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CROSSTALK

CrossTalk opposing view: the hypoxic ventilatory response does not include a central, excitatory hypoxia sensing component

Luc J. Teppema
Department of Anaesthesiology, Leiden University Medical Centre, Leiden, The Netherlands

Email: luc.teppema@kpnmail.nl

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Combining state-of-the-art (opto)genetic, molecular, viral transfection, imaging and staining techniques, Gourine and coworkers have constructed a framework with astrocytes as brain interoceptors particularly focusing on a putative role as central respiratory CO_2 and O_2 sensors (Gourine *et al.* 2005; Gourine & Kasparov, 2011; Angelova *et al.* 2015; Gourine & Funk, 2017; Rajani *et al.* 2018). In the opinion of this author, this work does not provide conclusive evidence for their hypothesis of an involvement of astrocytes as central O_2 sensors in the ventilatory response to hypoxia (HVR) especially in awake animals and humans.

Several animal species show immediate hypoventilation, hypercapnia and substantial reduction or entire absence of their HVR after bilateral carotid body denervation (CBD). Partial or complete restoration of the HVR is observed in rat, cat, piglet, goat and pony, all with different rates. This results from up-regulation of aortic bodies, release from cortical inhibition, recruitment of accessory glomus tissue in the trunk, neuroplastic changes consisting of axon regeneration, building alternative circuitries and recruiting central (including astrocytic) O_2 sensors that may be silent in carotid body-intact conditions (Tenny & Ou, 1977; Martin-Body *et al.* 1986; Olson *et al.* 1988; Roux *et al.* 2000; Teppema & Dahan, 2010; Mouradian *et al.* 2012). Surprisingly, Angelova *et al.* (2015) did not challenge CBD animals with acute hypoxia or NaCN infusion shortly (a few days) after surgery; after 10 weeks, hypoxic responses in these animals may thus be due to neuroplastic adaptations. In the conscious goat, isolated central hypoxia does not lead to a progressive increase in ventilation as occurs during chronic carotid body (CB) hypoxia (Weizhen *et al.* 1992). The central hypoxia-induced rise in ventilation in the awake dog depends on peripheral chemoreceptor integrity (Curran *et al.* 2000).

Up to two decades after CB removal or carotid endarterectomy, humans show no HVR (Wade *et al.* 1970; Swanson *et al.* 1978; Honda *et al.* 1979; Dahan *et al.* 2007). Exceptionally, on a hypercapnic background, a small response develops that can be ascribed to an involvement of the aortic bodies (Swanson *et al.* 1978; Timmers *et al.* 2003) but not to central mechanisms (Honda *et al.* 1979). Gourine and Funk (2017) propose an important stimulatory role of mitochondrial ROS in the HVR. To support their hypothesis involving a role of electron flow in the mitochondria in the HVR, they refer to patients with a mutation in the gene encoding succinate dehydrogenase (*SDHD*). These patients, however, do indeed show increased mitochondrial ROS (Cerecer-Gil *et al.* 2010) but *do not* have an abnormally large HVR but rather one at the lower end of normal (Dahan *et al.* 2007). Carotid body type I cells from $SDHD^{+/-}$ mice show an unaltered response to hypoxia, and mitochondrial complex II is not involved in oxygen sensing in these mice (note that total *SDHD* gene knockout is lethal in these mice; Piruat *et al.* 2004).

Utilizing barometric plethysmography, Gourine and coworkers tested their hypothesis *in vivo*. Even if air humidity and temperature in the plethysmograph are controlled, body temperature measured and the inspired CO_2 concentration maintained constant, the pressure signal may be influenced by frequency and airway resistance (Enhorning *et al.* 1998). Percentages of oxygen in the inspired air as the independent variable (i.e. the chamber O_2%) is not the same as the inspired P_{O_2} (Fig. 5 in Angelova *et al.* 2015), and has little predictive value as to the actual stimulus, in this case, the P_{aO_2}. Using arbitrary or relative units is not meaningful and can even be misleading. In other words, what would be of interest is the following: (1) to show blood gases or at least oxygen saturation (e.g. by using a tail probe); (2) to employ a useful index of the HVR such as $\dot{V}_A/\dot{V}_{CO_2}$ or $\dot{V}_A/\dot{V}_{O_2}$ (normalized to body weight; see also Olson *et al.* 1988; Morgan *et al.* 2014); and (3) precise control of the P_{aCO_2} Useful quantitative comparisons between groups require exposure to equal stimulus levels that reach their final values at equal rates. The hypoxic challenges in Angelova *et al.* (2015) were poikilocapnic, which is a confounding factor, given the CO_2 sensitivity of astrocytes (Gourine *et al.* 2005) and the known O_2–CO_2 interaction in the rat (Wilson & Teppema, 2016). Finally, Angelova *et al.* (2015) claim lower respiratory activity in PINK1-deficient mice, but failure of astrocytes to sense low oxygen in these mice does not lead to a reduced response to hypoxia (Fig. 7E in Angelova *et al.* 2015). In conclusion, the data of Gourine and coworkers convincingly show O_2 sensitivity of brain astrocytes and provide details of the resulting stimulus-tranduction cascade, involving ROS, the spread of Ca^{2+} waves and a role of ATP. Concerning the role of Ca^{2+}, could gap-junctional Ca^{2+} exchange between glial cells and retrotrapezoid (RTN) neurons excite the latter? And what is

Luc J. Teppema received his MSci (1978) and PhD (1984) degrees from the Catholic University of Nijmegen, The Netherlands (currently Radboud University). As a staff member at the Department of Anaesthesiology, Leiden University Medical Centre, his research focuses on the effects of hypercapnia and hypoxia on the control of breathing and the effects of anaesthetics, analgesics and carbonic anhydrase inhibitors.

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the effect of glial depolarization (previously qualified as 'glial impairment' by Holleran *et al.* 2000) and ATP on the release of H^+ by glia? Whether the reported increase in respiratory activity in CBD animals (10 weeks after surgery) is due to O_2 sensing by astrocytes remains to be determined, as is the case with an astrocytic role in the normal poikilocapnic (and consequently hypocapnic) and isocapnic HVR.

The human *poikilocapnic* response to mild acute hypoxemia (saturation ∼80%) is biphasic, consisting of an initial increase in ventilation, followed by a secondary fall and a rise in cerebral blood flow (CBF; Steinback & Poulin, 2007, 2016). The modest rise in CB activity will induce little central depression other than that caused by a local fall in P_{CO_2} and may account entirely for the small sustained rise in ventilation. Consequently, astrocytes are unlikely to be involved in maintaining an appropriate minute ventilation. If a modest decrease in brainstem P_{O_2} (which is smaller than in the arterial blood) would result in astrocytic release of ATP, then, apart from directly impacting RTN neurons, the lower P_{CO_2} would tend to reduce it. If, as claimed by Gourine *et al.* (2005) and Rajani *et al.* (2018), a prolonged astrocytic ATP release in the anaesthetized rat maintains phrenic activity in the depressing phase, why then is a similar release after CBD not able to augment respiratory activity (Gourine *et al.* 2005)? The data from the poikilocapnic studies in Angelova *et al.* (2015) were collected in the last 5 min of a 10 min lasting exposure, but the time courses of both stimuli and responses are not shown, so it is unclear to what extent these data include those from the depressing phase.

In the laboratory setting the HVR is often measured *isocapnically* to quantitatively estimate hypoxic sensitivity and O_2–CO_2 interaction effects. The isocapnic HVR is also biphasic, and the secondary fall (HVD) is related to a rise in cerebral blood flow combined with central depression (Teppema & Dahan, 2010). During HVD ventilation reaches a level ∼30% above control, maintained by the carotid bodies that operate at lower gain but do not show a biphasic response (Teppema & Dahan, 2010). In anaesthesia, however, HVD is uncoupled from carotid body activity (see Teppema & Dahan, 2010) and may involve a different role of ATP, as suggested in the rat (Rajani *et al.* 2018).

If the O_2 sensitivity of astrocytes is of less relevance to the awake ventilatory response to mild hypoxia, what then is its physiological significance? In the event of local brain hypoxia or ischaemia, an increase in ventilation would be counterproductive: the resulting fall in P_{aCO_2} would cause vasoconstriction and thus impede blood flow to the affected areas and other brain regions. A crucial role of astrocytes is sensing (and adapting) neural activity and metabolism, and translating this also into an adaptation of local vasomotor activity to defend the supply of oxygen and nutrients (Gordon *et al.* 2016; Mukandala *et al.* 2016). That O_2 sensitivity is a general property of astrocytes, rather than a distinctive feature of those located in the ventral medullary surface may suggest that although the latter are located in close association with the RTN, their primary role is to guard the supply of oxygen and nutrients by other means than by stimulating ventilation.

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Additional information

Competing interests

None declared.