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NO, SNO and low O_2

The respiratory control system responds to hypoxia by increasing the rate of breathing. The finding that nitric-oxide–related molecules might control the ventilatory response to hypoxia suggests possible moleculartargets for the treatment of apnea.

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Respiration is an automatic response regulated by a network of neurons in the hindbrain. These neurons control the respiratory muscles and produce pressure gradients that rhythmically move air in and out the lungs. One of the remarkable features of the respiratory control system is its capacity to adjust the rate of breathing to a variety of conditions—a characteristic first described more than a century ago. Thus, a rise in the partial pressure of carbonic anhydride or oxygen deprivation (hypoxia) increases the rate of ventilation, which then restores the partial pressures of oxygen and carbonic anhydride to their normal values. Failure to increase ventilation in response to hypoxia can be fatal; in particular, it can lead to respiratory distress in newborns and to obstructive sleep apnea (cessation of breathing) in adults.

The molecular mechanisms by which hypoxia leads to hyperventilation are poorly understood[1]. In the 13 September issue of *Nature*, Lipton *et al.*[2] make a convincing case that the ventilatory response to hypoxia is regulated by a group of unsuspected players, deoxyhemoglobin-derived S-nitrosothiols (SNOs).

Recent advances in our understanding of signaling through nitric oxide (NO) have provided a model whereby NO induces the S-nitrosylation of a single critical cysteine residue on different classes of proteins, through a S-nitrosothiol–thiol exchange reaction known as transnitrosation[3,4]. According to the model, SNOs—rather than NO—are the signaling molecules. Consequently, SNO biochemistry has become of central importance in elucidating how NO regulates cellular functions. Work carried out mainly by Stamler's group has demonstrated the formation of S-nitrosohemoglobin in the lung when red blood cells become oxygenated, and the release of the SNO group and formation of reactive SNO molecules, such as S-nitrosoglutathione (GSNO) during blood deoxygenation[5]. The anion exchange protein 1 (AE1) in the red blood-cell membrane appears to facilitate the transport of the SNO group from S-nitrosohemoglobin, across the erythrocyte membrane, to glutathione (GSH) dissolved in the plasma to form GSNO (ref. 6). The beauty of this mechanism is that signaling does not rely on the free diffusion of NO, but instead the generation of reactive SNOs molecules is confined to specific sites.

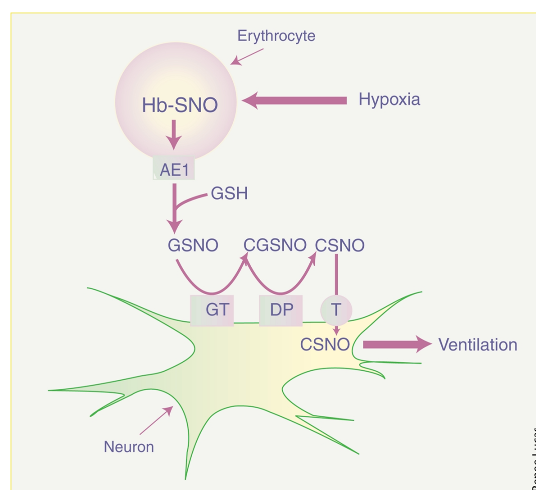

Fig. 1 Proposed mechanism by which GSNO controls the ventilatory response to hypoxia. In hypoxia, the SNO from S-nitrosohemoglobin (Hb-SNO) is transferred to GSH to form GSNO in the vicinity of the neurons of the nTS, through a process facilitated by the erythrocyte cell membrane anion exchange protein 1 (AE1). Breakdown of newly synthesized GSNO is then catalyzed by γGT. The CGSNO formed is then split by a membrane-bound dipeptidase (DP). The resulting S-nitrosocysteine (CSNO), through a transporter (T), is taken into the neuron where it initiates a signaling process that induces an increase in the rate of ventilation and restores the partial pressure of oxygen to normal values.

Based on the findings described above and on earlier work that connected NO with breathing and oxygen tension[7], as well as the observation that GSNO is present in the brain[8], Lipton *et al.* hypothesized that SNOs could be involved in the ventilatory response to hypoxia. To probe their hypothesis, the authors first showed that a number of naturally occurring SNOs increased ventilation in freely behaving conscious rats. In these experiments, Lipton *et al.* implanted a dual cannula in close proximity of the nucleus tractus solitarius (nTS) through a hole drilled into the occipital skull of the rats and, after the animals had recovered from the intervention, measured the ventilatory response to various agents. The authors found that injection of 1 nmol S-nitroso-L-cysteine (L-CSNO), S-nitrosocysteinyl-glycine (CGSNO) or GSNO, but not S-nitroso-D-cysteine (D-CSNO), induced a marked increase in the ventilation rate. Moreover, the injection of the synthetic NO donor S-nitroso-N-acetylpenicillamine, was found to have similar effects. The authors also demonstrated that changes in the respiratory rate in response to the SNOs tested were similar to the response observed after briefly exposing the whole animal to hypoxia. In summary, these elegant experiments demonstrate that SNOs mimic the physiological ventilatory response to hypoxia.

In a second set of experiments, Lipton *et al.* observed that deoxygenated blood reacted with GSH to form GSNO more efficiently than oxygenated blood. Then, they demonstrated that a low-mass compound derived from deoxygenated blood, identified as GSNO by mass spectrometry, could reproduce the ventilatory effect of hypoxia. Interestingly, the authors observed that pharmacological inhibition of γ-glutamyl transpeptidase (γGT, an enzyme that catalyzes the cleavage of the γ-glutamyl moiety of GSNO to form CGSNO) in the nTS abolished the ventilatory response induced by GSNO, but not the one induced by CGSNO. By generating a knockout mouse lacking γGT and showing that the mutant animals exhibit a marked alteration of hypoxic ventilatory recovery, Lipton *et al.* conclusively demonstrate that γGT is required for the normal ventilatory response to hypoxia.

The results of Lipton *et al.* reveal the importance of SNOs as signaling mole-

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cules that regulate the normal response to hypoxia *in vivo*. The authors also provide evidence that the metabolism of GSNO is similar to that of reduced glutathione. Therefore, revisiting GSH biochemistry might be helpful developing a model of how signaling through GSNO occurs (Fig. 1). Blood GSH is hydrolyzed by the membrane-bound enzyme γGT. The cysteinylglycine formed is split by membrane-bound dipeptidases, and the resulting cysteine is transported into a cell by a stereoselective transporter[9]). It is then possible that GSNO formed in the proximity of nTS during blood deoxygenation is first hydrolyzed by γGT to form CGSNO and then to CSNO by a membrane-bound dipeptidase (Fig. 1). Finally, through a stereoselective transporter, L-CSNO but not D-CSNO, would be taken inside the neuron to initiate the signaling process that leads to the control of ventilation, probably through the S-nitrosylation of specific target proteins. As CGSNO and CSNO are very reactive, unstable molecules, the confinement of the critical components (γGT, dipeptidase and the transporter) of GSNO metabolism to a specific membrane compartment may be critical for both specificity of targeting and propagation of the signal.

The components in the signaling pathway that have been elegantly elucidated by Lipton *et al*. will identify potential targets to inhibit or increase the effects of SNOs on the hypoxic response and thereby provide treatments for disorders such as sleep apnea.

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Linking ubiquitin, parkin and synphilin-1

One neuronal characteristic of Parkinson disease is the presence of Lewy bodies–intracytoplasmic accumulations of protein. A new study draws a molecular link between the formation of these bodies, the ubiquitin protein degradation system, parkin and synphilin-1. (pages 1144–1150)

Aaron Ciechanover

The ubiquitin- and proteasome-mediated degradation of certain intracellular proteins is fundamental to the regulation of basic cellular processes, such as cell-cycle progression, growth and differentiation, as well as the immune and inflammatory responses. The ubiquitin proteolytic system (UPS) also plays an important role in cellular quality control by removing mutated, misfolded, unassembled or post-translationally damaged proteins.

Recent work has implicated aberrations of the UPS in the pathogenesis of several neurodegenerative diseases and in the formation of the inclusion bodies that characterize many of these diseases. Inclusion bodies, also termed Lewy bodies (LBs), are proteinaceous cytoplasmic inclusions that are enriched in disease-characteristic proteins and ubiquitin, some of which is conjugated to these proteins. In this issue, Chung *et al.*[1] demonstrate that parkin, a ubiquitin-protein ligase that when mutated leads to autosomal recessive juvenile parkinsonism (AR-JP), normally conjugates ubiquitin to synphilin-1, an important component of Parkinson disease (PD) LBs. The study further attempts to link this normal function of parkin to the pathology that results from its inactivation. To understand this association, it is important to understand the basic functions and mode of action of the UPS (Fig. 1).

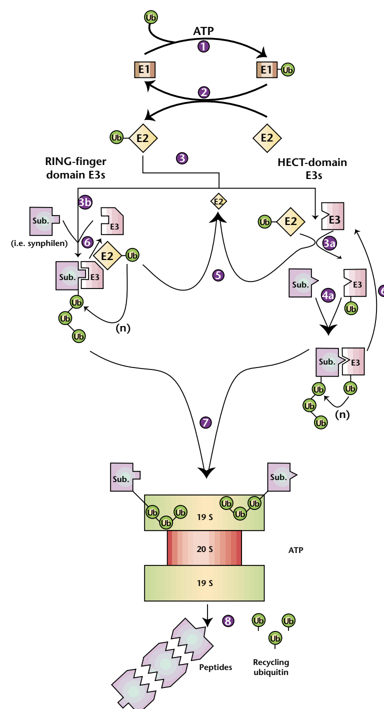

Fig. 1 The ubiquitin proteasome-mediated pathway. 1) Activation of ubiquitin by E1. 2) Transfer of the activated ubiquitin moiety from E1 to E2. 3) Ubiquitin is further transferred in one of two ways. 3a) In the case of HECT-domain ligases (E3s), ubiquitin generates a third, high-energy intermediate with the ligase. 4a) Following specific recognition of the substrate and generation of an E3–substrate complex, multiple ubiquitin moieties are successively transferred to generate a substrate-anchored polyubiquitin chain that serves as a recognition marker for the 26S proteasome. 3b) In the case of RING-finger domain E3s, a ternary complex is generated between the substrate, E3, and E2, and the activated ubiquitin moieties are transferred directly from E2 to the E3-bound substrate. 5 and 6) Recycling of the E2s and E3s, respectively. 7) Recognition of the polyubiquitin chain by the 19S subcomplex of the 26S proteasome. 8) Degradation of the substrate to generate peptides with release of free and reusable ubiquitin.

Degradation via the UPS involves two successive steps: tagging of the protein substrate by covalent attachment of multiple ubiquitin molecules, followed by degradation of the tagged protein by the 26S proteasome with release of free and reusable ubiquitin, a reaction catalyzed by ubiquitin C-terminal hydrolases (UCHs).

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