Editorial Commentary

NO Break-Ins at Water Gate

Bruce C. Kone

Nitric oxide (NO) is a gaseous free radical that functions as an endogenous mediator in diverse biological effects in numerous tissues. In the kidney and vasculature, these processes include the control of systemic and microvascular tone, the glomerular microcirculation, renal sodium excretion, and inflammatory responses in the glomerulus and tubulointerstitium, among many others. NO also impacts the renin–angiotensin and eicosanoid systems, endothelin, cytokines, and other key regulators of inflammation. Because of its potent chemical reactivity, high diffusibility, and the fact that, unlike most endogenous chemical signals, it cannot be stored or secreted, NO production by NO synthases is under complex, tight control to dictate specificity of its signaling and to limit toxicity to other cellular components. These controls serve to govern the timing, magnitude, and spatial distribution of NO release, and, in turn, specify the input signals that activate NO release and the effector functions of the molecule to target specific proteins.

The biological role of NO as an inter- and intracellular messenger molecule is greatly dependent on its effective concentration and bioavailability at sites of action. As a hydrophobic gas, NO has traditionally been thought to traverse freely across cell membranes without the need for a specific transport protein to facilitate diffusion. It is also known that NO may accumulate in cellular lipids and preferentially interact with molecules in lipid environments. Since cellular entry of NO has generally been regarded to be near diffusion-limited, many of the reactions of NO are thought to depend on the rate of collision between NO and its target molecules or functional groups. Proteins that are structurally or functionally dependent on amine, thiol, tyrosine, or heme groups or on ternary iron complexes represent potential direct of indirect targets of NO. In addition to cGMP-mediated effects, NO participates in redox chemistry to provide surrogate NO-like bioactivity that functions in the cGMP-independent control of numerous cellular functions. The formation of S-nitrosothiols, for example, results in allosteric receptor modification, inhibition of the activities of enzymes containing sulfhydryl groups, and downregulation of transcriptional activators. Accordingly, mechanisms that might facilitate and regulate NO entry into cells are important, because they govern the bioavailability and the diffusional distance NO can move. Membrane proteins might play an important role where cell membranes have a relatively low intrinsic gas permeability and/or where rates of gas transport are very high.

In this issue of Hypertension, Herrera et al provide the first evidence that an integral membrane protein, aquaporin (AQP)-1, facilitates diffusion of NO into cells and when reconstituted in lipid vesicles. Their evidence to support this conclusion is compelling. In CHO-K1 cells stably overexpressing AQP-1, NO permeability tracked with that of water, and, like water permeability, was dramatically inhibited by the AQP-1 antagonists mercuric chloride and dimethyl sulfoxide, suggesting that the cell membrane serves as a barrier to NO diffusion and that AQP-1 facilitates transfer of the gas. Kinetic analysis of NO flux showed it to be a saturable process with a $K_{m}$ of 0.5 μmol/L. Since NO concentrations in the blood are thought to be in the range of 0.1 to 10 μmol/L and the concentration of NO in the subendothelium of small arterioles has been estimated to be in the range of 250 to 500 nM, the AQP-1-dependent transport described is likely to be physiologically relevant, although this remains to be more rigorously studied. Herrera et al further showed that reconstitution of AQP-1 into lipid vesicles containing no other potential transport proteins enhanced NO influx, and, conversely, small interfering RNA knockdown of AQP-1 in endothelial cells depressed NO release. This latter result further suggests the intriguing possibility that NO transport via AQP-1 may be bidirectional. As Herrera and colleagues are quick to point out, AQP-1 null humans and mice are not hypertensive, as one might anticipate if NO release through the channel from the vascular endothelium were absent. Their argument that impaired entry of NO into erythrocytes lacking AQP-1 might limit the ability of hemoglobin to act as a biological sink for NO and counteract the hypertensive effect has merit and points to the difficulty investigators will face in studying this issue in vivo.

The idea that membrane transport proteins known for their transport of specific molecules might also transport small, volatile molecules is not new; that a membrane protein can transport NO to overcome a diffusion barrier is new. For example, several studies in mammalian cells have suggested that AQP-1 can mediate the transport of carbon dioxide and ammonia (reviewed in Ref.4), although data from others labs challenge whether this occurs in quantitatively significant amounts. Although AQP-1 knockout mice did not show differences in CO$_2$ exchange rates in lung and kidney,5 plants with reduced or enhanced expression of a corresponding AQP ortholog or treated with AQP inhibitors showed differences in CO$_2$ flux and CO$_2$-dependent functions, such as photosynthesis.6–8 Moreover, it has been established that the plasma membrane of some cells has extremely low permeability to volatile molecules, so that facilitative diffusion may be needed for more rapid transit of specific gasses. For example, the apical membrane of the thick ascending limb of Henle has been reported to have limited

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NH₃ permeability, and chief and parietal cells have apical membranes that have very low permeabilities to NH₃ and CO₂. Of course, unstirred layers in series with the membrane can pose an additional obstacle to the transport of permeant hydrophobic molecules. Studies in erythrocytes, for example, demonstrated that extracellular diffusion in the unstirred layer surrounding each erythrocyte, but not the erythrocyte membrane itself, causes the main resistance to NO uptake. It may be that transport via an integral membrane protein improves the efficiency of transport when needed.

The AQPs are a family of small, hydrophobic, evolutionarily conserved integral membrane proteins that are expressed in a specialized subset of mammalian tissues, including kidney, eye, choroid plexus, vasculature, lymph vessels, and muscle that require enhanced, osmotically driven water movement across the cell membrane. In the kidney, AQP-1 is extremely abundant in both apical and basolateral membranes of proximal tubules, the descending thin limb of Henle’s loop, and the endothelial cells lining nonfenestrated capillaries. It is critical for the countercurrent mechanism for urine concentration. Analysis of the crystal structure of AQP-1 and molecular-dynamic simulations of water movement through it reveals that AQP-1 exists as a tetramer with each subunit containing its own aqueous pore. The pore narrows to a diameter of ≈3 Å midway between the lipid bilayer leaflets, allowing tortuous, single-file passage of water through the pore. Given the very small differences in size between water and NO, passage of NO through the pore seems biophysically plausible.

The new results reported by Herrera et al. indicate that a novel function of AQP-1 may be to control specificity of NO action and bioavailability at certain cell types. These provocative findings also raise several interesting and biologically important questions. Is AQP-1 a selective or nonselective gas channel? If it is selective, what structural or regulatory features of the transport protein and the gas determine NO selectivity of the channel? If it is nonselective, can other physiologically relevant gases thought to traverse AQP-1 (such as CO₂) compete with NO for transport? Do changes in osmotic water flux produce changes in NO flux through AQP-1? Can other AQPs transport NO, and if not, what structural features in this class of proteins dictate NO permeability? Can NO transport through AQP-1 modify the channel’s function via S-nitrosylation of cysteine thiols, such as the mercury-sensitive cysteine-189 in the pore of human AQP-1? Confirmation and extension of the current findings may uncover additional versatility in the functions of both molecules and provide further insights into selective transfer of gases across biological membranes and the control of vascular tone and the extracellular fluid volume.

Disclosures

None.

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