COMMUNICATIONS ARISING

Physiology

The ventilatory response to hypoxia

Respiratory physiologists traditionally attribute the increased ventilatory response to hypoxia to increased discharge by the carotid-body chemoreceptor, which is transmitted by sensory processes to neurons in the medullary nucleus of the solitary tract[1]. However, Lipton *et al.* propose a radically new model[2] in which hypoxia causes haemoglobin to release molecules derived from nitric oxide, which then increase ventilation by directly stimulating solitary-tract neurons. Despite the apparent feasibility of this model[3–5], we show here that the observations of Lipton *et al.*[2] do not invalidate the classic carotid-body-mediated explanation of the hypoxic ventilatory response. We thus question the justification for a new model to account for hypoxia's effect on breathing.

Lipton and colleagues' proposal that molecules derived from nitric oxide, called *S*-nitrosothiols (SNOs), duplicate the physiological response to hypoxia is based on three observations. First, they observed an equivalent increase in ventilation after separate injections into the rat nucleus tractus solitarius (NTS) of equimolar concentrations of *S*-nitrosocysteinyl glycine (CGSNO), *S*-nitroso-L-cysteine (L-CSNO) and *S*-nitrosoglutathionine (GSNO). Second, injection of an extract of deoxygenated, but not oxygenated, blood reproduced the effect of these agents. Third, the response to 10% O_2 mirrored that produced by CGSNO, CSNO and GSNO, and the decay characteristics of the recovery of ventilation were identical in each case. No data are presented to support this final claim, but the authors' Figs 2 and 3 reveal widely different time constants of about 120 seconds after hypoxia, 60 s after CGSNO, and 240 s after blood extract.

If SNOs mediate the hypoxic ventilatory response (HVR), the effect of SNOs and hypoxia should disappear in response to blocking any step between the proposed release of SNOs at the NTS and the increased respiratory motor output. Acivicin, which blocks a step involving γ-glutamyl transpeptidase (γ-GT), abolished the ventilatory response to GSNO in rats, but the HVR persisted, as it did in mice genetically deficient in γ-GT (ref. 2). SNOs thus increase ventilation at the NTS through a γ-GT-dependent mechanism, but the HVR is independent of γ-GT; SNOs are therefore not essential for this response.

Lipton *et al.* also propose that SNOs are crucial for the return of ventilation to normal when air replaces hypoxic inspired gas[2], with SNOs effecting a residual stimulation of ventilation during this recovery phase. This suggestion is based on their findings that pretreatment with acivicin leads to a marked acceleration of ventilatory decay after hypoxia in rats, and that hypoxic ventilatory recovery is profoundly attenuated in knockout mice that lack γ-GT, which manifests itself as an undershoot in ventilation after 60 s.

We contend that key features of this evidence conflict with earlier work. Ventilation is shown as increasing continuously during 5 min of hypoxia in rats[2], whereas the typical response is a rapid increase in ventilation, then a decline as hypoxia continues; this well-known biphasic HVR occurs in many species, including humans[6] and, crucially, mice[7] and rats[8]. The slow decline in ventilation after a return to air[2] is also unusual because ventilation falls rapidly on termination of hypoxia in humans[9], lambs[10] and mice[7].

We suggest that the 3-litre plethysmograph used by Lipton *et al.*, through which gas passed at 8 litres per min, is likely to have slowed changes in gas concentration[2]. Consistent with this, the reported ramp-like increase in ventilation in response to hypoxia[2] is identical to that seen in lambs exposed to progressive hypoxia[10], and the 2-min return to baseline ventilation upon returning to air[2] suggests that hypoxia is persisting.

Furthermore, although the ventilatory undershoot after returning to air is considered to be abnormal[2], it is in fact well documented[7,9,10] and has been explained by the development of hypocapnia during the HVR[9]. The duration of hypoxic exposure and the associated fall in blood and tissue CO_2 levels influence whether undershoot occurs[9] — so without information on blood gases[2], a chemical-control explanation for the presence or absence of undershoot cannot be discounted.

We suggest that attribution of an important role to SNOs in the HVR is not justified on the basis of Lipton and colleagues' present results, a conclusion that is supported by a wealth of evidence that the response to hypoxaemia is negligible in animals and humans with denervated or resected carotid bodies[11].

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1. Heymans, C. & Neil, E. *Reflexogenic Areas of the Cardiovascular System* (Churchill, London, 1958).
2. Lipton, A. J. *et al. Nature* **413,** 171–174 (2001).
3. Lipton, S. A. *Nature* **413,** 118–121 (2001).
4. Butcher, J. *Lancet* **358,** 897 (2001).
5. Mato, J. M., Avila, M. A. & Corrales, F. J. *Nature Med.* **7,** 1107–1108 (2001).
6. Easton, P. A., Slykerman, L. J. & Anthonisen, N. R. *J. Appl. Physiol.* **61,** 906–911 (1986).
7. Robinson, D. M. *et al. J. Appl. Physiol.* **88,** 1907–1914 (2000).
8. Maxova, H. & Vizek, M. *Physiol. Res.* **50,** 327–331 (2001).
9. Badr, M. S., Skatrud, J. B. & Dempsey, J. A. *J. Appl. Physiol.* **73,** 1958–1971 (1992).
10. Bureau, M. A. *et al. J. Appl. Physiol.* **61,** 836–842 (1986).
11. Honda, Y. *J. Appl. Physiol.* **73,** 1–8 (1992).

Gozal et al. *reply* — We do not challenge the 'classic' theory of peripheral-chemoreceptor-mediated HVR. However, this offers no insight into the mediators of the time-domain components of HVR, such as ventilatory short-term potentiation (VSTP)[1]. VSTP is critical to respiratory-system stability[2] and is unrelated to the activity of the peripheral chemoreceptor[3,4], so other factors may have a role in HVR[5,6]. One such factor involves SNO signalling in brainstem neurons. SNOs could be formed by neuronal nitric oxide synthase (NOS, activated by afferents from peripheral chemoreceptors) and by erythrocyte deoxygenation. Indeed, erythrocyte deoxygenation could be signalled to peripheral chemoreceptors through SNO formation.

When concentrations of SNO delivered to the dorsocaudal brainstem match the magnitude of peak HVR, the time constants of VSTP after hypoxia and SNO are similar (our unpublished results). Thus VSTP, a principal component of HVR, is critically dependent on SNO formation and its subsequent activity in brainstem structures. These results are supported by data that demonstrate the NOS-dependency of both VSTP and long-term facilitation following hypoxia[7].

On the contributions of hypocapnia and ramp-presentation of the hypoxic stimulus to the ventilatory undershoot, following cessation of hypoxic gas administration: isocapnic hypoxia and step presentation of hypoxic stimuli to γ-GT-knockout and wild-type mice confirm the anticipated absence and presence of VSTP, respectively (our unpublished results).

S-nitrosoglutathione may not be the only SNO that signals HVR, because other SNOs are also formed during erythrocyte deoxygenation and NOS activation[8,9]. We used GSNO as a reporter SNO, but found that other L-isomeric SNOs mimic HVR, as indicated by the large increase in HVR in humans treated with *N*-acetyl-L-cysteine[10].

Peripheral chemoreceptors are therefore important in initiating the ventilatory response to hypoxia, and SNO signalling is crucial in determining the characteristics of the HVR. It is not necessary to assume that these two features are mutually exclusive.

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1. Powell, F. L. *et al. Resp. Physiol.* **112,** 123–134 (1998).
2. Eldridge, F. L. & Gill-Kumar, P. *Resp. Physiol.* **40,** 49–63 (1980).
3. Gallman, E. A. & Millhorn, D. E. *J. Physiol.* **395,** 333–347 (1988).
4. Georgopoulos, D. *et al. Chest* **107,** 150–155 (1995).
5. Dahan, A. *et al. J. Physiol.* **488,** 803–813 (1995).
6. Menendez, A. A. *et al. J. Appl. Physiol.* **86,** 1478–1482 (1999).
7. Kline, D. D. *et al. J. Physiol.* **539,** 309–315 (2002).
8. Pawloski, J. R. *et al. Nature* **409,** 622–626 (2001).
9. Lipton, S. A. *et al. Nature* **364,** 626–632 (1993).
10. Hildebrandt, W. *et al. Blood* **99,** 1552–1555 (2002).