. Appl. Physiol. 108, 1304–1310.

He, L., Dinger, B., Sanders, K., Hoidal, J., Obeso, A., Fidone, S., Gonzalez, C., 2005. Effect of p47^{phox} gene-deletion on reactive oxygen species (ROS) production and oxygen sensing in mouse carotid body chemoreceptor cells. Am. J. Physiol. Lung Cell Mol. Physiol. 289, 916–924.

Iizuka, T., Kanegasaki, S., Makino, R., Tanaka, T., Ishimura, Y., 1985. Studies on neutrophil b-type cytochrome *are situ* by low temperature absorption spectroscopy. J. Biol. Chem. 260, 12049–12053.

Iturriaga, R., Alcayaga, J., 2004. Neurotransmission in the carotid body: transmitters and modulators between glomus cells and petrosal ganglion nerve terminals. Brain Res. Brain Res. Rev. 47, 46–53.

Iturriaga, R., Alcayaga, J., Gonzalez, C., 2009. Neurotransmitters in carotid body function: the case of dopamine. Adv. Exp. Med. Biol. 648, 137–143.

Jelic, S., Le Jemtel, T.H., 2008. Inflammation, oxidative stress, and the vascular endothelium in obstructive sleep apnea. Trends Cardiovasc. Med. 18, 253–260.

Kahn, B.B., Alquier, T., Carling, D., Hardie, D.G., 2005. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 1, 15–25.

Kemp, P.J., Peers, C., Lewis, A., 2003. Oxygen sensing by human recombinant large conductance, calcium-activated potassium channels. Regulation by acute hypoxia. Adv. Exp. Med. Biol. 536, 209–215.

Kim, D., Cavanaugh, E.J., Kim, I., Carroll, J.L., 2009. Heteromeric TASK-1/TASK-3 is the major oxygen-sensitive background K+ channel in rat carotid body glomus cells. J. Physiol. 587, 2963–2975.

Kummer, W., Acker, H., 1995. Immunohistochemical demonstration of four subunits of neutrophil NAD(P)H oxidase in type I cells of carotid body. J. Appl. Physiol. 78, 1904–1909.

Kääb, S., Miguel-Velado, E., López-López, J.R., Pérez-García, M.T., 2005. Down regulation of Kv3.4 channels by chronic hypoxia increases acute oxygen sensitivity in rabbit carotid body. J. Physiol. 566 (Pt 2), 395–408.

Lahiri, S., Iturriaga, R., Mokashi, A., Ray, D.K., Chugh, D., 1993. CO reveals dual mechanisms of chemoreception in the cat carotid body. Res. Physiol. 94, 227–240.

Lassègue, B., Griendling, K.K., 2010. NADPH oxidases: functions and pathologies in the vasculature. Arterioscler. Thromb. Vasc. Biol. 30, 653–661.

Lee, Y.M., Kim, B.J., Chun, Y.S., So, I., Choi, H., Kim, M.S., Park, J.W., 2006. NOX4 as an oxygen sensor to regulate TASK-1 activity. Cell Signal. 18, 499–507.

Lewis, A., Peers, C., Ashford, M.L., Kemp, P.J., 2002. Hypoxia inhibits human recombinant large conductance, Ca(2+)-activated K(+) (maxi-K) channels by a mechanism which is membrane delimited and Ca(2+) sensitive. J. Physiol. 540, 771–780.

Lopez-Barneo, J., Lopez-Lopez, J.R., Ureña, J., Gonzalez, C., 1988. Chemotransduction in the carotid body: K^+ current modulated by PO2 in type I chemoreceptor cells. Science 241, 580–582.

Lopez-Lopez, J.R., Gonzalez, C., 1992. Tissue course of K^+ current inhibition by low oxygen in chemoreceptors cells of adult rabbit carotid body. Effects of carbon monoxide. FEBS Lett. 299, 251–254.

McCartney, C.E., McClafferty, H., Huibant, J.M., Rowan, E.G., Shipston, M.J., Rowe, I.C., 2005. A cysteine-rich motif confers hypoxia sensitivity to mammalian large conductance voltage- and Ca-activated K (BK) channel alpha-subunits. Proc. Natl. Acad. Sci. U.S.A. 102, 17870–21786.

Michelakis, E.D., Hampl, V., Nsair, A., Wu, X., Harry, G., Haromy, A., Gurtu, R., Archer, S.L., 2002. Diversity in mitochondrial function explains differences in vascular oxygen sensing. Circ. Res. 90, 1307–1315.

Montero, M., Alonso, M.T., Carnicero, E., Cuchillo-Ibáñez, I., Albillos, A., García, A.G., García-Sancho, J., Alvarez, J., 2000. Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca^{2+} transients that modulate secretion. Nat. Cell Biol. 2, 57–61.

Moudgil, R., Michelakis, E.D., Archer, S.L., 2005. Hypoxic pulmonary vasoconstriction. J. Appl. Physiol. 98, 390–403.

Murphy, M.P., 2009. How mitochondria produce reactive oxygen species. Biochem. J. 417, 1–13.

Nakahira, K., Kim, H.P., Geng, X.H., Nakao, A., Wang, X., Murase, N., Drain, P.F., Wang, X., Sasidhar, M., Nabel, E.G., Takahashi, T., Lukacs, N.W., Ryter, S.W., Morita, K., Choi, A.M., 2006. Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. J. Exp. Med. 203, 2377–2389.

Nielsen, A.M., Bisgard, G.E., Vidruk, E.H., 1988. Carotid chemoreceptor activity during acute and sustained hypoxia in goats. J. Appl. Physiol. 65, 1796–1802.

Nohl, H., Gille, L., Staniek, K., 2005. Intracellular generation of reactive oxygen species by mitochondria. Biochem. Pharmacol. 69, 719–723.

Oakley, F.D., Abbott, D., Li, Q., Engelhardt, J.F., 2009. Signaling components of redox active endosomes: the redoxosomes. Antioxid. Redox Signal. 11, 1313–1333.

Obeso, A., Almaraz, L., Gonzalez, C., 1985. Correlation between adenosine triphosphate levels, dopamine release and electrical activity in the carotid body: support for the metabolic hypothesis of chemoreception. Brain Res. 348, 64–68.

Obeso, A., Gonzalez, C., Dinger, B., Fidone, S., 1989. Metabolic activation of carotid body glomus cells by hypoxia. J. Appl. Physiol. 67, 484–487.

Obeso, A., Gonzalez, C., Dinger, B., Fidone, S., 1993. Effect of low O_2 on glucose uptake in the rabbit carotid body. J. Appl. Physiol. 74, 2387–2393.

Obeso, A., Rocher, A., Herreros, B., Gonzalez, C., 1997. Oxygen consumption and energy metabolism of the carotid body. In: Gonzalez, C. (Ed.), The Carotid Body Chemoreceptors. Springer-Verlag, NY, pp. 31–45.

Ortega-Sáenz, P., Levitsky, K.L., Marcos-Almaraz, M.T., Bonilla-Henao, V., Pascual, A., López-Barneo, J., 2010. Carotid body chemosensory responses in mice deficient of TASK channels. J. Gen. Physiol. 135 (April 4), 379–392.

Osipenko, O.N., Tate, R.J., Gurney, A.M., 2000. Potential role for kv3. 1b channels as oxygen sensors. Circ. Res. 86, 534–540.

Pardal, R., Ludewig, U., Garcia-Hirschfeld, J., Lopez-Barneo, J., 2000. Secretory responses of intact glomus cells in thin slices of rat carotid body to hypoxia and tetraethylammonium. Proc. Natl. Acad. Sci. U.S.A. 97, 2361–2366.

Park, S.J., Chun, Y.S., Park, K.S., Kim, S.J., Choi, S.O., Kim, H.L., Park, J.W., 2009. Identification of subdomains in NADPH oxidase-4 critical for the oxygen-dependent regulation of TASK-1 K^+ channels. Am. J. Physiol. Cell Physiol. 297, C855–864.

Parkos, C.A., Dinauer, M.C., Walker, L.E., Allen, R.A., Jesaitis, A.J., Orkin, S.H., 1988. Primary structure and unique expression of the 22-kilodalton light chain of human neutrophil cytochrome b. Proc. Natl. Acad. Sci. U.S.A. 15, 3319–3323.

Paulsen, C.E., Carroll, K.S., 2010. Orchestrating redox signaling networks through regulatory cysteine switches. ACS Chem. Biol. 5, 47–62.

Peers, C., Wyatt, C.N., 2007. The role of maxiK channels in carotid body chemotransduction. Respir. Physiol. Neurobiol. 157, 75–82.

Peng, Y.J., Nanduri, J., Yuan, G., Wang, N., Deneris, E., Pendyala, S., Natarajan, V., Kumar, G.K., Prabhakar, N.R., 2009. NADPH oxidase is required for the sensory plasticity of the carotid body by chronic intermittent hypoxia. J. Neurosci. 29, 4903–4910.

Perez-Garcia, M.T., Lopez-Lopez, J.R., Gonzalez, C., 1999. Kvbeta1.2 subunit coexpression in HEK293 cells confers O2 sensitivity to kv4.2 but not to Shaker channels. J. Gen. Physiol. 113, 897–907.

Rathore, R., Zheng, Y.M., Niu, C.F., Liu, Q.H., Korde, A., Ho, Y.S., Wang, Y.X., 2008. Hypoxia activates NADPH oxidase to increase [ROS]i and [Ca2+]i through the mitochondrial ROS-PKCepsilon signaling axis in pulmonary artery smooth muscle cells. Free Radic. Biol. Med. 45, 1223–1231.

Rathore, R., Zheng, Y.M., Li, X.Q., Wang, Q.S., Liu, Q.H., Ginnan, R., Singer, H.A., Ho, Y.S., Wang, Y.X., 2006. Mitochondrial ROS-PKCepsilon signaling axis is uniquely involved in hypoxic increase in [Ca2+]i in pulmonary artery smooth muscle cells. Biochem. Biophys. Res. Commun. 351, 784–790.

Reeve, H.L., Michelakis, E., Nelson, D.P., Weir, E.K., Archer, S.L., 2001. Alterations in a redox oxygen sensing mechanism in chronic hypoxia. J. Appl. Physiol. 90, 2249–2256.

Rico, A.J., Prieto-Lloret, J., Gonzalez, C., Rigual, R., 2005. Hypoxia and acidosis increase the secretion of catecholamines in the neonatal rat adrenal medulla: an in vitro study. Am. J. Physiol. Cell Physiol. 289, C1417–C1425.

Ridge, J.W., 1972. Hypoxia and the energy charge of the cerebral adenylate pool. Biochem. J. 127, 351–355.

Riesco-Fagundo, A., Pérez-García, M.T., Gonzalez, C., López-López, J.R., 2001. O_2 modulates large-conductance Ca^{2+}-dependent K^+ channels of rat chemoreceptor cells by a membrane-restricted and CO-sensitive mechanism. Circ. Res. 89, 430–436.

Roepstorff, C., Thiele, M., Hillig, T., Pilegaard, H., Richter, E.A., Wojtaszewski, J.F., Kiens, B., 2006. Higher skeletal muscle alpha2AMPK activation and lower energy charge and fat oxidation in men than in women during submaximal exercise. J. Physiol. 574, 125–138.

Roy, A., Rozanov, C., Mokashi, A., Daudu, P., Al-mehdi, A.B., Shams, H., Lahiri, S., 2000. Mice lacking in gp91 phox subunit of NAD(P)H oxidase showed glomus cell [Ca(2+)](i) and respiratory responses to hypoxia. Brain Res. 872, 188–193.

Ruderman, N.B., Xu, X.J., Nelson, L., Cacicedo, J.M., Saha, A.K., Lan, F., Ido, Y., 2010. AMPK and SIRT1: a long-standing partnership? Am. J. Physiol. Endocrinol. Metab. 298, E751–E760.

Rumsey, W.L., Iturriaga, R., Spergel, D., Lahiri, S., Wilson, D.F., 1991. Optical measurements of the dependence of chemoreception on oxygen pressure in the cat carotid body. Am. J. Physiol. 261, C614–C622.

Rumsey, W.L., Lahiri, S., Iturriaga, R., Mokashi, A., Spergel, D., Wilson, D.F., 1992. Optical measurements of oxygen and electrical measurements of oxygen chemoreception in the cat carotid body. Adv. Exp. Med. Biol. 317, 387–395.

Sanchez, D., López-López, J.R., Pérez-García, M.T., Sanz-Alfayate, G., Obeso, A., Ganfornina, M.D., Gonzalez, C., 2002. Molecular identification of Kvalpha subunits that contribute to the oxygen-sensitive K(+) current of chemoreceptor cells of the rabbit carotid body. J. Physiol. 542, 369–382.

Sanders, K.A., Sundar, K.M., He, L., Dinger, B., Fidone, S., Hoidal, J.R., 2002. Role of components of the phagocytic NADPH oxidase in oxygen sensing. J. Appl. Physiol. 93, 1357–1364.

Santodomingo, J., Vay, L., Camacho, M., Hernández-Sanmiguel, E., Fonteriz, R.I., Lobatón, C.D., Montero, M., Moreno, A., Alvarez, J., 2008. Calcium dynamics in bovine adrenal medulla chromaffin cell secretory granules. Eur. J. Neurosci. 28, 1265–1274.

Sanz-Alfayate, G., Obeso, A., Agapito, M.T., Gonzalez, C., 2001. Reduced to oxidized glutathione ratios and oxygen sensing in calf and rabbit carotid body chemoreceptor cells. J. Physiol. 537, 209–220.

Saran, M., Bors, W., 1994. Signalling by $O_2^{\bullet-}$ and $NO^{\bullet}$: how far can either radical, or any specific reaction product, transmit a message under in vivo conditions? Chem. Biol. Interact. 90, 35–45.

Seidler, F.J., Slotkin, T.A., 1985. Adrenomedullary function in the neonatal rat: responses to acute hypoxia. J. Physiol. 358, 1–16.

Sekiguchi, F., Seo, M., Sunano, S., 2003. Contraction of arterial smooth muscle of normotensive and spontaneously hypertensive rats by manganese(III) tetrakis(1-methyl-4-pyridyl)porphyrin (MnTMPyP). J. Pharmacol. Sci. 92, 163–165.

Steinberg, G.R., Kemp, B.E., 2009. AMPK in health and disease. Physiol. Rev. 89, 1025–1078.

Streit, B.R., Blanc, B., Lukat-Rodgers, G.S., Rodgers, K.R., DuBois, J.L., 2010. How active-site protonation state influences the reactivity and ligation of the heme in chlorite dismutase. J. Am. Chem. Soc. 132, 5711–5724.

Tamayo, L., López-López, J.R., Castañeda, J., González, C., 1997. Carbon monoxide inhibits hypoxic pulmonary vasoconstriction in rats by a cGMP-independent mechanism. Pflugers Arch. 434, 698–704.

Taylor, C.T., 2008. Mitochondria and cellular oxygen sensing in the HIF pathway. Biochem. J. 409, 19–26.

Ushio-Fukai, M., 2009. Compartmentalization of redox signaling through NADPH oxidase-derived ROS. Antioxid. Redox Signal. 11, 1289–1299.

Verna, A., Talib, N., Roumy, M., Pradet, A., 1990. Effects of metabolic inhibitors and hypoxia on the ATP, ADP and AMP content of the rabbit carotid body in vitro: the metabolic hypothesis in question. Neurosci. Lett. 116, 156–161.

Wang, Y.X., Zheng, Y.M., 2010. ROS-dependent signaling mechanisms for hypoxic Ca(2+) responses in pulmonary artery myocytes. Antioxid. Redox Signal. 12, 611–623.

Ward, J.P., 2008. Oxygen sensors in context. Biochim. Biophys. Acta 1777, 1–14.

Waypa, G.B., Schumacker, P.T., 2008. Oxygen sensing in hypoxic pulmonary vasoconstriction: using new tools to answer an age-old question. Exp. Physiol. 93, 133–138.

Waypa, G.B., Chandel, N.S., Schumacker, P.T., 2001. Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing. Circ. Res. 88, 1259–1266.

Waypa, G.B., Marks, J.D., Guzy, R., Mungai, P.T., Schriewer, J., Dokic, D., Schumacker, P.T., 2010. Hypoxia triggers subcellular compartmental redox signaling in vascular smooth muscle cells. Circ. Res. 106, 526–535.

Waypa, G.B., Schumacker, P.T., 2005. Hypoxic pulmonary vasoconstriction: redox events in oxygen sensing. J. Appl. Physiol. 98, 404–414.

Waypa, G.B., Schumacker, P.T., 2006. Role for mitochondrial reactive oxygen species in hypoxic pulmonary vasoconstriction. Novartis Found. Symp. 272, 176–192.

Weir, E.K., Archer, S.L., 1995. The mechanism of acute hypoxic pulmonary vasoconstriction: the tale of two channels. FASEB J. 9, 183–189.

Weissmann, N., Zeller, S., Schäfer, R.U., Turowski, C., Ay, M., Quanz, K., Ghofrani, H.A., Schermuly, R.T., Fink, L., Seeger, W., Grimminger, F., 2006. Impact of mitochondria and NADPH oxidases on acute and sustained hypoxic pulmonary vasoconstriction. Am. J. Respir. Cell Mol. Biol. 34, 505–513.

Westermann, M., Leutbecher, H., Meyer, H.W., 1999. Membrane structure of caveolae and isolated caveolin-rich vesicles. Histochem. Cell Biol. 111, 71–81.

Whalen, W.J., Nair, P., 1976. Factors affecting the tissue PO_2 in the carotid body of the cat. In: Paintal, A.S. (Ed.), Morphology and Mechanisms of Chemoreceptors. Navchetan Press, Delhi, pp. 91–102.

Whalen, W.J., Nair, P., 1983. Oxidative metabolism and tissue PO_2 of the carotid body. In: Acker, H., O'Regan, R.G. (Eds.), Physiology of the Peripheral Arterial Chemoreceptors. Elsevier, Amsterdam, pp. 117–132.

Williams, S.E., Brazier, S.P., Baban, N., Telezhkin, V., Müller, C.T., Riccardi, D., Kemp, P.J., 2008. A structural motif in the C-terminal tail of slo1 confers carbon monoxide sensitivity to human BK Ca channels. Pflugers Arch. 456, 561–572.

Williams, S.E., Wootton, P., Mason, H.S., Bould, J., Iles, D.E., Riccardi, D., Peers, C., Kemp, P.J., 2004. Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. Science 306, 2093–2097.

Woods, A., Johnstone, S.R., Dickerson, K., Leiper, F.C., Fryer, L.G., Neumann, D., Schlattner, U., Wallimann, T., Carlson, M., Carling, D., 2003. LKB1 is the upstream kinase in the AMP-activated protein kinase cascade. Curr. Biol. 13, 2004–2008.

Wu, W., Platoshyn, O., Firth, A.L., Yuan, J.X., 2007. Hypoxia divergently regulates production of reactive oxygen species in human pulmonary and coronary artery smooth muscle cells. Am. J. Physiol. Lung Cell Mol. Physiol. 293, L952–L959.

Wyatt, C.N., Mustard, K.J., Pearson, S.A., Dallas, M.L., Atkinson, L., Kumar, P., Peers, C., Hardie, D.G., Evans, A.M., 2007. AMP-activated protein kinase mediates carotid body excitation by hypoxia. J. Biol. Chem. 282, 8092–8098.

Wyatt, C.N., Wright, C., Bee, D., Peers, C., 1995. O_2-sensitive K^+ currents in carotid body chemoreceptor cells from normoxic and chronically hypoxic rats and their roles in hypoxic chemotransduction. Proc. Natl. Acad. Sci. U.S.A. 92, 295–299.

Yu, J.H., Kim, K.H., Kim, D.G., Kim, H., 2007. Diphenyleneiodonium suppresses apoptosis in cerulein-stimulated pancreatic acinar cells. Int. J. Biochem. Cell Biol. 39, 2063–2075.