Examining the reaction of NO and H_2S and the possible cross-talk between the two signaling pathways

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Small Molecule Signaling

The small, low molecular weight molecules nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) are all endogenously generated in mammalian systems via regulated enzymatic catalysis (1). Along with dioxygen (O_2), these small molecules, as well as their derived species, constitute a unique and expanding class of signaling species with important biological function. These species play critical roles in numerous biological processes, including regulation of enzyme activity, protein structure and function, and cellular defense (1). The initial idea that low molecular weight species can be biosynthesized enzymatically for the purpose of regulating vital signaling function came from work on NO. It has been unequivocally demonstrated that NO is made via the actions of nitric oxide synthase (NOS) enzymes. Biosynthesized NO then activates the iron-heme-containing enzyme soluble guanylate cyclase (sGC) via coordination to the iron-heme, leading to increases in the second messenger cGMP (2). In the vascular system, the NO-sGC-cGMP pathway elicits smooth muscle relaxation and this pathway is a major mechanism for controlling vascular tone. Importantly, the discovery of NO as an endogenously generated small-molecule signaling species evolved from an initial understanding of its target, sGC, and the fact that exogenous/pharmacological addition of NO (or NO-donors) led to enzyme activation. The discovery that NO was biosynthesized for this purpose was a watershed moment in the understanding of physiological signaling because it represented a unique biological pathway whereby a small, freely diffusible, nonionic molecule, previously known mostly as a toxic species, could be synthesized, diffuse to its target, sGC, and elicit a biological response. Following this seminal work, other endogenously generated small-molecule species, such as CO and H_2S, have also been shown to possess important signaling properties. However, unlike NO, whose biological target was known, the mechanisms by which these other signaling molecules elicit their function are not firmly established. Indeed, the signaling functions of molecules, such as CO and H_2S, do not necessarily need to have a specific target, as is the case for NO, but may instead have more global effects via interactions with numerous targets and disruption of established processes (*vide infra*). To be sure, NO-mediated signaling may go beyond simple activation of sGC (although this is clearly its most established signaling function) as other more "global" and less specific processes, such as thiol modification or oxidant formation (via interaction with other molecules) have been proposed to be important in the overall biological actions of NO (3).

Integrated View of Small Molecule Signaling

The fundamental chemistry of NO, CO, H_2S, O_2, and derived species dictates their biological targets and lifetimes. At first glance, it appears that all of these molecules share some important chemical reactivities that may serve as clues to their biological functions (4). Clearly, all these species are capable of coordinating metals, especially ironhemes. Indeed, the primary targets for NO and O_2 are ironheme proteins (sGC and cytochrome *c* oxidase, respectively) and the toxicity of high levels of CO and H_2S have been reported to be a result of interactions with ironheme proteins (hemoglobin and cytochrome *c* oxidase). These simple facts allude to possible interactions between these species at these and other targets. Other possible biological targets these species may also share are thiols and thiol proteins. Many signaling functions associated with O_2 (mostly via O_2-derived species, such as H_2O_2) are reported to occur via thiol protein modification, and NO-mediated thiol modification (again, mostly via NO-derived species) is well established and may also have important biological roles. Of course, H_2S is the simplest biological thiol and its interactions with other, more complex thiols via redox exchange processes has been reported to be an important aspect of its biological actions. Although CO will not directly react with thiols, its ability to disrupt O_2 and NO-dependent processes and metabolism may indirectly affect thiol proteins (5).

Another important aspect of the chemistry of NO, O_2, and H_2S (and derived species) is their ability to interact with each other: for example, both NO and H_2S are unstable in the presence of O_2 leading to oxidized nitrogen oxides and oxidized sulfur compounds (4). Moreover, as already mentioned, NO-derived species can modify thiols, which of course include H_2S. In a biological system, second-order (and higher) kinetics associated with many of these chemical interactions typically precludes their relevance because the concentrations of these signaling species are typically very low. However, some reactions possess very high rate constants, and colocalized and simultaneous generation of the signaling species may allow these interactions to occur. A prime example of this is the reaction of NO with the O_2-derived species superoxide (O_2^-). It is established that the simultaneous presence/generation of NO and O_2^- leads to the destruction of both species, via the initial formation of peroxynitrite ($ONOO^-$), and their associated biological activity (6). The products of these possible chemical interactions often possess chemical reactivity that is distinct from the precursors. Again, the interaction of NO with O_2^- is a good example of this. Both NO and O_2^- are weak one-electron oxidants, whereas $ONOO^-$ has the ability to elicit potent hydroxyl radical-like oxidative reactivity (HO·).

The above discussion indicates that examination of the physiological signaling of

Author contributions: C.L.B. and J.M.F. wrote the paper.

The authors declare no conflict of interest.

See companion article on page E4651.

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any of these species must be done with ample consideration for the others. That is, the fact that these species potentially interact with each other via direct chemical reactions or by competing at distinct targets necessitates the need for all species to be considered simultaneously. Although it is typical (and important) to delineate the signaling pathways and mechanisms of the individual species (i.e., NO, CO, H_2S, O_2), a realistic understanding of their in vivo signaling requires the realization of the possible interactions between them. That is, it is becoming increasingly clear that these molecules have the ability to exhibit cross-talk and represent an integrated signaling system that needs to be viewed as a whole. In line with this idea, the paper by Cortese-Krott et al. in PNAS (7) examines the chemical and biological cross-talk between the pathways involving H_2S and NO. Interestingly, these authors determine that administration of H_2S to Wistar rats caused a marked decrease in circulating NO levels in the blood, which was followed by a decrease in blood pressure. This finding is consistent with the idea that scavenging of NO by H_2S occurs, followed by subsequent downstream NO release. In addition, inhibition of the NO-producing enzyme, NO-synthase, prolonged the effects of H_2S, while also increasing its toxicity. Taking these data together, it appears that NO and H_2S interact with each other, raising the possibility of connected, integrated signaling pathways.

Because NO-mediated modification of thiols is well established, previous examination of the chemical interaction of NO with the simplest biological thiol, H_2S, has been reported. Many speculate this reaction to result in thionitrous acid (HSNO) generation; however, because of the instability of HSNO, little is known about this species (7). In contrast, Cortese-Krott et al. found that reactions involving H_2S and NO result in three primary sulfur–nitrogen species: nitrosopersulfide ($ONSS^-$), polysulfides (HS_n^-), and dinitrososulfite (SULFI/NO). Of these, $ONSS^-$ was the primary species capable of releasing NO, as verified by chemiluminescence detection. SULFI/NO also displayed the ability to release NO and nitroxyl (HNO); however, these effects were weak. Furthermore, $ONSS^-$ was resistant to nucleophilic attack by other thiols and cyanide anion. Therefore, because of its extended lifetime, its ability to resist nucleophilic attack, and its ability to release NO, $ONSS^-$ (in comparison with HSNO) seems to be a candidate as a NO transporter or extended NO-releasing species. The idea that biological NO sources exist (besides direct NOS generation) with targeted release at distant sites or extended kinetics is not new (8, 9), but $ONSS^-$ appears to be a viable candidate for this role.

It should be noted that the NO/H_2S reactions studied by Cortese-Krott et al. (7) are not completely straightforward and somewhat speculative at this time. Purification and identification of the products of such reactions are troublesome because many sulfur species undergo spontaneous secondary reactions, leading to more complex reaction mixtures. Some speculate that these secondary reactions cannot be avoided and, more importantly, must be considered to provide insight on the reactivity of the overall system (10). For example, Cortese-Krott et al. (7) found that addition of polysulfides to reactions involving H_2S and NO enhanced the formation of the $ONSS^-$ product. This is interesting because polysulfide formation is also a result of H_2S and NO reactivity, indicating that polysulfides may not only be a product of reaction, but they may also be an intermediate along the reaction pathway. Others have also observed similar results when studying sulfide reactivity, leading to the notion that oxidized sulfide species, such as polysulfides, may be important species in reactions involving sulfides, and therefore must be considered in such chemistry (11).

In addition to complex reaction mixtures, the reactivity of sulfides can also have significant concentration and pH dependencies. Many sulfide species have low pK_a values, and thus are deprotonated at physiological pH. The protonation state of sulfides can drastically affect the nature of their abilities to act as either nucleophiles or electrophiles. Furthermore, as previously indicated, many sulfur species undergo secondary reactions. It could be speculated that such secondary reactions could be limited by the use of low concentrations or more pronounced at higher ones. The studies of Cortese-Krott et al. (7) show that low concentrations of H_2S in the presence of the NO donor, spermine NONOate (Sper/NO), prevented stimulation of sGC and did not alter cGMP levels. However, high concentrations of H_2S caused a significant increase in the level of cGMP. This result indicates that at low H_2S concentrations, NO is scavenged and thus unavailable for sGC activation, whereas an opposite effect is witnessed at higher H_2S concentration.

In summary, the paper by Cortese-Krott et al. (7) highlights the need to examine the the small-molecule signaling species NO, H_2S, O_2, and CO in an integrated fashion to fully appreciate the intimate details of their fascinating signaling mechanisms/pathways. The mere fact that they share biological targets and have the ability to interact chemically with each other necessitates studies such as this. The generation of numerous reaction products from this physiological chemistry with differing activities and kinetics raises some very important possibilities that may extend the biological scope and kinetics of these species. Clearly, further work is warranted as this is likely the "tip of the iceberg" with regards to understanding the wide degree of biologically relevant NO/H_2S/O_2/CO interactions possible. In addition, mechanisms by which nature controls and regulates this potentially complex chemistry will be important in future research endeavors. Finally, this study reinforces the idea that polysulfur species are important and underappreciated entities in biological signaling. Although they have been known in the plant and nutritional chemistry worlds for some time as beneficial components of, for example, the alliums (garlic, onions, and so forth) (12), their relevance in mammalian physiology is only now beginning to be appreciated.

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