

1 On the existence of a central respiratory oxygen sensor

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4 Abbreviated title: **CNS oxygen sensor**

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35 **Abstract**

36

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

52

53 **Introduction**

54

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

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

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

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

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96 **Animal Studies**

97

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

105 reduction of ventilation is traditionally attributed to the hypoxia-induced depression of
106 the brainstem respiratory circuits (although there is evidence that in the awake state
107 hypoxic ventilatory decline is also dependent on the carotid body input (e.g. see (33)).
108

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109 Indeed, the majority of central neurons respond to hypoxia with a reduction in
110 excitability. However, in the early 90s Sun and Reis (57; 58) demonstrated that some
111 CNS neurons, in particular pre-sympathetic neurons of the brainstem, increase their
112 discharge in low PO_2 conditions and trigger generalized increases in sympathetic nerve
113 activity during brain hypoxia. Subsequent experimental studies performed using *in*
114 *vitro*, *in situ* and anesthetized animal models demonstrated that in addition to pre-
115 sympathetic neurons, brainstem respiratory control circuits are sensitive to, and can be
116 activated by, decreases in local parenchymal PO_2 or cytotoxic hypoxia (10; 38; 45; 47;
117 49; 52; 55; 56; 61). These studies demonstrated biphasic responses of the isolated (in
118 an *in vitro* brainstem slice) central respiratory network to hypoxia, quantified PO_2
119 sensitivity, described differential neuronal responses and suggested some plausible
120 mechanisms of neuronal oxygen sensing (e.g. heme oxygenase-dependent hypoxia-
121 induced depolarization of cultured brainstem neurons (10)).
122

122

123 These experimental studies conducted *in vitro* support a central stimulatory component
124 of the hypoxic ventilatory response, however, whether these mechanisms (activated *in*
125 *vitro*) are relevant to the physiological responses that are involved in the homeostatic
126 regulation of brain oxygenation by the respiratory network remains a contentious
127 issue. Few investigators working in more intact preparations (e.g. *in vivo*) consider that
128 the responses of the respiratory network to hypoxia/anoxia *in vitro* are physiologically
129 relevant. This is a valid criticism because baseline conditions of the *in vitro*
130 preparations are characterized by extreme hyperoxia and the strength of the hypoxic
131 stimulus varies with tissue depth since O_2 delivery is via diffusion (discussed in detail in
132 (17)).
133

133

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

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

143

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

157

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

166

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

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

181

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

196

197 **Human Studies**

198

199 In humans, denervation of the carotid body chemoreceptors abolishes the hypoxic
200 ventilatory response (for comprehensive reviews of the literature see (60; 63)). Most
201 of these studies were conducted in small numbers of patients following bilateral
202 resection of healthy carotid bodies to treat bronchial asthma or chronic obstructive
203 pulmonary disease (25; 26; 37; 62; 66; 69). Following denervation, these patients also
204 failed to show the characteristic rapid decline in ventilation that occurs in response to
205 rapid administration of 100% oxygen (the Dejours test) (37). Ventilatory responses to
206 CO_2 were also reduced, typically by ~20-30% (for a review see (63)). However, more
207 extreme respiratory deficits were reported shortly after the denervation surgery,
208 including 75% reductions in ventilatory CO_2 sensitivity that recovered gradually over 2-
209 3 years (11).

210

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

223

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

236

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

243

244 **Central Respiratory Oxygen Sensor**

245

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

251

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

262

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

272

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

280 proximity to the brainstem respiratory networks, including the rhythm generating
281 circuits of the pre-Bötzinger complex that are sensitive to, and potently excited by ATP
282 (20; 21; 23; 29; 30; 35; 36).

283

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

292

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

299

300 **Summary**

301

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

315

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

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References

1. **Angelova PR, Kasymov V, Christie I, Sheikhabaei 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 CO₂ sensitivity. *PLoS Med* 4: e239, 2007.
12. **Daristotle L, Engwall MJ, Niu WZ and Bisgard GE.** Ventilatory effects and interactions with change in PaO₂ 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 O₂ 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.

- 390 18. **Gautier H and Bonora M.** Possible alterations in brain monoamine metabolism during
391 hypoxia-induced tachypnea in cats. *J Appl Physiol Respir Environ Exerc Physiol* 49: 769-
392 777, 1980.
- 393 19. **Gjedde A.** Cerebral blood flow change in arterial hypoxemia is consistent with negligible
394 oxygen tension in brain mitochondria. *Neuroimage* 17: 1876-1881, 2002.
- 395 20. **Gourine AV.** On the peripheral and central chemoreception and control of breathing: an
396 emerging role of ATP. *J Physiol* 568: 715-724, 2005.
- 397 21. **Gourine AV, Atkinson L, Deuchars J and Spyer KM.** Purinergic signalling in the
398 medullary mechanisms of respiratory control in the rat: respiratory neurones express the
399 P2X₂ receptor subunit. *J Physiol* 552: 197-211, 2003.
- 400 22. **Gourine AV, Kasymov V, Marina N, Tang F, Figueiredo MF, Lane S,**
401 **Teschemacher AG, Spyer KM, Deisseroth K and Kasparov S.** Astrocytes Control
402 Breathing Through pH-Dependent Release of ATP. *Science* 329: 571-575, 2010.
- 403 23. **Gourine AV, Llaudet E, Dale N and Spyer KM.** Release of ATP in the ventral medulla
404 during hypoxia in rats: role in hypoxic ventilatory response. *J Neurosci* 25: 1211-1218,
405 2005.
- 406 24. **Hodges MR and Forster HV.** Respiratory neuroplasticity following carotid body
407 denervation: Central and peripheral adaptations. *Neural Regen Res* 7: 1073-1079, 2012.
- 408 25. **Holton P and Wood JB.** The effects of bilateral removal of the carotid bodies and
409 denervation of the carotid sinuses in two human subjects. *J Physiol* 181: 365-378, 1965.
- 410 26. **Honda Y, Watanabe S, Hashizume I, Satomura Y, Hata N, Sakakibara Y and**
411 **Severinghaus JW.** Hypoxic chemosensitivity in asthmatic patients two decades after
412 carotid body resection. *J Appl Physiol Respir Environ Exerc Physiol* 46: 632-638, 1979.
- 413 27. **Huckstepp RT, Id BR, Eason R, Spyer KM, Dicke N, Willecke K, Marina N, Gourine**
414 **AV and Dale N.** Connexin hemichannel-mediated CO₂-dependent release of ATP in the
415 medulla oblongata contributes to central respiratory chemosensitivity. *J Physiol* 588:
416 3901-3920, 2010.
- 417 28. **Huckstepp RT, Llaudet E and Gourine AV.** CO₂-Induced ATP-Dependent Release of
418 Acetylcholine on the Ventral Surface of the Medulla Oblongata. *PLoS One* 11: e0167861,
419 2016.
- 420 29. **Huxtable AG, Zwicker JD, Alvares TS, Ruangkittisakul A, Fang X, Hahn LB, Posse**
421 **de CE, Baker GB, Ballanyi K and Funk GD.** Glia contribute to the purinergic
422 modulation of inspiratory rhythm-generating networks. *J Neurosci* 30: 3947-3958, 2010.
- 423 30. **Huxtable AG, Zwicker JD, Poon BY, Pagliardini S, Vrouwe SQ, Greer JJ and Funk**
424 **GD.** Tripartite purinergic modulation of central respiratory networks during perinatal
425 development: the influence of ATP, ectonucleotidases, and ATP metabolites. *J Neurosci*
426 29: 14713-14725, 2009.
- 427 31. **Kasischke KA, Lambert EM, Panepento B, Sun A, Gelbard HA, Burgess RW,**
428 **Foster TH and Nedergaard M.** Two-photon NADH imaging exposes boundaries of
429 oxygen diffusion in cortical vascular supply regions. *J Cereb Blood Flow Metab* 31: 68-81,
430 2011.
- 431 32. **Kasymov V, Larina O, Castaldo C, Marina N, Patrushev M, Kasparov S and**
432 **Gourine AV.** Differential sensitivity of brainstem versus cortical astrocytes to changes in
433 pH reveals functional regional specialization of astroglia. *J Neurosci* 33: 435-441, 2013.
- 434 33. **Kimura H, Tanaka M, Nagao K, Niijima M, Masuyama S, Mizoo A, Uruma T,**
435 **Tatsumi K, Kuriyama T, Masuda A, Kobayashi T and Honda Y.** A new aspect of the
436 carotid body function controlling hypoxic ventilatory decline in humans. *Appl Human Sci*
437 17: 131-137, 1998.
- 438 34. **Kumar P and Prabhakar NR.** Peripheral chemoreceptors: function and plasticity of the
439 carotid body. *Compr Physiol* 2: 141-219, 2012.
- 440 35. **Lorier AR, Huxtable AG, Robinson DM, Lipski J, Housley GD and Funk GD.** P2Y₁
441 receptor modulation of the pre-Botzinger complex inspiratory rhythm generating network
442 in vitro. *J Neurosci* 27: 993-1005, 2007.
- 443 36. **Lorier AR, Lipski J, Housley GD, Greer JJ and Funk GD.** ATP sensitivity of
444 preBotzinger complex neurones in neonatal rat in vitro: mechanism underlying a P2
445 receptor-mediated increase in inspiratory frequency. *J Physiol* 586: 1429-1446, 2008.

- 446 37. **Lugliani R, Whipp BJ, Seard C and Wasserman K.** Effect of bilateral carotid-body
447 resection on ventilatory control at rest and during exercise in man. *N Engl J Med* 285:
448 1105-1111, 1971.
- 449 38. **Marina N, Ang R, Machhada A, Kasymov V, Karagiannis A, Hosford PS, Mosienko**
450 **V, Teschemacher AG, Vihko P, Paton JF, Kasparov S and Gourine AV.** Brainstem
451 hypoxia contributes to the development of hypertension in the spontaneously
452 hypertensive rat. *Hypertension* 65: 775-783, 2015.
- 453 39. **Marina N, Tang F, Figueiredo M, Mastitskaya S, Kasimov V, Mohamed-Ali V,**
454 **Roloff E, Teschemacher AG, Gourine AV and Kasparov S.** Purinergic signalling in
455 the rostral ventro-lateral medulla controls sympathetic drive and contributes to the
456 progression of heart failure following myocardial infarction in rats. *Basic Res Cardiol* 108:
457 317, 2013.
- 458 40. **Martin-Body RL, Robson GJ and Sinclair JD.** Restoration of hypoxic respiratory
459 responses in the awake rat after carotid body denervation by sinus nerve section. *J*
460 *Physiol* 380: 61-73, 1986.
- 461 41. **Marty N, Dallaporta M, Foretz M, Emery M, Tarussio D, Bady I, Binnert C,**
462 **Beermann F and Thorens B.** Regulation of glucagon secretion by glucose transporter
463 type 2 (glut2) and astrocyte-dependent glucose sensors. *J Clin Invest* 115: 3545-3553,
464 2005.
- 465 42. **Miller MJ and Tenney SM.** Hypoxia-induced tachypnea in carotid-deafferented cats.
466 *Respir Physiol* 23: 31-39, 1975.
- 467 43. **Moss IR.** Respiratory responses to single and episodic hypoxia during development:
468 mechanisms of adaptation. *Respir Physiol* 121: 185-197, 2000.
- 469 44. **Ndubuizu O and LaManna JC.** Brain tissue oxygen concentration measurements.
470 *Antioxid Redox Signal* 9: 1207-1219, 2007.
- 471 45. **Neubauer JA and Sunderram J.** Oxygen-sensing neurons in the central nervous
472 system. *J Appl Physiol (1985)* 96: 367-374, 2004.
- 473 46. **Olson EB, Vidruk EH and Dempsey JA.** Carotid-Body Excision Significantly Changes
474 Ventilatory Control in Awake Rats. *J Appl Physiol* 64: 666-671, 1988.
- 475 47. **Pena F, Parkis MA, Tryba AK and Ramirez JM.** Differential contribution of pacemaker
476 properties to the generation of respiratory rhythms during normoxia and hypoxia.
477 *Neuron* 43: 105-117, 2004.
- 478 48. **Prabhakar NR.** Sensing hypoxia: physiology, genetics and epigenetics. *J Physiol* 591:
479 2245-2257, 2013.
- 480 49. **Ramirez JM, Quellmalz UJ, Wilken B and Richter DW.** The hypoxic response of
481 neurones within the in vitro mammalian respiratory network. *J Physiol* 507: 571-582,
482 1998.
- 483 50. **Robinson DM, Kwok H, Adams BM, Peebles KC and Funk GD.** Development of the
484 ventilatory response to hypoxia in Swiss CD-1 mice. *J Appl Physiol (1985)* 88: 1907-
485 1914, 2000.
- 486 51. **Roux JC, Pequignot JM, Dumas S, Pascual O, Ghilini G, Pequignot J, Mallet J and**
487 **Denavit-Saubie M.** O₂-sensing after carotid chemodenervation: hypoxic ventilatory
488 responsiveness and upregulation of tyrosine hydroxylase mRNA in brainstem
489 catecholaminergic cells. *Eur J Neurosci* 12: 3181-3190, 2000.
- 490 52. **Ruangkittisakul A and Ballanyi K.** Anoxia response in physiological potassium of the
491 isolated inspiratory center in calibrated newborn rat brainstem slices. *Adv Exp Med Biol*
492 758: 91-98, 2012.
- 493 53. **Smith PG and Mills E.** Restoration of reflex ventilatory response to hypoxia after
494 removal of carotid bodies in the cat. *Neuroscience* 5: 573-580, 1980.
- 495 54. **Soliz J, Joseph V, Soulage C, Becskei C, Vogel J, Pequignot JM, Ogunshola O and**
496 **Gassmann M.** Erythropoietin regulates hypoxic ventilation in mice by interacting with
497 brainstem and carotid bodies. *J Physiol* 568: 559-571, 2005.
- 498 55. **Solomon IC.** Glutamate neurotransmission is not required for, but may modulate,
499 hypoxic sensitivity of pre-Botzinger complex in vivo. *J Neurophysiol* 93: 1278-1284,
500 2005.

- 501 56. **Solomon IC, Edelman NH and Neubauer JA.** Pre-Botzinger complex functions as a
502 central hypoxia chemosensor for respiration in vivo. *J Neurophysiol* 83: 2854-2868,
503 2000.
- 504 57. **Sun MK, Jeske IT and Reis DJ.** Cyanide excites medullary sympathoexcitatory neurons
505 in rats. *Am J Physiol* 262: R182-R189, 1992.
- 506 58. **Sun MK and Reis DJ.** Hypoxia selectively excites vasomotor neurons of rostral
507 ventrolateral medulla in rats. *Am J Physiol* 266: R245-R256, 1994.
- 508 59. **Swanson GD, Whipp BJ, Kaufman RD, Aqleh KA, Winter B and Bellville JW.** Effect
509 of hypercapnia on hypoxic ventilatory drive in carotid body-resected man. *J Appl Physiol*
510 *Respir Environ Exerc Physiol* 45: 871-877, 1978.
- 511 60. **Teppema LJ and Dahan A.** The ventilatory response to hypoxia in mammals:
512 mechanisms, measurement, and analysis. *Physiol Rev* 90: 675-754, 2010.
- 513 61. **Thoby-Brisson M and Ramirez JM.** Role of inspiratory pacemaker neurons in
514 mediating the hypoxic response of the respiratory network in vitro. *J Neurosci* 20: 5858-
515 5866, 2000.
- 516 62. **Timmers HJ, Karemaker JM, Wieling W, Marres HA, Folgering HT and Lenders**
517 **JW.** Baroreflex and chemoreflex function after bilateral carotid body tumor resection. *J*
518 *Hypertens* 21: 591-599, 2003.
- 519 63. **Timmers HJ, Wieling W, Karemaker JM and Lenders JW.** Denervation of carotid
520 baro- and chemoreceptors in humans. *J Physiol* 553: 3-11, 2003.
- 521 64. **Turovsky E, Karagiannis A, Abdala AP and Gourine AV.** Impaired CO₂ sensitivity of
522 astrocytes in a mouse model of Rett syndrome. *J Physiol* 593: 3159-3168, 2015.
- 523 65. **Turovsky E, Theparambil SM, Kasymov V, Deitmer JW, Del Arroyo AG, Ackland**
524 **GL, Corneveaux JJ, Allen AN, Huentelman MJ, Kasparov S, Marina N and Gourine**
525 **AV.** Mechanisms of CO₂/H⁺ Sensitivity of Astrocytes. *J Neurosci* 36: 10750-10758, 2016.
- 526 66. **Vermeire P, de BW, van MR, Bal J and van KW.** Carotid body resection in patients
527 with severe chronic airflow limitation. *Bull Eur Physiopathol Respir* 23 Suppl 11: 165s-
528 166s, 1987.
- 529 67. **Weil JV.** Variation in human ventilatory control-genetic influence on the hypoxic
530 ventilatory response. *Respir Physiol Neurobiol* 135: 239-246, 2003.
- 531 68. **Wells JA, Christie IN, Hosford PS, Huckstepp RT, Angelova PR, Vihko P, Cork SC,**
532 **Abramov AY, Teschemacher AG, Kasparov S, Lythgoe MF and Gourine AV.** A
533 critical role for purinergic signalling in the mechanisms underlying generation of BOLD
534 fMRI responses. *J Neurosci* 35: 5284-5292, 2015.
- 535 69. **Whipp BJ and Ward SA.** Physiologic changes following bilateral carotid-body resection
536 in patients with chronic obstructive pulmonary disease. *Chest* 101: 656-661, 1992.
- 537 70. **Zwicker JD, Rajani V, Hahn LB and Funk GD.** Purinergic modulation of preBotzinger
538 complex inspiratory rhythm in rodents: the interaction between ATP and adenosine. *J*
539 *Physiol* 589: 4583-4600, 2011.
- 540 71. **Zylka MJ, Sowa NA, Taylor-Blake B, Twomey MA, Herrala A, Voikar V and Vihko**
541 **P.** Prostatic Acid Phosphatase Is an Ectonucleotidase and Suppresses Pain by Generating
542 Adenosine. *Neuron* 60: 111-122, 2008.
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545 **Figure legends**

546

547 **Figure 1** | Hypothesized cellular mechanisms underlying central oxygen sensitivity. The
548 astroglial signalling cascade triggered by hypoxia involves mitochondrial depolarization,
549 facilitated formation of reactive oxygen species (ROS), lipid peroxidation, activation of
550 phospholipase C (PLC), IP₃ receptors, release of Ca²⁺ from the intracellular stores and
551 enhanced vesicular release of ATP. Hypoxia may also alter opening probability of
552 connexin (Cx) hemichannels permeable to ATP and lactate. Released ATP acts in
553 autocrine and paracrine manner, spreads astroglial Ca²⁺ signals within the neuropil and
554 enhances respiratory and sympathetic activities via excitation of the respiratory rhythm
555 generating circuits of the pre-Bötzinger complex (preBötC) and sympathoexcitatory
556 (pre-sympathetic) C1 neurons of the ventrolateral medulla oblongata.

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558

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



