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Forum Editorial

Quartet Signal Transducers in Gas Biology

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DIFFERENT GASEOUS MOLECULES, such as molecular oxygen (O_2), nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S), have been shown to play important roles in executing signal transduction in biological systems. These quartet molecules share unique physicochemical properties with each other and exert their biological activities through mechanisms distinct from those of other signaling molecules. First, as summarized in a review of this forum (22), these molecules are highly membrane-permeable and thus serve as a substance that readily conveys the signal from one site to another in autocrine, paracrine, and/or juxtacrine manners. Second, they exert their biological actions via interactions with macromolecules through multiple ways: covalent binding of gases to prosthetic metal complexes in their receptor proteins, noncovalent binding to the regulatory subunits of the proteins, and their space occupancy in and around the protein structure that leads to a decrease in accessibility of other gases to the functionally critical regions.

Regarding the aforementioned quartet gases, they share an important feature, that is, their elimination from biological systems by heme proteins. This is because of the nature of the gases to be captured by prosthetic ferrous heme of the proteins. In mammals, hemoglobin (Hb) in erythrocytes constitutes a major protein-sink compartment to scavenge these gases, and thus accounts for a determinant of lifetime of the gases *in vivo*. NO and CO can be captured by the ferrous heme of Hb *in vivo*. Their binding to the heme is far tighter than that of O_2, but not irreversible. When the concentration of NO is sufficient, the gas reacts with the heme to be degraded into NO_3^- in the presence of oxygen. Under these circumstances, Hb is oxidized into metHb, but this is rapidly reduced and recycled into Hb through the reaction of metHb reductase in erythrocytes. As a result, NO is metabolized into its soluble and oxidized form that can be excreted into urine or sweat. According to previous studies (17), NO also binds to cysteine residues of Hb and circulates until Hb in erythrocytes is exposed to hypoxia; in this case, NO is excreted from erythrocytes and helps microvascular relaxation site-specifically at hypoxic regions. Such a concept together with recent studies showing the hypoxia-sensing ability of erythrocytes to trigger NO-dependent vasorelaxation led us to hypothesize that Hb in the cells not only serves as an oxygen carrier, but also accounts for the circulating O_2 sensor (1).

CO also binds to the ferrous heme of Hb, but undergoes quite different processes for metabolism from those seen in NO. This process stabilizes the structure of Hb without causing oxygen activation seen in the NO-mediated process. HbCO in erythrocytes can thus stay in circulation and gradually releases CO when the cells pass through alveolar capillary vessels where the local oxygen concentration is sufficient enough to trigger dissociation of CO from Hb to the airway. Such a nature of the gases allows us to understand the fact that heme proteins constitute an important class of the receptor for utilization and reception of O_2, NO, and CO, although direct evidence that H_2S utilizes one of the heme proteins to transduce its signals *in vivo* has not been provided yet. A complex interaction between multiple gases and one receptor protein is also the case when we understand how NO and CO regulate the activity of the heme-containing soluble guanylate cyclase (sGC) *in vivo* (3). Like NO, CO is believed to serve as an activator of sGC that causes vasorelaxation. Novel monoclonal antibodies that can sense function-associated conformational changes in sGC revealed that CO serves as a partial antagonist of the NO-activated enzyme (8). Systemic hypertension caused by gene transfection of the CO-generating heme oxygenase into vascular smooth muscle cells also supports this concept (6). Considering that the availability of NO *in vivo* is greatly influenced by local oxygen tension or by formation of superoxide (7, 12, 16, 18), the CO-producing heme oxygenase system serves as another mechanism for limiting the NO-mediated signal transduction. The modulatory actions of CO on sGC are distinct from those that we first observed in liver microcirculation, where CO constitutively generated in hepatocytes activates sGC in hepatic stellate cells and thereby relaxes sinusoidal tone under steady-state conditions (19, 20). Such a double-faced action of the gas seems to depend on local concentrations of NO: CO appears to modulate sGC activities only when the enzyme

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is substantially activated by NO, while activating it when local NO concentration is low (*e.g.,* liver) (13, 21). CO overproduced by stress-inducible heme oxygenase can not only alter the activity of sGC, but also change that of cytochrome P450 monooxygenases in the liver (11, 14). Previous studies revealed novel actions of CO on spermatogenesis (14) and modulation of inflamed cells such as monocytes/macrophages, while the molecular targets for these events that offer the binding site of CO remain largely unknown (13, 23); further studies on sGC-independent pathways to trigger diverse actions of this gas could shed light on discovery of novel signal transducing systems by the gas.

Besides the prosthetic heme of these proteins, those containing non-heme metal complexes also serve as another important class of the gas receptors. Prolyl hydroxylases for hypoxia-inducible factor 1α are such examples that can sense a reduction of the oxygen supply in cells and thereby give this transcriptional factor a specific structure that limits its accessibility from cytoplasm to nuclei to convey its mission to genes (4). CO is an important carbon source for microorganisms. They utilize the gas to be converted to methane and acetyl-CoA through the reaction of CO dehydrogenase that possesses molybdenum for the catalytic center exerting CO reception (2). Because of its reactivity as a radical species, NO reacts with thiol, and this reaction not only serves as an important signal transducing mechanism through this gas, but also offers a large compartment for NO sink *in vivo* (5, 7). Recent advances in intravital visualization of the gas molecule revealed that microvascular endothelium is such a sink compartment for NO that can store the gas independently of the local activities of NO synthases (4, 10, 21).

This forum issue provides an opportunity to discuss how the gases transduce their signals in mammals. At present, the whole picture of mechanisms through which gases regulate homeostasis of biological systems remains largely unknown. However, the articles in this forum shed light on future directions of Gas Biology in which we attempt to understand how multiple gases conduct the function of proteomics as a whole. This new area deserves further study provided that details of the atomic basis of the gas–protein interactions become available through advanced spectrophotometrical analyses and the complex roles of symbiotic bacteria as a generator and consumer of the signaling gases are unraveled.

REFERENCES

1. Ellsworth ML, Forrester T, Ellis CG, and Dietrich HH. The erythrocyte as a regulator of vascular tone. *Am J Physiol Heart Circ Physiol* 269: H2155–H2161, 1995.
2. Ferry JG. CO dehydrogenase. *Annu Rev Microbiol* 49: 305–333, 1995.
3. Friebe A, Schultz G, and Koesling D. Sensitizing soluble guanylate cyclase to become a highly CO-sensitive enzyme. *EMBO J* 15: 6863–6868, 1996.
4. Goda N, Dozier SJ, and Johnson RS. HIF-1 in cell cycle regulation, apoptosis, and tumor progression. *Antioxid Redox Signal* 5: 467–473, 2003.
5. Gow AJ, Chen Q, Hess DT, Day BJ, Ischiropoulos H, and Stamler JS. Basal and stimulated protein *S*-nitrosylation in multiple cell types and tissues. *J Biol Chem* 277: 9637–9640, 2002.
6. Imai T, Morita T, Shindo T, Nagai R, Yazaki Y, Kurihara H, Suematsu M, and Katayama S. Vascular smooth muscle cell-directed overexpression of heme oxygenase-1 elevates blood pressure through attenuation of nitric oxide-induced vasodilation in mice. *Circ Res* 89: 55–62, 2001.
7. Inoue M, Sato E, Nishikawa M, Park A-M, Kira Y, Imada I, and Utsumi K. Cross talk of nitric oxide, oxygen radicals, and superoxide dismutase regulates the energy metabolism and cell death and determines the fates of aerobic life. *Antioxid Redox Signal* 5: 475–484, 2003.
8. Kajimura M, Shimoyama M, Tsuyama S, Suzuki T, Kozaki S, Takenaka S, Tsubota K, Oguchi Y, and Suematsu M. Visualization of gaseous monoxide reception by soluble guanylate cyclase in the rat retina. *FASEB J* 17: 506–508, 2003.
9. Kashiba M, Kajimura M, Goda N, and Suematsu M. From O_2 to H_2S: a landscape view of gas biology. *Keio J Med* 51: 1–10, 2002.
10. Kashiwagi S, Kajimura M, Yoshimura Y, and Suematsu M. Nonendothelial source of nitric oxide in arterioles but not in venules: alternative source revealed in vivo by diaminofluorescein microfluorography. *Circ Res* 91: e55–e64, 2002.
11. Kyokane T, Norimizu S, Taniai H, Yamaguchi T, Takeoka S, Tsuchida E, Naito M, Nimura Y, Ishimura Y, and Suematsu M. Carbon monoxide from heme catabolism protects against hepatobiliary dysfunction in endotoxin-treated rat liver. *Gastroenterology* 120: 1227–1240, 2001.
12. Moncada S, Palmer RMJ, and Higgs EA. Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol Rev* 43: 109–142, 1991.
13. Morita T, Imai T, Yamaguchi T, Sugiyama T, Katayama S, and Yoshino G. Induction of heme oxygenase-1 in monocytes suppresses angiotensin II-elicited chemotactic activity through inhibition of CCR2: role of bilirubin and carbon monoxide generated by the enzyme. *Antioxid Redox Signal* 5: 439–447, 2003.
14. Norimizu S, Kudo A, Kajimura M, Ishikawa K, Taniai H, Yamaguchi T, Fujii K, Arii S, Nimura Y, and Suematsu M. Carbon monoxide stimulates mrp2-dependent excretion of bilirubin-IXα into bile in the perfused rat liver. *Antioxid Redox Signal* 5: 449–456, 2003.
15. Ozawa N, Goda N, Makino N, Yamaguchi T, Yoshimura Y, and Suematsu M. Leydig cell-derived heme oxygenase-1 regulates apoptosis of premeiotic germ cells in response to stress. *J Clin Invest* 109: 457–467, 2002.
16. Schaefer S, Kajimura M, Tsuyama S, Uchida K, Sato E, Inoue M, Suematsu M, and Watanabe K. Aberrant utilization of nitric oxide and regulation of soluble guanylate cyclase in rat diabetic retinopathy. *Antioxid Redox Signal* 5: 457–465, 2003.
17. Stamler JS, Jia L, Eu JP, McMahon TJ, Demchenko IT, Bonaventura J, Gernert K, and Piantadosi CA. Blood flow regulation by *S*-nitrosohemoglobin in the physiological oxygen gradient. *Science* 276: 2034–2037, 1997.

18. Suematsu M, Kurose I, Asako H, Miura S, and Tsuchiya M. In vivo visualization of oxyradical-dependent photoemission during endothelium-granulocyte interaction in microvascular beds treated with platelet-activating factor. *J Biochem (Tokyo)* 106: 355–360, 1989.
19. Suematsu M, Kashiwagi S, Sano T, Goda N, Shinoda Y, and Ishimura Y. Carbon monoxide as an endogenous modulator of hepatic vascular perfusion. *Biochem Biophys Res Commun* 205: 1333–1337, 1994.
20. Suematsu M, Goda N, Sano T, Kashiwagi S, Egawa T, Shinoda Y, and Ishimura Y. Carbon monoxide: an endogenous modulator of sinusoidal tone in the perfused rat liver. *J Clin Invest* 96: 2431–2437, 1995.
21. Suematsu M, Suganuma K, and Kashiwagi S. Mechanistic probing of gaseous signal transduction in microcirculation. *Antioxid Redox Signal* 5: 485–492, 2003.
22. Wang R. The gasotransmitter role of hydrogen sulfide. *Antioxid Redox Signal* 5: 493–501, 2003.
23. Willis D, Moore AR, Frederick R, and Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. *Nat Med* 2: 87–89, 1996.

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