On the existence of a central respiratory oxygen sensor

Abbreviated title: **CNS oxygen sensor**

Alexander V Gourine[1] and Gregory D Funk[2]

[1]*Centre for Cardiovascular and Metabolic Neuroscience, Department of Neuroscience, Physiology & Pharmacology, University College London, London WC1E 6BT, United Kingdom;*

[2]*Department of Physiology, Women and Children's Health Research Institute, Neuroscience and Mental Health Institute, University of Alberta, Edmonton T6G 2E1, Canada;*

[CA]Corresponding author: Alexander V Gourine PhD, Centre for Cardiovascular and Metabolic Neuroscience, Department of Neuroscience, Physiology & Pharmacology, University College London, London WC1E 6BT, United Kingdom; Tel +44 20 7679 6480; Email: a.gourine@ucl.ac.uk

Keywords: astrocyte, brainstem, carotid body, chemosensitivity, hypoxia, hypoxic ventilatory response, oxygen.

Conflict of interest: The authors declare no competing financial interests.

Acknowledgements: Results of the authors' experimental studies described in this review article were obtained with generous support of The Wellcome Trust and British Heart Foundation. A.V.G is a Wellcome Trust Senior Research Fellow (Refs. 095064 and 200893).

Abstract

A commonly held view that dominates both the scientific and educational literature is that in terrestrial mammals the central nervous system lacks a physiological hypoxia sensor capable of triggering increases in lung ventilation in response to decreases in PO_2 of the brain parenchyma. Indeed, a normocapnic hypoxic ventilatory response has never been observed in humans following bilateral resection of the carotid bodies. In contrast, almost complete or partial recovery of the hypoxic ventilatory response after denervation/removal of the peripheral respiratory oxygen chemoreceptors has been demonstrated in many experimental animals when assessed in an awake state. In this essay we review the experimental evidence obtained using *in vitro* and *in vivo* animal models, results of human studies, and discuss potential mechanisms underlying the effects of CNS hypoxia on breathing. We consider experimental limitations and discuss potential reasons why the recovery of the hypoxic ventilatory response has not been observed in humans. We review recent experimental evidence suggesting that the lower brainstem contains functional respiratory oxygen sensitive elements capable of stimulating respiratory activity independently of peripheral chemoreceptor input.

Introduction

The high metabolic rate of the brain associated with the activities of millions of nerve cells processing information requires constant, optimal nutrient and oxygen supply, as well as effective removal of carbon dioxide and other metabolic waste products. Adequate oxygenation of the arterial blood supplying the brain is monitored by specialized respiratory oxygen sensors located in the carotid bifurcation (carotid bodies) and, in some species, the aortic arch (aortic bodies). These peripheral chemoreceptors detect decreases in the arterial PO_2 and transmit chemosensory information to the brainstem respiratory centers, triggering adaptive changes in breathing (34; 48). This simple 'textbook' view on chemosensory control of breathing implies that detection of the arterial partial pressure of O_2 (PO_2) at the level of the peripheral chemoreceptor is sufficient to ensure appropriate oxygenation of all regions of the brain. However, significant gradients of brain tissue oxygen levels have been demonstrated at normal arterial PO_2 (15; 31) supporting the contentious idea that many central neurons may operate in a low oxygen environment (19; 44). Being located "upstream" from the central nervous system, arterial respiratory

chemoreceptors are obviously not able to detect and respond to significant regional differences in brain oxygenation or local brain hypoxia. Moreover, all species of terrestrial mammals studied so far survive surgical denervation or removal of the peripheral oxygen chemoreceptors with no major adverse physiological consequences. While hypoxic stress at sea level is rare, peripherally chemodenervated experimental animals and humans can tolerate hypoxia that might be common during sleep in a host of disparate disease states.

A commonly held view that dominates both the scientific and educational literature is that the central nervous system lacks a physiological oxygen sensor capable of stimulating the brainstem respiratory network and lung ventilation in response to decreases in the PO_2 of the brain parenchyma. Indeed, no recovery of the normocapnic hypoxic ventilatory response has ever been reported in humans following bilateral resection of the carotid bodies (63). In contrast, significant evidence from experimental animal studies demonstrates almost complete or partial recovery of the hypoxic ventilatory response after surgical denervation/removal of the peripheral respiratory oxygen chemoreceptors (1; 2; 12-14; 18; 40; 42; 46; 51; 53; 54).

In this short review article we discuss the experimental evidence obtained in studies of the hypoxic ventilatory response using animal models (*in vitro* and *in vivo*) as well as human subjects with denervated peripheral respiratory oxygen sensors. We consider potential reasons why the recovery of the hypoxic ventilatory response has not been observed in human studies. We also discuss recent experimental evidence suggesting that the lower brainstem contains functional oxygen sensitive elements capable of stimulating breathing independently of peripheral chemoreceptor input.

Animal Studies

In mammals, acute moderate hypoxia generally induces a biphasic hypoxic ventilatory response, which consists of an initial strong increase in the respiratory effort (within the first minute of exposure to hypoxia) (phase I) followed by a secondary reduction in respiratory activity (phase II, roll-off or hypoxic ventilatory decline) over the next several minutes reaching a new steady state level of ~25-40% above the baseline (43; 60). The initial increase in the respiratory activity is believed to be primarily triggered by activation of the peripheral respiratory oxygen chemoreceptors while the secondary

reduction of ventilation is traditionally attributed to the hypoxia-induced depression of the brainstem respiratory circuits (although there is evidence that in the awake state hypoxic ventilatory decline is also dependent on the carotid body input (e.g. see (33)).

Indeed, the majority of central neurons respond to hypoxia with a reduction in excitability. However, in the early 90s Sun and Reis (57; 58) demonstrated that some CNS neurons, in particular pre-sympathetic neurons of the brainstem, increase their discharge in low PO_2 conditions and trigger generalized increases in sympathetic nerve activity during brain hypoxia. Subsequent experimental studies performed using *in vitro*, *in situ* and anesthetized animal models demonstrated that in addition to pre-sympathetic neurons, brainstem respiratory control circuits are sensitive to, and can be activated by, decreases in local parenchymal PO_2 or cytotoxic hypoxia (10; 38; 45; 47; 49; 52; 55; 56; 61). These studies demonstrated biphasic responses of the isolated (in an *in vitro* brainstem slice) central respiratory network to hypoxia, quantified PO_2 sensitivity, described differential neuronal responses and suggested some plausible mechanisms of neuronal oxygen sensing (e.g. heme oxygenase-dependent hypoxia-induced depolarization of cultured brainstem neurons (10)).

These experimental studies conducted *in vitro* support a central stimulatory component of the hypoxic ventilatory response, however, whether these mechanisms (activated *in vitro*) are relevant to the physiological responses that are involved in the homeostatic regulation of brain oxygenation by the respiratory network remains a contentious issue. Few investigators working in more intact preparations (e.g. *in vivo*) consider that the responses of the respiratory network to hypoxia/anoxia *in vitro* are physiologically relevant. This is a valid criticism because baseline conditions of the *in vitro* preparations are characterized by extreme hyperoxia and the strength of the hypoxic stimulus varies with tissue depth since O_2 delivery is via diffusion (discussed in detail in (17)).

In anaesthetized animal models hypoxia triggers a prototypical biphasic hypoxic ventilatory response, which relies on the integrity of the peripheral respiratory oxygen sensors for its full expression. Acute denervation of the carotid bodies abolishes the initial stimulatory effect of hypoxia and the hypoxic ventilatory response often manifests as a depression of central respiratory drive (e.g. see (23)). In contrast, unanaesthetized experimental animals (e.g. Figure 2 in (9)) and humans with

(chronically) denervated carotid bodies (e.g. Figure 3 in (59)) increase and/or maintain the respiratory activity during the acute hypoxic challenge above and/or at the normoxic level and do not exhibit depression of ventilation in response to CNS hypoxia.

Earlier experimental studies involving chronic denervation of the peripheral oxygen chemoreceptors and assessment of the respiratory activity demonstrated almost complete or partial recovery of the hypoxic ventilatory response in awake animals, including dogs (13), cats (18; 42), ponies (2; 3), goats (12), and rats (40; 46; 51). More recent studies in rodents also reported (but did not comment on this particular aspect) robust respiratory responses to hypoxia in awake mice and rats with chronically denervated carotid bodies (14; 54). The latest studies of the mechanisms underlying peripheral oxygen sensitivity using transgenic animal models reported that during the early postnatal period in mice, chemosensitive carotid body glomus cells do not express critical components of the hypoxia-sensitive signalling pathway (e.g., olfactory receptor Olfr78 which is activated by lactate produced during hypoxia; in the absence of Olfr78 isolated carotid bodies are reported to be insensitive to hypoxia) (6). Yet, neonatal mice display robust and sustained hypoxic ventilatory responses (4; 50).

A number of potential mechanisms may account for the development of the hypoxic ventilatory response in the absence of the carotid body chemoreceptor input. In some species subsidiary peripheral chemoreceptors (e.g. aortic bodies) may take over and compensate for the loss of the carotid body afferent activity (for a review see (24)). Recent data in rodents provide strong evidence that the hypoxic ventilatory response that develops in conditions of peripheral chemoreceptor denervation is centrally mediated and involves activation of oxygen sensitive glial cells leading to the enhanced activity of the brainstem respiratory network (1).

Careful scrutiny of the literature reveals that the use of anesthesia is a potential confounding factor. If there is a central stimulatory component of the hypoxic ventilatory response, it appears to be very sensitive to suppression by general anesthesia. Indeed, a majority of studies that reported recovery of a hypoxic ventilatory response after carotid body denervation were conducted in unanaesthetized animals. A recent study measured changes in ventilation in the same cohort of peripherally chemodenervated rats (10 weeks after carotid body ablation) in awake state and under general anesthesia (1). In these rats, the hypoxia-induced arousal

response (5; 42) was markedly reduced following carotid body denervation indicating that the afferent input from the peripheral oxygen chemoreceptors was absent/impaired. Yet, while awake these animals displayed a robust hypoxic ventilatory response that was dramatically reduced under general anesthesia (urethane) (1), suggesting that the signaling mechanisms underlying central respiratory oxygen sensitivity are inhibited by anesthetic agents.

Two studies specifically investigated the effect of CNS hypoxia on the respiratory activity in unanaesthetized sleeping dogs (9) and awake goats (12). Both studies reported significant (~30% in dogs and ~75% in goats) increases in ventilation during CNS hypoxia, despite the fact that the carotid body respiratory chemoreceptors were maintained normocapnic/normoxic by means of vascular isolation and separate perfusion. In both studies the hypoxic ventilatory response induced by CNS hypoxia was mediated entirely by increased respiratory frequency, indicating an effect of low PO_2 on the respiratory rhythm-generating circuits. In dogs, however, once the carotid bodies were acutely denervated, CNS hypoxia failed to trigger increases in ventilation suggesting that tonic (permissive) peripheral chemoreceptor input (provided by normoxic/normocapnic carotid bodies) is required for central oxygen-sensitive mechanisms to stimulate breathing (9). In goats, a significant proportion (~40%) of the ventilatory response to CNS hypoxia was preserved following carotid body denervation (12).

Human Studies

In humans, denervation of the carotid body chemoreceptors abolishes the hypoxic ventilatory response (for comprehensive reviews of the literature see (60; 63)). Most of these studies were conducted in small numbers of patients following bilateral resection of healthy carotid bodies to treat bronchial asthma or chronic obstructive pulmonary disease (25; 26; 37; 62; 66; 69). Following denervation, these patients also failed to show the characteristic rapid decline in ventilation that occurs in response to rapid administration of 100% oxygen (the Dejours test) (37). Ventilatory responses to CO_2 were also reduced, typically by ~20-30% (for a review see (63)). However, more extreme respiratory deficits were reported shortly after the denervation surgery, including 75% reductions in ventilatory CO_2 sensitivity that recovered gradually over 2-3 years (11).

A key point is that the majority of human studies involving carotid body denervation have been conducted in patients suffering from chronic lung disease, which may (by itself) significantly impact chemoreflex function. Two studies (16; 62) recruited individuals who had undergone bilateral carotid body tumor resection, but were otherwise free of pulmonary disease. The hypoxic ventilatory response was abolished in seven bilaterally carotid body-resected subjects studied by Fatemian and colleagues (16). Similarly, eight patients recruited by Timmers and colleagues (62) failed to mount a significant ventilatory response to hypoxia under normocapnic conditions. However, two of these eight subjects responded to hypoxia with an increase in ventilation when the stimulus was applied in hypercapnic conditions (62), confirming similar observations in carotid body resected patients with a history of bronchial asthma (59).

Importantly, patients recruited in both of these studies (16; 62) underwent carotid body resection to remove tumors caused by a mutation of the gene encoding for succinate dehydrogenase complex subunit D (SDHD, part of cytochrome b_{588} of the mitochondrial respiratory chain complex II). Healthy age- and sex-matched volunteers were used as controls. However, the hypoxic ventilatory response in healthy humans may vary by a factor of twenty due to various genetic factors (60; 67). Tumor-free carriers of the SDHD mutation (with intact carotid bodies) display a reduced isocapnic hypoxic ventilatory response at the very low end of the “normal” range (60). Could the reduction of the hypoxic ventilatory response in conditions of SDHD mutation reflect impaired central respiratory oxygen sensitivity in these individuals, a possibility consistent with the proposed key role of the mitochondrial hypoxia-sensitive mechanism (discussed below)?

Thus, studies of the hypoxic ventilatory response following bilateral resection of the carotid bodies in humans are somewhat hampered by either the underlying chronic lung disease or genetic factors which may alter chemoreflex function. Therefore, it will remain unknown whether the hypoxic ventilatory response in young and healthy humans would recover over time following bilateral carotid body denervation in a manner similar to that documented in many experimental animals.

Central Respiratory Oxygen Sensor

Growing evidence from *in vitro* and *in vivo* experimental animal models suggests that the central stimulatory effect of hypoxia on breathing is mediated by a mechanism that operates within the brainstem regions harboring neuronal circuits responsible for the generation of respiratory rhythm and pattern. Recent data suggest that this oxygen-sensitive mechanism is not neuronal in nature (1; 38).

The hypoxic ventilatory response that remains in rats following denervation of peripheral oxygen chemoreceptors is abolished when the ventral regions of the medulla oblongata are transduced to express a potent ectonucleotidase - transmembrane prostatic acid phosphatase (TMPAP) (1). Catalytic activity of TMPAP effectively blocks purinergic signalling mechanisms by preventing vesicular accumulation of ATP and by promoting rapid degradation of extracellular purines (39; 68; 71). Mammalian brain cells that are not electrically excitable but show calcium excitability – glial cells (astrocytes and microglia) – release ATP as the major signaling molecule through which they communicate with neighboring glia, neurons and other brain cell types. Thus, glial cells were hypothesized to be responsible for sensing brain hypoxia.

Consistent with this hypothesis, the hypoxic ventilatory response of both carotid body intact and peripherally chemodenervated rats was significantly reduced when brainstem astrocytes were selectively targeted to express the light chain of tetanus toxin (TeLC) (1). TeLC cleaves certain SNARE proteins required for vesicular docking and fusion, effectively blocking vesicular release of gliotransmitters (including ATP) by astrocytes (1; 8). Interestingly, bilateral TeLC expression in brainstem astrocytes (in carotid body intact rats) and bilateral carotid body ablation (in rats expressing control transgene) resulted in quantitatively similar reductions in the magnitude of the hypoxic ventilatory response (see Figure 7 in (1)).

Subsequent studies demonstrated that astrocytes are able to sense physiologically-relevant decreases in brain parenchymal PO_2 a few mmHg below normal brain oxygenation level (1). The astroglial signalling cascade triggered by hypoxia involves mitochondrial depolarization, facilitated formation of free radicals, activation of phospholipase C, IP_3 receptors, release of Ca^{2+} from the intracellular stores and enhanced vesicular release of ATP (Figure 1). Real-time biosensor measurements *in vivo* and *in vitro* (23) demonstrated hypoxia-induced release of ATP within and in close

proximity to the brainstem respiratory networks, including the rhythm generating circuits of the pre-Bötzinger complex that are sensitive to, and potently excited by ATP (20; 21; 23; 29; 30; 35; 36).

An important role of reactive oxygen species in this signalling pathway is supported by a number of earlier reports that demonstrated the stimulatory effect of CNS hyperoxia (which would be expected to increase free radical production) on ventilation in awake, decerebrate or anaesthetized experimental animals with denervated carotid bodies (see (12) and references therein). The bimodal shape of the relationship between lung ventilation and the arterial PO_2 (in conditions of isolated separately perfused normoxic carotid body) correlates very well with the relationship between reactive oxygen species formation and intracellular PO_2 (Figure 2).

Thus, a significant body of evidence collectively suggests that the central stimulatory effect of hypoxia on breathing is mediated by the actions of ATP released by activated astrocytes intermingled with the neuronal networks responsible for the generation of the respiratory activity. Hypoxia-induced excitation of brainstem pre-sympathetic neurons also appears to be indirect, mediated by prior release and actions of ATP and lactate (38).

Summary

The existence of a brain hypoxia sensor capable of stimulating breathing is not universally accepted. Many investigators question the physiological relevance of the anoxia/hypoxia-evoked responses observed in the *in vitro* preparations of the neonatal rodent brainstem. In addition, human data suggest that functional respiratory oxygen sensitivity is an exclusive function of the carotid body chemoreceptors, as no recovery of the normocapnic hypoxic ventilatory response has been reported from studies conducted in individuals with bilaterally resected carotid bodies. Yet, significant recovery of the hypoxic ventilatory response after peripheral chemoreceptor denervation has been observed in all experimental animals studied so far (ponies, goats, dogs, cats, rats and mice). That the respiratory responses in experimental animals with denervated carotid bodies are markedly suppressed by general anesthesia suggests that the operation of the physiological central respiratory oxygen sensitive mechanism is readily inhibited by anesthetic agents.

Astrocytes are able to detect various sensory modalities (22; 27; 28; 32; 41; 64; 65) and appear to be functionally specialized as CNS oxygen sensors tuned to detect physiological decreases in brain oxygenation (1). When activated in hypoxic conditions, brainstem astrocytes release ATP, which stimulates the respiratory neuronal circuits leading to increases in lung ventilation. The variability in the degree to which the hypoxic ventilatory response recovers after peripheral chemodenervation may reflect species differences in the level of endogenous ectonucleotidase activity of the brainstem parenchyma (70). Future research may include studies of the ectonucleotidase activity in the human brain, and, in experimental animal models, analysis of the association between changes in the brainstem ectonucleotidase activity and recovery of the hypoxic ventilatory response over time after denervation of the carotid body chemoreceptors. It is plausible that in humans brainstem ectonucleotidase activity is relatively high, resulting in rapid degradation of ATP released during brain hypoxia and enhanced production of adenosine (which may contribute to the hypoxic ventilatory decline). We propose that during systemic hypoxia in human subjects with denervated carotid bodies, the central respiratory oxygen sensitive mechanism effectively maintains lung ventilation by counteracting the hypoxia-induced depression of breathing, although in the absence of the peripheral chemoreceptor input it is not sufficiently potent to evoke increases in ventilation above the normoxic baseline.

References

1. **Angelova PR, Kasymov V, Christie I, Sheikhbahaei S, Turovsky E, Marina N, Korsak A, Zwicker J, Teschemacher AG, Ackland GL, Funk GD, Kasparov S, Abramov AY and Gourine AV**. Functional Oxygen Sensitivity of Astrocytes. *J Neurosci* 35: 10460-10473, 2015.
2. **Bisgard GE, Forster HV and Klein JP**. Recovery of Peripheral Chemoreceptor Function After Denervation in Ponies. *J Appl Physiol* 49: 964-970, 1980.
3. **Bisgard GE, Forster HV, Orr JA, Buss DD, Rawlings CA and Rasmussen B**. Hypoventilation in ponies after carotid body denervation. *J Appl Physiol* 40: 184-190, 1976.
4. **Bissonnette JM and Knopp SJ**. Developmental changes in the hypoxic ventilatory response in C57BL/6 mice. *Respir Physiol* 128: 179-186, 2001.
5. **Bowes G, Townsend ER, Kozar LF, Bromley SM and Phillipson EA**. Effect of carotid body denervation on arousal response to hypoxia in sleeping dogs. *J Appl Physiol Respir Environ Exerc Physiol* 51: 40-45, 1981.
6. **Chang AJ, Ortega FE, Riegler J, Madison DV and Krasnow MA**. Oxygen regulation of breathing through an olfactory receptor activated by lactate. *Nature* 527: 240-244, 2015.
7. **Clanton TL**. Hypoxia-induced reactive oxygen species formation in skeletal muscle. *J Appl Physiol (1985)* 102: 2379-2388, 2007.
8. **Coco S, Calegari F, Pravettoni E, Pozzi D, Taverna E, Rosa P, Matteoli M and Verderio C**. Storage and release of ATP from astrocytes in culture. *Journal of Biological Chemistry* 278: 1354-1362, 2003.
9. **Curran AK, Rodman JR, Eastwood PR, Henderson KS, Dempsey JA and Smith CA**. Ventilatory responses to specific CNS hypoxia in sleeping dogs. *J Appl Physiol (1985)* 88: 1840-1852, 2000.
10. **D'Agostino D, Mazza E Jr and Neubauer JA**. Heme oxygenase is necessary for the excitatory response of cultured neonatal rat rostral ventrolateral medulla neurons to hypoxia. *Am J Physiol Regul Integr Comp Physiol* 296: R102-R118, 2009.
11. **Dahan A, Nieuwenhuijs D and Teppema L**. Plasticity of central chemoreceptors: effect of bilateral carotid body resection on central CO_2 sensitivity. *PLoS Med* 4: e239, 2007.
12. **Daristotle L, Engwall MJ, Niu WZ and Bisgard GE**. Ventilatory effects and interactions with change in PaO_2 in awake goats. *J Appl Physiol (1985)* 71: 1254-1260, 1991.
13. **Davenport HW, Brewer G, Chambers AH and Goldschmidt S**. The Respiratory Responses to Anoxemia of Unanesthetized Dogs with Chronically Denervated Aortic and Carotid Chemoreceptors and Their Causes. *American Journal of Physiology* 148: 406-416, 1947.
14. **Del Rio R, Marcus NJ and Schultz HD**. Carotid Chemoreceptor Ablation Improves Survival in Heart Failure Rescuing Autonomic Control of Cardiorespiratory Function. *Journal of the American College of Cardiology* 62: 2422-2430, 2013.
15. **Devor A, Sakadzic S, Saisan PA, Yaseen MA, Roussakis E, Srinivasan VJ, Vinogradov SA, Rosen BR, Buxton RB, Dale AM and Boas DA**. "Overshoot" of O_2 is required to maintain baseline tissue oxygenation at locations distal to blood vessels. *J Neurosci* 31: 13676-13681, 2011.
16. **Fatemian M, Nieuwenhuijs DJ, Teppema LJ, Meinesz S, van der Mey AG, Dahan A and Robbins PA**. The respiratory response to carbon dioxide in humans with unilateral and bilateral resections of the carotid bodies. *J Physiol* 549: 965-973, 2003.
17. **Funk GD and Greer JJ**. The rhythmic, transverse medullary slice preparation in respiratory neurobiology: contributions and caveats. *Respir Physiol Neurobiol* 186: 236-253, 2013.

18. **Gautier H and Bonora M**. Possible alterations in brain monoamine metabolism during hypoxia-induced tachypnea in cats. *J Appl Physiol Respir Environ Exerc Physiol* 49: 769-777, 1980.
19. **Gjedde A**. Cerebral blood flow change in arterial hypoxemia is consistent with negligible oxygen tension in brain mitochondria. *Neuroimage* 17: 1876-1881, 2002.
20. **Gourine AV**. On the peripheral and central chemoreception and control of breathing: an emerging role of ATP. *J Physiol* 568: 715-724, 2005.
21. **Gourine AV, Atkinson L, Deuchars J and Spyer KM**. Purinergic signalling in the medullary mechanisms of respiratory control in the rat: respiratory neurones express the $P2X_2$ receptor subunit. *J Physiol* 552: 197-211, 2003.
22. **Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S, Teschemacher AG, Spyer KM, Deisseroth K and Kasparov S**. Astrocytes Control Breathing Through pH-Dependent Release of ATP. *Science* 329: 571-575, 2010.
23. **Gourine AV, Llaudet E, Dale N and Spyer KM**. Release of ATP in the ventral medulla during hypoxia in rats: role in hypoxic ventilatory response. *J Neurosci* 25: 1211-1218, 2005.
24. **Hodges MR and Forster HV**. Respiratory neuroplasticity following carotid body denervation: Central and peripheral adaptations. *Neural Regen Res* 7: 1073-1079, 2012.
25. **Holton P and Wood JB**. The effects of bilateral removal of the carotid bodies and denervation of the carotid sinuses in two human subjects. *J Physiol* 181: 365-378, 1965.
26. **Honda Y, Watanabe S, Hashizume I, Satomura Y, Hata N, Sakakibara Y and Severinghaus JW**. Hypoxic chemosensitivity in asthmatic patients two decades after carotid body resection. *J Appl Physiol Respir Environ Exerc Physiol* 46: 632-638, 1979.
27. **Huckstepp RT, Id BR, Eason R, Spyer KM, Dicke N, Willecke K, Marina N, Gourine AV and Dale N**. Connexin hemichannel-mediated CO_2-dependent release of ATP in the medulla oblongata contributes to central respiratory chemosensitivity. *J Physiol* 588: 3901-3920, 2010.
28. **Huckstepp RT, Llaudet E and Gourine AV**. CO_2-Induced ATP-Dependent Release of Acetylcholine on the Ventral Surface of the Medulla Oblongata. *PLoS One* 11: e0167861, 2016.
29. **Huxtable AG, Zwicker JD, Alvares TS, Ruangkittisakul A, Fang X, Hahn LB, Posse de CE, Baker GB, Ballanyi K and Funk GD**. Glia contribute to the purinergic modulation of inspiratory rhythm-generating networks. *J Neurosci* 30: 3947-3958, 2010.
30. **Huxtable AG, Zwicker JD, Poon BY, Pagliardini S, Vrouwe SQ, Greer JJ and Funk GD**. Tripartite purinergic modulation of central respiratory networks during perinatal development: the influence of ATP, ectonucleotidases, and ATP metabolites. *J Neurosci* 29: 14713-14725, 2009.
31. **Kasischke KA, Lambert EM, Panepento B, Sun A, Gelbard HA, Burgess RW, Foster TH and Nedergaard M**. Two-photon NADH imaging exposes boundaries of oxygen diffusion in cortical vascular supply regions. *J Cereb Blood Flow Metab* 31: 68-81, 2011.
32. **Kasymov V, Larina O, Castaldo C, Marina N, Patrushev M, Kasparov S and Gourine AV**. Differential sensitivity of brainstem versus cortical astrocytes to changes in pH reveals functional regional specialization of astroglia. *J Neurosci* 33: 435-441, 2013.
33. **Kimura H, Tanaka M, Nagao K, Niijima M, Masuyama S, Mizoo A, Uruma T, Tatsumi K, Kuriyama T, Masuda A, Kobayashi T and Honda Y**. A new aspect of the carotid body function controlling hypoxic ventilatory decline in humans. *Appl Human Sci* 17: 131-137, 1998.
34. **Kumar P and Prabhakar NR**. Peripheral chemoreceptors: function and plasticity of the carotid body. *Compr Physiol* 2: 141-219, 2012.
35. **Lorier AR, Huxtable AG, Robinson DM, Lipski J, Housley GD and Funk GD**. $P2Y_1$ receptor modulation of the pre-Botzinger complex inspiratory rhythm generating network in vitro. *J Neurosci* 27: 993-1005, 2007.
36. **Lorier AR, Lipski J, Housley GD, Greer JJ and Funk GD**. ATP sensitivity of preBotzinger complex neurones in neonatal rat in vitro: mechanism underlying a P2 receptor-mediated increase in inspiratory frequency. *J Physiol* 586: 1429-1446, 2008.

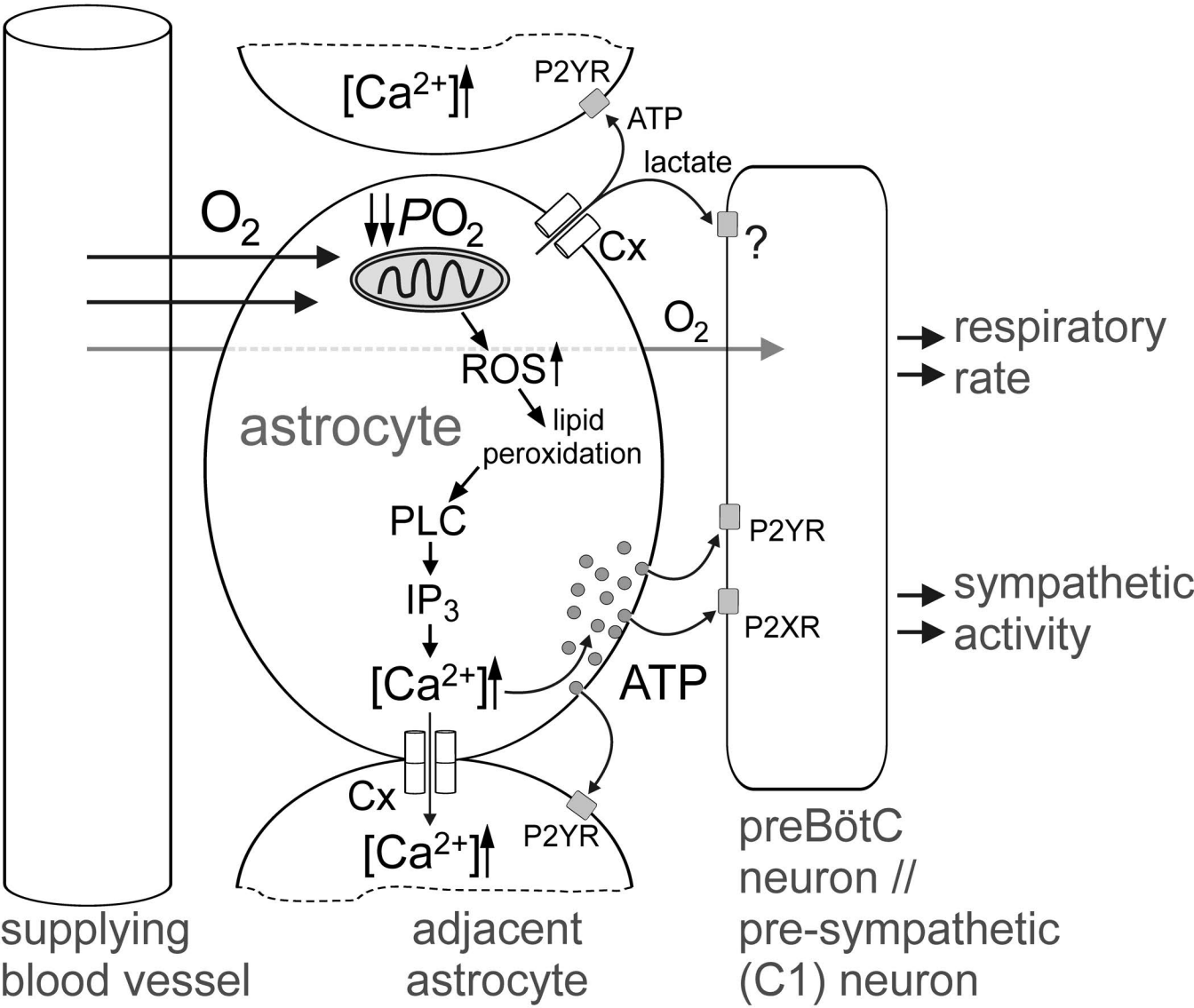
37. **Lugliani R, Whipp BJ, Seard C and Wasserman K**. Effect of bilateral carotid-body resection on ventilatory control at rest and during exercise in man. *N Engl J Med* 285: 1105-1111, 1971.
38. **Marina N, Ang R, Machhada A, Kasymov V, Karagiannis A, Hosford PS, Mosienko V, Teschemacher AG, Vihko P, Paton JF, Kasparov S and Gourine AV**. Brainstem hypoxia contributes to the development of hypertension in the spontaneously hypertensive rat. *Hypertension* 65: 775-783, 2015.
39. **Marina N, Tang F, Figueiredo M, Mastitskaya S, Kasimov V, Mohamed-Ali V, Roloff E, Teschemacher AG, Gourine AV and Kasparov S**. Purinergic signalling in the rostral ventro-lateral medulla controls sympathetic drive and contributes to the progression of heart failure following myocardial infarction in rats. *Basic Res Cardiol* 108: 317, 2013.
40. **Martin-Body RL, Robson GJ and Sinclair JD**. Restoration of hypoxic respiratory responses in the awake rat after carotid body denervation by sinus nerve section. *J Physiol* 380: 61-73, 1986.
41. **Marty N, Dallaporta M, Foretz M, Emery M, Tarussio D, Bady I, Binnert C, Beermann F and Thorens B**. Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. *J Clin Invest* 115: 3545-3553, 2005.
42. **Miller MJ and Tenney SM**. Hypoxia-induced tachypnea in carotid-deafferented cats. *Respir Physiol* 23: 31-39, 1975.
43. **Moss IR**. Respiratory responses to single and episodic hypoxia during development: mechanisms of adaptation. *Respir Physiol* 121: 185-197, 2000.
44. **Ndubuizu O and LaManna JC**. Brain tissue oxygen concentration measurements. *Antioxid Redox Signal* 9: 1207-1219, 2007.
45. **Neubauer JA and Sunderram J**. Oxygen-sensing neurons in the central nervous system. *J Appl Physiol (1985)* 96: 367-374, 2004.
46. **Olson EB, Vidruk EH and Dempsey JA**. Carotid-Body Excision Significantly Changes Ventilatory Control in Awake Rats. *J Appl Physiol* 64: 666-671, 1988.
47. **Pena F, Parkis MA, Tryba AK and Ramirez JM**. Differential contribution of pacemaker properties to the generation of respiratory rhythms during normoxia and hypoxia. *Neuron* 43: 105-117, 2004.
48. **Prabhakar NR**. Sensing hypoxia: physiology, genetics and epigenetics. *J Physiol* 591: 2245-2257, 2013.
49. **Ramirez JM, Quellmalz UJ, Wilken B and Richter DW**. The hypoxic response of neurones within the in vitro mammalian respiratory network. *J Physiol* 507: 571-582, 1998.
50. **Robinson DM, Kwok H, Adams BM, Peebles KC and Funk GD**. Development of the ventilatory response to hypoxia in Swiss CD-1 mice. *J Appl Physiol (1985)* 88: 1907-1914, 2000.
51. **Roux JC, Pequignot JM, Dumas S, Pascual O, Ghilini G, Pequignot J, Mallet J and Denavit-Saubie M**. O_2-sensing after carotid chemodenervation: hypoxic ventilatory responsiveness and upregulation of tyrosine hydroxylase mRNA in brainstem catecholaminergic cells. *Eur J Neurosci* 12: 3181-3190, 2000.
52. **Ruangkittisakul A and Ballanyi K**. Anoxia response in physiological potassium of the isolated inspiratory center in calibrated newborn rat brainstem slices. *Adv Exp Med Biol* 758: 91-98, 2012.
53. **Smith PG and Mills E**. Restoration of reflex ventilatory response to hypoxia after removal of carotid bodies in the cat. *Neuroscience* 5: 573-580, 1980.
54. **Soliz J, Joseph V, Soulage C, Becskei C, Vogel J, Pequignot JM, Ogunshola O and Gassmann M**. Erythropoietin regulates hypoxic ventilation in mice by interacting with brainstem and carotid bodies. *J Physiol* 568: 559-571, 2005.
55. **Solomon IC**. Glutamate neurotransmission is not required for, but may modulate, hypoxic sensitivity of pre-Botzinger complex in vivo. *J Neurophysiol* 93: 1278-1284, 2005.

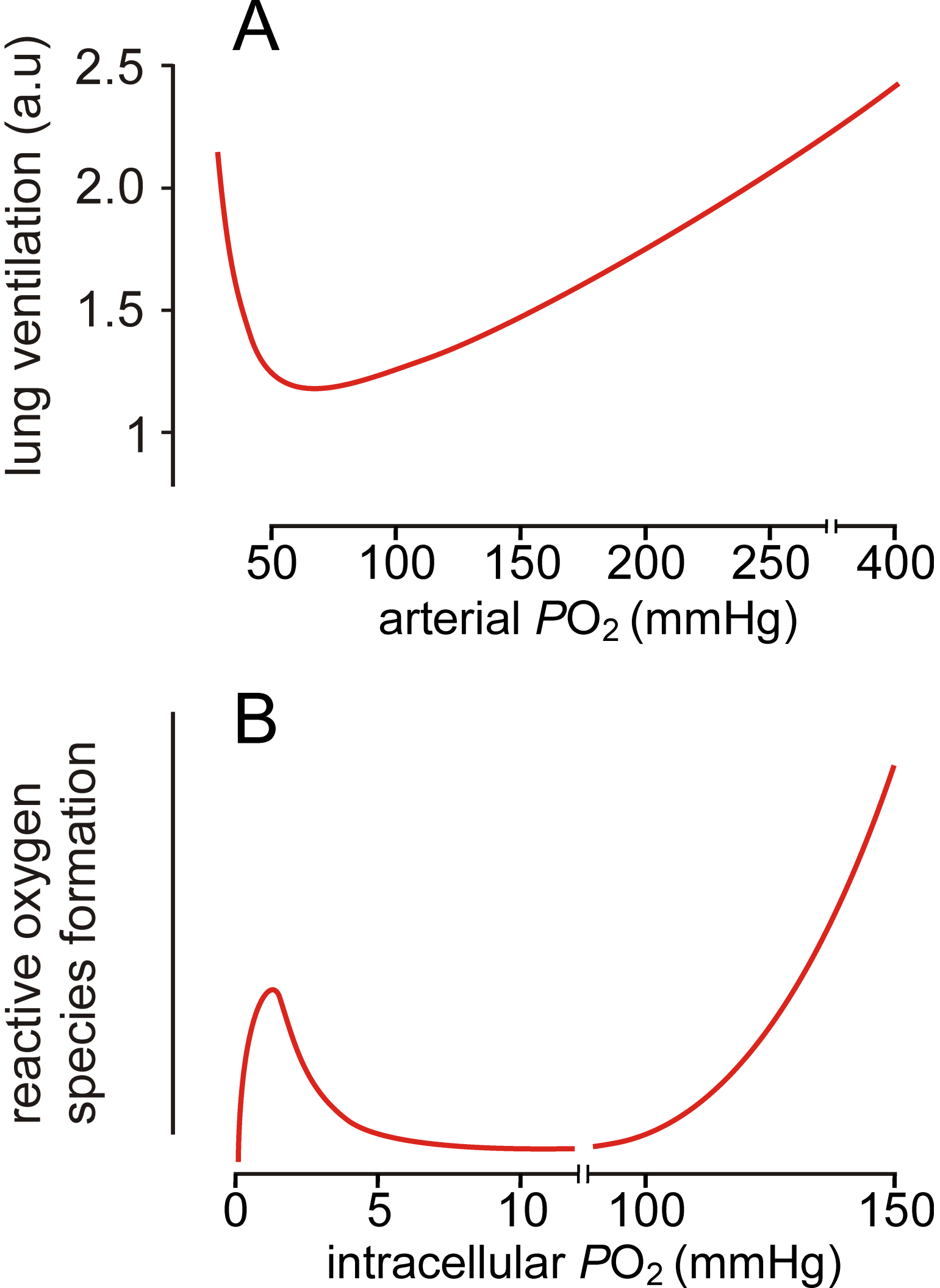
56. **Solomon IC, Edelman NH and Neubauer JA**. Pre-Botzinger complex functions as a central hypoxia chemosensor for respiration in vivo. *J Neurophysiol* 83: 2854-2868, 2000.
57. **Sun MK, Jeske IT and Reis DJ**. Cyanide excites medullary sympathoexcitatory neurons in rats. *Am J Physiol* 262: R182-R189, 1992.
58. **Sun MK and Reis DJ**. Hypoxia selectively excites vasomotor neurons of rostral ventrolateral medulla in rats. *Am J Physiol* 266: R245-R256, 1994.
59. **Swanson GD, Whipp BJ, Kaufman RD, Aqleh KA, Winter B and Bellville JW**. Effect of hypercapnia on hypoxic ventilatory drive in carotid body-resected man. *J Appl Physiol Respir Environ Exerc Physiol* 45: 871-877, 1978.
60. **Teppema LJ and Dahan A**. The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. *Physiol Rev* 90: 675-754, 2010.
61. **Thoby-Brisson M and Ramirez JM**. Role of inspiratory pacemaker neurons in mediating the hypoxic response of the respiratory network in vitro. *J Neurosci* 20: 5858-5866, 2000.
62. **Timmers HJ, Karemaker JM, Wieling W, Marres HA, Folgering HT and Lenders JW**. Baroreflex and chemoreflex function after bilateral carotid body tumor resection. *J Hypertens* 21: 591-599, 2003.
63. **Timmers HJ, Wieling W, Karemaker JM and Lenders JW**. Denervation of carotid baro- and chemoreceptors in humans. *J Physiol* 553: 3-11, 2003.
64. **Turovsky E, Karagiannis A, Abdala AP and Gourine AV**. Impaired CO_2 sensitivity of astrocytes in a mouse model of Rett syndrome. *J Physiol* 593: 3159-3168, 2015.
65. **Turovsky E, Theparambil SM, Kasymov V, Deitmer JW, Del Arroyo AG, Ackland GL, Corneveaux JJ, Allen AN, Huentelman MJ, Kasparov S, Marina N and Gourine AV**. Mechanisms of CO_2/H^+ Sensitivity of Astrocytes. *J Neurosci* 36: 10750-10758, 2016.
66. **Vermeire P, de BW, van MR, Bal J and van KW**. Carotid body resection in patients with severe chronic airflow limitation. *Bull Eur Physiopathol Respir* 23 Suppl 11: 165s-166s, 1987.
67. **Weil JV**. Variation in human ventilatory control-genetic influence on the hypoxic ventilatory response. *Respir Physiol Neurobiol* 135: 239-246, 2003.
68. **Wells JA, Christie IN, Hosford PS, Huckstepp RT, Angelova PR, Vihko P, Cork SC, Abramov AY, Teschemacher AG, Kasparov S, Lythgoe MF and Gourine AV**. A critical role for purinergic signalling in the mechanisms underlying generation of BOLD fMRI responses. *J Neurosci* 35: 5284-5292, 2015.
69. **Whipp BJ and Ward SA**. Physiologic changes following bilateral carotid-body resection in patients with chronic obstructive pulmonary disease. *Chest* 101: 656-661, 1992.
70. **Zwicker JD, Rajani V, Hahn LB and Funk GD**. Purinergic modulation of preBotzinger complex inspiratory rhythm in rodents: the interaction between ATP and adenosine. *J Physiol* 589: 4583-4600, 2011.
71. **Zylka MJ, Sowa NA, Taylor-Blake B, Twomey MA, Herrala A, Voikar V and Vihko P**. Prostatic Acid Phosphatase Is an Ectonucleotidase and Suppresses Pain by Generating Adenosine. *Neuron* 60: 111-122, 2008.

Figure legends

Figure 1| Hypothesized cellular mechanisms underlying central oxygen sensitivity. The astroglial signalling cascade triggered by hypoxia involves mitochondrial depolarization, facilitated formation of reactive oxygen species (ROS), lipid peroxidation, activation of phospholipase C (PLC), IP_3 receptors, release of Ca^{2+} from the intracellular stores and enhanced vesicular release of ATP. Hypoxia may also alter opening probability of connexin (Cx) hemichannels permeable to ATP and lactate. Released ATP acts in autocrine and paracrine manner, spreads astroglial Ca^{2+} signals within the neuropil and enhances respiratory and sympathetic activities via excitation of the respiratory rhythm generating circuits of the pre-Bötzinger complex (preBötC) and sympathoexcitatory (pre-sympathetic) C1 neurons of the ventrolateral medulla oblongata.

Figure 2| (**A**) Relationship between lung ventilation and the arterial PO_2 in 11 awake goats whose carotid bodies were isolated and separately perfused with normocapnic-normoxic blood (schematic adapted from the data reported in Ref. 12); (**B**) Bimodal distribution of reactive oxygen species formation as a function of intracellular PO_2 (schematic adapted from Ref. 7).


[Ca2+]
P2YR
ATP
lactate
O2
PO2
Cx
?
O2
respiratory
rate
ROS
astrocyte
lipid
peroxidation
PLC
IP3
P2YR
P2XR
sympathetic
activity
[Ca2+]
ATP
Cx
[Ca2+]
P2YR
supplying
blood vessel
adjacent
astrocyte
preBötC
neuron //
pre-sympathetic
(C1) neuron


A
lung ventilation (a.u)
2.5
2.0
1.5
1
50
100
150
200
250
400
arterial PO_2 (mmHg)
B
reactive oxygen species formation
0
5
10
100
150
intracellular PO_2 (mmHg)