Fifty Years of Progress in Carotid Body Physiology – *Invited Article*

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Abstract Research on arterial chemoreceptors, particularly on the carotid body, has been fruitful in the last fifty years, to which this review is addressed. The functional anatomy of the organ appears to be well established. The biophysical bases by which glomus cells transduce chemical changes in the *milieu intérieur* (hypoxia, hypercapnia, acidosis) into electrical and biochemical changes in glomus cells have received much attention. Physical changes (in temperature, flow and osmolarity) are also detected by the carotid body. Electrical coupling between glomus cells themselves appears as very extensive. Sustentacular cells classically considered as ensheathing glia for glomus cells and nerve endings now appear to behave as stem cells precursors for glomus cells under chronic hypoxic conditions. Many papers have been devoted to transmitters released from glomus cells (acetylcholine, dopamine, ATP) and well as to their effects upon chemosensory nerve activity. Chemosensory neurons have been explored from generation of action potentials at peripheral nerve endings, passing to properties of perikarya at petrosal ganglia and finally at characterization of synaptic transmission at solitary tract nuclei. There is abundant literature on ventilatory and cardiovascular reflexes elicited from arterial chemoreceptors. The transient effects of sudden and brief withdrawal of chemosensory discharges by hyperoxia also provide clues on the role played by carotid bodies in the homeostasis of full organisms.

Keywords Carotid body physiology · Progress in research · Research last 50 years

1 Prolegomena

January 31, 1743 appears to be the date of the first historically available document about the anatomy of the carotid body. The thesis of Louis Taube was presented in the lab of the great German physiologist, Albrecht von Haller. Over 200 years

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followed without much significant work. In 1926 and especially in 1928, the eminent Spanish histologist Fernando De Castro presented his studies and his thoughts on the function of the carotid body. Though Corneille Heymans was awarded the 1938 Nobel Prize in Physiology or Medicine, his work was based on both the anatomy presented in De Castro's work and on his hypothesis as to its physiological function (for refs. see Eyzaguirre et al., 1983).

2 Organization of the Carotid Body

Several electron-microscopic studies in the 1960's showed that glomus cells (De Castro's epithelioid cells) were rich in dense-core granules and abundantly supplied with apposing nerve endings containing synaptic-like vesicles, suggesting from morphological grounds that glomus cells were some sort of endocrine cells controlled by efferent nerve fibers. Then, Arthur Hess performed several electron-microscopic studies in normally innervated, fully denervated and solely deafferented carotid bodies, demonstrating that glomus cells had reciprocal synapses with apposing nerve endings, which were part of the peripheral processes of sensory neurons with perikarya located in the petrosal ganglion, thus confirming De Castro's earlier hypothesis (see refs. in Eyzaguirre et al., 1983).

The electron microscopic studies performed by Donald McDonald showed that the carotid body was vascularly supplied by convoluted capillaries in the center of glomoids (glomus cells islets) and straight capillaries and arterio-venous anastomoses at the periphery of the organ (see McDonald, 1981), thus providing a vascular system capable of regulating blood flow through the parenchyma of the carotid body.

The autonomic innervation of the carotid body was also a matter of much debate, but the elegant microscopic studies of Alain Verna led to the conclusion that most sympathetic fibers arriving from the superior cervical ganglion were innervating carotid body blood vessels and that a parasympathetic innervation of the organ was scarce or absent (see Verna, 1997). However, autonomic neurons located in microganglia along the glossopharyngeal nerve may provide efferent inhibition to the carotid body (Campanucci and Nurse, 2007).

Sustentacular cells (expressing the glial marker GFAP) were for long time considered only as enveloping bags for glomus cells and nerve endings appositions. But, clustered glomus cells in culture (retaining their sustentacular envelope) behave differently from isolated cells. Moreover, recent observations by López-Barneo and coworkers suggest that -when exposed to prolonged hypoxia- sustentacular cells may also behave as stem cells precursors for glomus cells (see Pardal et al., 2007).

3 Testing Carotid Body Physiological Responses

The initial information on carotid body physiology was obtained by the elicitation of ventilatory chemoreflexes in preparations in toto, a procedure that was followed by the recording of carotid nerve chemosensory discharges in situ. Thus, Mulligan

and Lahiri (1981) showed that every agent acting as inhibitor of cytochrome oxidase (such as cyanide) or uncoupler of electron transport (such as 2,4-dinitrophenol) was an effective stimulant for carotid body chemosensory discharges. Attempts to perfuse the carotid body in situ with saline resulted in short-lived preparations, but perfusing the organ with blood was more successful (O'Regan, 1979). Nevertheless, it was not possible to establish whether carotid body responses to a single chemical stimulus (e.g., hypoxia) or to a given pharmacological agent (e.g., nicotine) resulted from their direct effects on chemoreceptors themselves or were secondary effects associated with changes in other chemoreceptor natural stimuli or to changes in blood flow through carotid body tissue.

The above problems were solved by Eyzaguirre and coworkers through the use of an isolated preparation of the carotid body in vitro superfused with saline solutions flowing at a controlled rate, and in which one controlled change in chemical constituents or physical conditions would be introduced at a time without affecting other variables of the experiment. This type of preparation revealed that the carotid body was a "multimodal" receptor, responding directly to various chemical changes (oxygen and carbon dioxide tensions; hydrogen ion and potassium concentrations) as well as to physical changes (temperature, osmolarity and fluid flow) that may occur under physiological conditions (see Eyzaguirre et al., 1983).

Further advances have been made by studies on carotid body tissue slices in vitro, cells acutely dissociated from excised carotid bodies, carotid body cells in tissue culture, petrosal ganglion cells in tissue culture, and co-cultures of carotid body and sensory ganglion cells. However, cultured glomus cells, and for that matter preparations in vitro, do not necessarily behave the same as glomus cells in the animal, and studies on carotid bodies in situ or in whole animals -in which the natural environment of the carotid body is better preserved- are still required to validate current observations made on isolated preparations in vitro.

4 Glomus Cell Responses to Chemical Stimuli

Since another review is addressed to the effects of "natural" chemical stimuli on membrane potential and ion channels of glomus cells, we will briefly mention that different stimuli (hypoxia, hypercapnia, acidity) depolarize about half of glomus cells while the others undergo hyperpolarization, an effect conditioned by the presence or absence of sustentacular cells (see Eyzaguirre et al., 1989).

The demonstration by José López-Barneo and Constancio González and their co-workers that hypoxia produces a reversible inhibition of a transient K^+ current in glomus cells (López-Barneo et al., 1988) initiated a prolific area of research on oxygen-dependent ionic channels. The studies performed by Chris Peers and Keith Buckler had been particularly clarifying on this issue (see Peers and Buckler, 1995).

It has been found that glomus cells are dye and electrically coupled between themselves, through gap junctions revealed by high resolution electron microscopy

and biochemical characterization of connexins. Chemoreceptor stimulants (acute and chronic hypoxia, hypercapnia, acidity, cholinergic agents and dopamine) uncouple most glomus cells, a change accompanied by cell depolarization and decreased amplitude of junction channels activity. Coupling is mostly resistive from glomus cells to nerve endings, but it is mostly capacitive from nerve endings to glomus cells (see Eyzaguirre, 2007). Thus, slow electric events originating in the glomus cells can be transferred to the nerve endings.

We know from several studies that hypoxia and acidity increase the intracellular concentration of calcium ($[Ca^{2+}]_i$). This may be due to calcium inflow through the glomus cell membrane (González et al., 1994), but it may also come from intracellular stores (Biscoe and Duchen, 1990). An acute increase in calcium concentration is required for excitation-secretion coupling to occur, as shown in every place in which it has been studied, and therefore it will be necessary for release of transmitters to convey excitation from glomus cells to sensory nerve endings. Nevertheless, carotid body preparations bathed in zero $[Ca^{2+}]_o$ -that should eliminate transmitter release- show a reduced chemosensory nerve discharge, but it is still there.

5 Chemical Transmission Between Glomus Cells and Chemosensory Nerve Endings

Several observations showing that destruction of glomus cells by criocoagulation or ischemia suppresses the chemoreceptive properties of the carotid body, and that regenerating nerve fibers acquire chemosensory activity when they come into contact with glomus cells (see Eyzaguirre et al., 1983) indicated that glomus cells were indeed required for hypoxic chemoreception. Furthermore, intracellular recordings from carotid nerve terminals showed small depolarizing potentials in unstimulated preparations, which upon stimulation with NaCN or ACh increased their frequency to the point of fusion, resulting in a larger total depolarization accompanying the increased sensory discharge (see Eyzaguirre et al., 1983). At a time when chemical transmission between nervous structures appeared to have defeated electrical transmission, a frantic search for chemical transmitters between glomus cells and nerve endings was initiated.

The **cholinergic hypothesis** was based on several observations accumulated during the first half of the 20th century (for refs. see Eyzaguirre et al., 1983). The idea of acetylcholine (ACh) playing the role of transmitter between glomus cells and chemosensory nerve endings gained acceptance by the bioassays revealing the persistence of ACh within the carotid body after denervation of the organ (Eyzaguirre and Koyano, 1965), suggesting that ACh was probably stored within glomus cells. More recently, Shirahata and Fitzgerald (1996) -using high performance liquid chromatography-electrochemical detection- confirmed the release of ACh from both cat and pig cultured glomus cells in vitro incubated in hypoxic solutions.

The possibility that ACh effects on the carotid body were vascularly mediated was discarded by the persistence of such effects on cat carotid bodies superfused in vitro (Eyzaguirre and Koyano, 1965). The sensitivity to ACh and nicotine of petrosal ganglion cells provided further support to the idea that chemosensory neurons were indeed cholinoceptive (Fitzgerald et al., 2000; Alcayaga et al., 1998). Furthermore, Fitzgerald and Shirahata (1994) reported that selective perfusion of cat's carotid bifurcation with a mixture of cholinergic antagonists (α-bungarotoxin, mecamylamine and atropine) reduces the chemosensory response of the carotid body to hypoxia, while carotid sinus barosensory discharges were unaffected.

The **dopaminergic hypothesis** was based on the detection of high levels of dopamine (DA) within the carotid bodies of all mammalian species studied (see Eyzaguirre et al., 1983). The observation by Fidone et al. (1982) that ^{3}H-DA outflow was highly correlated with the degree of hypoxia to which rabbit carotid bodies superfused in vitro had been exposed revealed that hypoxia releases DA from glomus cells and gave strong support to the idea that DA was the chemical transmitter between such cells and sensory nerve endings. The later availability of voltammetric-amperometric techniques employing carbon fibre electrodes made possible the fast temporal resolution of DA release from the carotid body, confirming that hypoxia was a strong stimulus for DA release from the organ, but revealing that such DA release was slower than chemosensory excitation (Donnelly, 1993; Buerk et al., 1995; Iturriaga et al., 1996).

The main problem with the dopaminergic hypothesis is that DA inhibits carotid body chemosensory discharges in most preparations and depresses ventilation in most mammalian species, with the possible exception of the rabbit. The transient increases in carotid body chemosensory discharges or ventilation observed after administration of dopaminergic blockers suggest that endogenous DA released from glomus cells may serve as an inhibitory modulating agent (see Zapata, 1997).

The **purinergic hypothesis**. The changing levels of ATP within carotid body tissues and the chemosensory excitatory effects observed upon its administration were initially considered as part of ATP metabolic role, but the demonstration of membrane receptors for ATP in other tissues made necessary to consider its possible role as transmitter between glomus cells and sensory nerve endings. Thus, $P2X_2$ and $P2X_3$ receptors subunits were detected in afferent nerve terminals surrounding clusters of glomus cells, as well as in the perikarya of many petrosal ganglion neurons (Prasad et al., 2001). Recently, Conde and Monteiro (2006) report that incubated carotid bodies release larger amounts of ATP when exposed to hypoxic media than when exposed to normoxic and hyperoxic media.

McQueen et al. (1998) reported that ATP evokes cardiorespiratory effects in rats, but that its antagonists suramin and PPADS did not affect carotid body chemosensory responses to cyanide-induced hypoxia or to asphyxia, while Rong et al. (2003) reported that PPADS reduces hypoxia-induced carotid nerve discharge in mice.

The **cholinergic-purinergic hypothesis**. Colin Nurse and co-workers reported that synapses re-established in co-cultures of rat's glomus cells with sensory ganglion neurons had their hypoxic-induced discharges only partially blocked by

nicotinic cholinergic antagonists (hexamethonium or mecamylamine), or by P2X purinergic blocker suramin, but that such evoked activity was suppressed by a mixture of mecamylamine and suramin, leading to the proposal that ACh and ATP were co-released from glomus cells during hypoxic stimulation, and thus responsible for the ensuing excitation of the juxtaposed sensory neurons (Zhang et al., 2000; Nurse and Zhang, 2001). While ACh and ATP were confirmed as strong stimuli for chemosensory discharges recorded from cat's carotid bodies in situ and in vitro, and these responses were respectively blocked by mecamylamine and suramin, the combination of such antagonists did not suppress chemosensory excitation evoked by hypoxic stimulation (see Zapata, 2007).

6 Chemoreflexes Originated from the Carotid Bodies

The **respiratory response** to carotid body stimulation seems to be the most obvious and the most powerful of the chemoreflex responses. Increases in tidal volume and frequency, end expiratory volume, airways secretions, and airways resistance are the most prominent respiratory responses to hypoxic, hypercapnic or acidotic stimulation of the carotid bodies. The Wisconsin group led by Bisgard, Dempsey, and Forster and more recently by Mitchell has contributed further to these studies with unanesthetized goats and other species. References to the abundant literature on this issue can be found in the reviews by Fitzgerald and Lahiri (1986), and Fitzgerald and Shirahata (1997).

A problem to be solved was the role played by the carotid bodies under resting (normoxic, eucapnic) conditions. Pierre Dejours (1957) proposed to test such "chemosensory drive" by applying an abrupt and brief ventilation of pure oxygen to silence arterial chemoreceptors and therefore withdraw chemoreflex influences. This Dejours' maneuver results in an almost immediate but brief decrease in tidal volume and/or respiratory rate. This hyperoxia-evoked transient hypoventilation was absent in animals after bilateral sectioning of carotid sinus nerves, as well as in humans previously subjected to bilateral glomectomy (Honda et al., 1979). It must be mentioned that awake dogs remain hypercapnic for at least 19 days after removal of their carotid bodies (Rodman et al., 2001).

The **cardiovascular responses** to carotid body stimulation have been a subject of controversy. They are less intense and somehow slower than respiratory responses, they are modified by respiratory responses, and some of them may be secondary to respiratory responses. It must be mentioned that Andrzej Trzebski and associates in Warsaw reported a transient reduction in arterial pressure upon breathing 100% O_2 in hypertensive patients. In view of recent studies by Schultz and coworkers (2007) showing the prominent role played by the carotid bodies in chronic heart failure, it might be helpful to review some of the findings from the mid-20th century. Whereas stimulation of the carotid sinus as, for example, in high blood pressure acts as a brake on sympathetic activity, carotid body stimulation

increases sympathetic output. Carotid body stimulation initially produces bradycardia which upon continued stimulation is followed by tachycardia. The impact of carotid body stimulation on cardiac contractility is still under debate. Experimental evidence supports both an increase and a decrease, differences arising from methods of measurement, species under study, state of wakefulness, sleep, or anesthesia, and control of ventilatory conditions (see reviews by Eyzaguirre et al., 1983; Fitzgerald and Shirahata, 1997).

Carotid body-stimulated increases in sympathetic output constrict the vasculature in most, but not all vascular beds. Carotid body stimulation blunts the classical hypoxic pulmonary vasoconstrictor response. It also provokes a dilation in the bronchial vasculature. Coronary circulation is certainly impacted by carotid body stimulation, but this is a classical example of the difficulty of predicting the outcome of the stimulation due to the presence of other reflex responses impacting on the vascular resistance. The increases in sympathetic output also produce a decrease in venous capacitance, which will in turn increase venous return and therefore cardiac output. The impact of carotid body stimulation on the cerebral vasculature remains perhaps the most controversial area. However, it appears that chemoreceptor stimulation does reduce vascular resistance in the neurohypophysis and in the eye. (See Fitzgerald and Shirahata, 1997).

7 Concluding Remarks

In summary, we can say that the last 50 years have produced a *dramatic advance* (see Figs. 1, 2 and 3) in our understanding of:

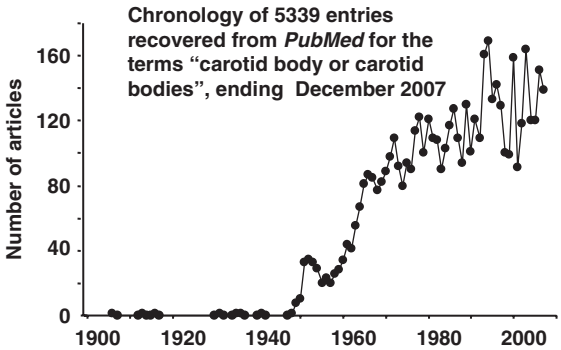

Fig. 1 Chronology of 5339 entries recovered from *PubMed* (service of the National Library of Medicine) for the terms "carotid body or carotid bodies", for the period ending at December 2007. For the same period, only 269 papers appeared for the terms "aortic body or aortic bodies", and 2604 for the term "arterial chemoreceptors". Number of papers on the ordinate; year of publication on the abscissa. The first 5 papers (from 1906 to 1935) are reports on carotid body tumors

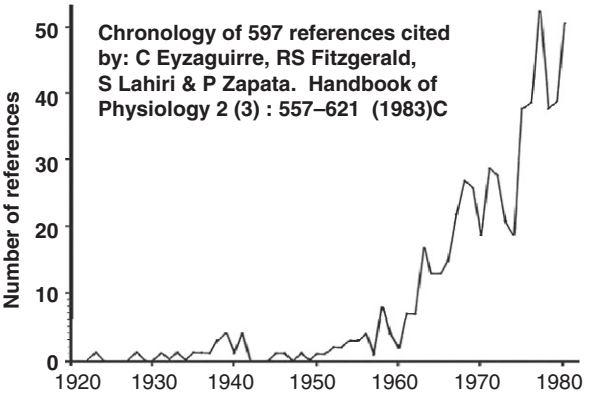

Fig. 2 Chronology of 597 papers cited as references for the chapter on "Arterial chemoreceptors" of the "Handbook of Physiology" (Section 2, Volume 3), printed in 1983. Number of papers on the ordinate; year of publication on the abscissa. The first reference corresponds to a paper by MW Gerard and PR Billingsley (1923) on the innervation of the carotid body. The second paper (1928) is De Castro's foundational contribution to this topic

Levels:	**Studies:**
Molecules	O_2-dependent ion channels
Organelles	DA uptake in isolated granules from glomus cells
Cells	Membrane potential changes in glomus cells
	Intracellular Ca^{++} changes in glomus cells
Tissue	Gap junctions between glomus cells
	Glomus cells to nerve endings transmission
Organ	Functional organization of the CB
	O_2 consumption of CB in vitro
System	Ventilatory reflexes originated from CBs
	Cardiovascular adjustments evoked from CB
Organism	Arousal evoked from CBs excitation
	'Defense' reaction evoked from CBs
Environmental	CB adjustment at high altitude
	CB function after chronic hypoxic challenges
Populations	Genetic traits in CB tumours
	Geographical distribution of CB tumors

Fig. 3 Levels of studies performed on the carotid body. Most studies on molecules, organelles, organ and tissue had been performed along the last 50 years, as well as those on environmental and populations levels

- what carotid body's impact is on the organism's need to maintain or reestablish homeostasis;
- what mechanisms are involved in generating the neural traffic from the carotid body to the nucleus tractus solitarius;
- which activity, passed on to various other nuclei in the brainstem and down the cord, initiates and sustains homeostasis-benefitting reflex responses;
- Not all controversies, conflicts of data, contradictory interpretations have been resolved, but at least an overall general agreement regarding the basic events required to increase CB outward neural traffic to the nucleus tractus solitarius seems to exist;
- Up to the middle of the 20th century most of today's remaining questions weren't even being asked.

References

Alcayaga J, Iturriaga R, Varas R, Arroyo J, Zapata P (1998) Selective activation of carotid nerve fibres by acetylcholine applied to the cat petrosal ganglion in vitro. *Brain Res* 786: 47–54

Biscoe TJ, Duchen MR (1990) Responses of type I cells dissociated from the rabbit carotid body to hypoxia. *J Physiol* 428: 39–59

Buerk DG, Lahiri S, Chugh D, Mokashi A (1995) Electrochemical detection of rapid DA release kinetics during hypoxia in perfused-superfused cat CB. *J Appl Physiol* 78: 830–7

Campanucci VA, Nurse CA (2007) Autonomic innervation of the carotid body: Role in efferent inhibition. *Respir Physiol Neurobiol* 157: 83–92

Conde SV, Monteiro EC (2006) Profiles for ATP and adenosine release at the carotid body in response to O_2 concentrations. *Adv Exp Med Biol* 580: 179–84

Dejours P (1957) Methodological importance of the study of a living organism at the initial phase of interruption of a physiological equilibrium. *C R Hebd Seances Acad Sci* 245: 1946–8

Donnelly DF (1993) Electrochemical detection of catecholamine release from rat carotid body in vitro. *J Appl Physiol* 74: 2330–7

Eyzaguirre C (2007) Electric synapses in the carotid body-nerve complex. *Respir Physiol Neurobiol* 157: 116–122

Eyzaguirre C, Fitzgerald RS, Lahiri S, Zapata P (1983) Arterial chemoreceptors. In: *American Physiological Society: Handbook of Physiology, sect. 2: The Cardiovascular System, vol. 3: Peripheral Circulation and Organ Blood Flow*. Baltimore, MD: Williams & Wilkins, pp. 557–621

Eyzaguirre C, Koyano H (1965) Effects of hypoxia, hypercapnia, and pH on the chemoreceptor activity of the carotid body in vitro. *J Physiol* 178: 385–409

Eyzaguirre C, Koyano H, Taylor JR (1965) Presence of acetylcholine and transmitter release from carotid body chemoreceptors. *J Physiol* 178: 463–76

Eyzaguirre C, Monti-Bloch L, Baron M, Hayashida Y, Woodbury JW (1989) Changes in glomus cell membrane properties in response to stimulants and depressants of carotid nerve discharge. *Brain Res* 477: 265–279

Fidone S, González C, Yoshizaki K (1982) Effects of low oxygen on the release of dopamine from the rabbit carotid body in vitro. *J Physiol* 333: 93–110

Fitzgerald RS, Lahiri S (1986) Reflex responses to chemoreceptor stimulation. In: *American Physiological Society (eds) Handbook of Physiology*, sect. 3, vol. 2, pp. 313–362

Fitzgerald RS, Shirahata M (1994) Acetylcholine and carotid body excitation during hypoxia in the cat. *J Appl Physiol* 76: 1566–1574

Fitzgerald RS, Shirahata M (1997) Systemic responses elicited by stimulating the carotid body: primary and secondary mechanisms. In: González C (ed) *The Carotid Body Chemoreceptors.* Berlin: Springer-Verlag, pp. 171–191

Fitzgerald RS, Shirahata M, Wang HY (2000) Acetylcholine is released from in vitro cat carotid bodies during hypoxic stimulation. *Adv Exp Med Biol* 475: 485–94

González C, Almaraz L, Obeso A, Rigual R (1994) Carotid body chemoreceptors: From natural stimuli to sensory discharges. *Physiol Rev* 74: 829–898

Honda Y, Watanabe S, Hashizume I, Satomura Y, Hata N, Sakakibara Y, Severinghaus JW (1979) Hypoxic chemosensitivity in asthmatic patients two decades after carotid body resection. *J Appl Physiol* 46: 632–8

Iturriaga R, Alcayaga J, Zapata P (1996) Dissociation of hypoxia-induced chemosensory responses and catecholamine efflux in cat carotid body superfused in vitro. *J Physiol* 497: 551–64

López-Barneo J, López-López JR, Ureña J, González C (1988) Chemotransduction in the carotid body: K^+ current modulated by PO_2 in type I chemoreceptor cells. *Science* 241: 580–582

McDonald DM (1981) Peripheral chemoreceptors: structure-function relationships of the carotid body. In: Hornbein TF (ed) *Regulation of Breathing. Lung Biology in Health and Disease*, vol 17. NY: Marcel Dekker. pp. 105–319

McQueen DS, Bond SM, Moores C, Chessell I, Humphrey PP, Dowd E (1998) Activation of P_2X receptors for adenosine triphosphate evokes cardiorespiratory reflexes in anaesthetized rats. *J Physiol* 507: 84–55

Mulligan E, Lahiri S (1981) Dependence of carotid chemoreceptor stimulation by metabolic agents on P_aO_2 and P_aCO_2. *J Appl Physiol* 50: 884–891

Nurse CA, Zhang M (2001) Synaptic mechanisms during re-innervation of rat arterial chemoreceptors in co-culture. *Comp Biochem Physiol A - Mol Integr Physiol* 130: 241–251

O'Regan RG (1979) Responses of the chemoreceptors of the cat carotid body perfused with cell-free solutions. *Ir J Med Sci* 148: 78–85

Pardal R, Ortega-Sáenz P, Durán R, López-Barneo J (2007) Glia-like stem cells sustain physiologic neurogenesis in the adult mammalian carotid body. *Cell* 131: 364–377

Peers C, Buckler KJ (1995) Transduction of chemostimuli by the type I carotid body cell. *J Memb Biol* 144: 1–9

Prasad M, Fearon IM, Zhang M, Laing M, Vollmer C, Nurse CA (2001) Expression of P_2X_2 and P_2X_3 receptor subunits in rat carotid body afferent neurones: role in chemosensory signalling. *J Physiol* 537: 667–77

Rodman JR, Curran AK, Henderson KS, Dempsey JA, Smith CA (2001) Carotid body denervation in dogs: eupnea and the ventilatory response to hyperoxic hypercapnia. *J Appl Physiol* 91: 328–35. Erratum in: *J Appl Physiol* 91(5): following table of contents

Rong W, Gourine AV, Cockayne DA, Xiang Z, Ford APDW, Spyer KM, Burnstock G (2003) Pivotal role of nucleotide $P2X_2$ receptor subunit of the ATP-gated ion channel mediating ventilatory responses to hypoxia. *J Neurosci* 23: 11315–11321.

Schultz HD, Li YL, Ding Y (2007) Arterial chemoreceptors and sympathetic nerve activity: implications for hypertension and heart failure. *Hypertension* 50: 6–13

Shirahata M, Fitzgerald RS (1996) Release of acetylcholine from cultured cat and pig glomus cells. *Adv Exp Med Biol* 410: 233–237

Verna A (1997) The mammalian carotid body: morphological data. In: González C (ed) *The Carotid Body Chemoreceptors*. Berlin: Springer; Austin, TX: Landes, pp. 1–29

Zapata P (1997) Chemosensory activity in the carotid nerve: Effects of pharmacological agents. In: González C (ed) *The Carotid Body Chemoreceptors*. Berlin: Springer-Verlag, pp. 119–146

Zapata P (2007) Is ATP a suitable co-transmitter in carotid body arterial chemoreceptors? *Respir Physiol Neurobiol* 157: 106–115

Zhang M, Zhong HJ, Vollmer C, Nurse CA (2000) Co-release of ATP and ACh mediates hypoxic signalling at rat carotid body chemoreceptors. *J Physiol, Lond* 525: 143–158