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Editorial:

# Embracing Sulfide and CO to Understand Nitric Oxide Biology

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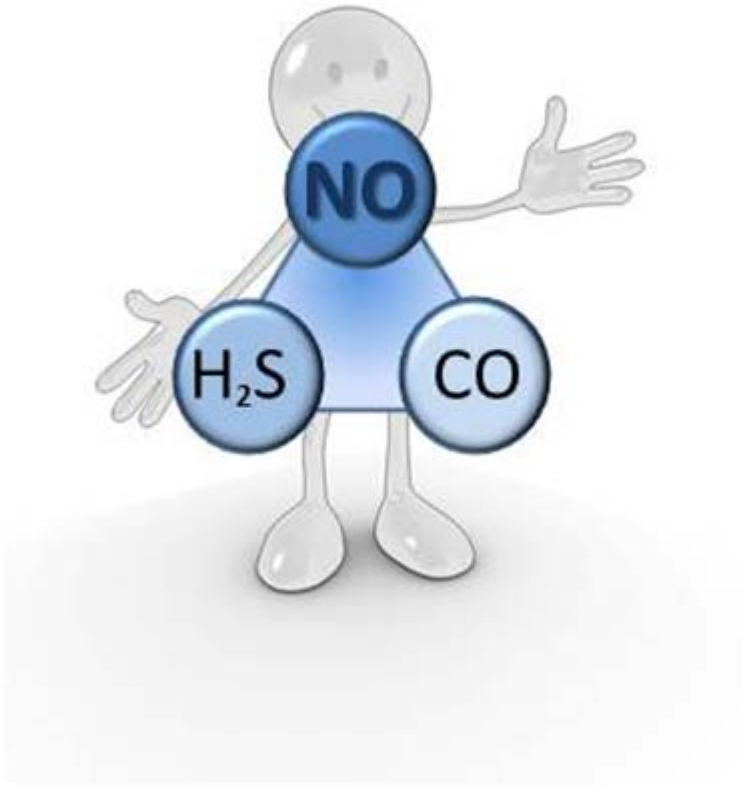
Research in the field of nitric oxide (NO) biology has come a long way from its humble beginnings as an 'endothelium-dependent relaxing factor' (EDRF) to the ubiquitous cell messenger, modulator and regulator of cellular activity it is recognized today. Those of us who had the good fortune to witness the development of this field of research over the years will remember the moments where the cross-talk of NO with other biomolecules became a central feature of the NO signalling process. Some of you may even have had a similar "deja-vu" feeling as we experienced while listening to the other individual's lecture at one of those themed integrated physiology meetings. At the occasion of one such meeting in Glasgow in 2010 the two of us couldn't help wondering why NO and Sulfide appeared so similar when viewed through the looking glass of "hypoxic tolerance". This Editorial serves to explain the Nitric Oxide Society Board Members' decision to expand the scope of our "house journal" to include the biology and chemistry of two other signalling molecules, a decision most of us feel is timely and justified as the field has evolved.

NO was the first of a new class of signalling molecules, followed at 8-10 year intervals by carbon monoxide (CO) and hydrogen sulphide ($H_2S$) and now collectively known as "gasotransmitters". Unfortunately, that term turned out to be somewhat of a misnomer which continues to invite conceptual misunderstandings. While all three substances are indeed gasses at STP, in cells they do not move about and signal in the form of tiny gas puffs. When produced in tissues and biological fluids they are 'solutes' and may use sophisticated transport mechanisms to cross cellular membranes. This would seem to make it easier to target biological actions to specific sites while avoiding production of waste, which is bioenergetically costly to eliminate or recycle. Formation of the latter would seem inevitable if NO was to reach its target simply by random diffusion. All three substances have in common that they are very small, interact with other biomolecules (in particular metals and hemes, although this does not necessarily translate into a change in biological activity), were known as atmospheric pollutants and toxic entities long before their endogenous production was established, and that lower rates of formation can protect tissues from damage. In the case of NO and CO this has been shown to result in either enzyme activation or inhibition. Although slow in the absence of catalysts, NO and $H_2S$ also react with oxygen ($O_2$) to form an array of metabolites

with distinct biological properties and chemical reactivities. The availability of NO is dependent on the relative rates of NO formation and trapping by oxygenated hemes, co-generated reactive oxygen species and perhaps $H_2S$. Thus, the formation of secondary reactive nitrogen oxide species such as peroxynitrite ($ONOO^-$), nitrogen dioxide ($NO_2$), and dinitrogen trioxide ($N_2O_3$) all depends on the relative flux rates of generation of individual reactants, nearby antioxidant enzyme expression/activity, and even the presence of carbon dioxide/bicarbonate ($CO_2/HCO_3^-$). As whether this wasn't complicated enough already, there is ample cross-talk between many of these species at multiple levels, including direct chemical interactions leading to formation of new reaction products, reciprocal interaction at the functional level affecting production and metabolism of either signalling entities, and other modulatory processes due to post-translational modification of proteins involved in down-stream signalling.

It has been known for some time that NO can react with thiols to form S-nitrosothiols. This editorial is neither the place to discuss the various reactions that account for the formation of those products in biology nor to get into trouble by citing the wrong papers/group that made important contributions to this or that area first, but one thing seems to be clear: the post-translational modification of critical protein sulfhydryl groups by S-nitrosation is becoming increasingly recognized as an important means of regulating cellular activity, perhaps similar in significance for the tonic actions of NO as stimulation of soluble guanylyl cyclase activity and inhibition of mitochondrial function is for its acute effects. While $H_2S$ may be seen by some as "just another" transmitter, it also happens to be the smallest reduced thiol with a unique chemical reactivity. So, no wonder it cross-talks with NO at multiple levels. Thiolates are among the most nucleophilic reactants in cells and heavily invested in governing protein structure and function. It is perhaps not surprising that thiol reactivity can be modified in many other ways, including sulfhydryl oxidation, nitrosation, nitration, thiolation, and guanylation. Add to this the possibility that multiple sulphur-oxy species and polysulfides may be formed that can react either with NO directly or one of its metabolites, and it becomes obvious why it can become challenging to keep track of what exactly is happening in biological systems at the molecular level.



At the beginning, everything looked relatively straigthforward: $H_2S$, formed by two enzymes of the trans-sulfuration pathway, either inhibited complex IV of the respiratory chain, leading to cellular "power failure", or elicited its biological effect through modification of $K_{ATP}$ channel activity. Soon thereafter, however, observations appeared in the literature that described either enhanced release or inhibition of NO, involvement of nitroxyl, sulfhydration (formation of a mixed disulfide with SH, giving rise to perthiols) and other ways of tweaking cellular activity levels. Recent studies have even shown that $H_2S$ can be a substrate in the respiratory chain, donate electrons for ATP synthesis and protect mitochondrial integrity. Moreover, we now know of several other pathways that can give rise to $H_2S$, yet how exactly $H_2S$ exerts its biological action has become far less clear. To be frank, in most cases we don't even know which molecular entity mediates its biological effects. Since at physiological pH the majority of it is present in the form of the hydrosulfide anion ($HS^-$), it may well be that this powerful nucleophile, and not its conjugate acid $H_2S$, is the major carrier of biological activity (which, if true, would probably disqualify it from being a "gasotransmitter"). To this end it is interesting to note that polysulfides are increasingly gaining attention as possible signalling entities in their own right - time will tell which sulfur species accounts for the majority of the biological activity of "$H_2S$".

NO research has greatly benefitted from the early mechanistic elucidation of its biosynthetic route, the identification of the three isoenzymes of the NO-synthase family, and the availability of specific NOS inhibitors. The early years were characterised by the excitement of identifying one system after another to be under tonic control of NO. In the early days of NO research, it was often sufficient to monitor what happens following application of a NOS inhibitor to a given biological system and demonstrate partial/full reversal of whatever effect observed following NO replenishment by a NO-donor to produce a paper. In part this was possible because so many biological processes are under control of NO, one way or another. Clearly, those days are long gone, but the $H_2S$ field never had the opportunity to use a single enzyme inhibitor to probe for the involvement of $H_2S$ in a given mechanism/cell signalling pathway, and many of the inhibitors available are either not very potent or rather unspecific.

NO research has also benefitted from a thorough systematic assessment of its biological chemistry in the past two decades. By comparison, the chemical biology of $H_2S$ (or maybe (poly)sulfide?) is still in its infancy. Like many years before in the NO field, there is a lack of sufficiently sensitive and specific analysis techniques around to reliably quantify the different sulfur species in biological systems in order to get a proper handle on the signalling process. That kinetics of release and consumption are important for availability has been appreciated early on in the NO field, but does not seem to be common knowledge in the $H_2S$ field. Several $H_2S$ donors have been synthesized, but only few reports have appeared that compared their biological effects with those of simple sulfide salts. Several new probes for $H_2S$ have been developed recently, but no good scavengers have emerged yet. There is a lot to do, and we are likely to witness a couple of surprises along the way.

By comparison, research in the field of CO biology appears to be more straightforward. For a start, CO is not redox active, which helps simplifying matters. The biological activity of CO is largely dominated by metal coordination chemistry (classic organometallic chemistry with established model systems) and its production from heme is subject to less complicated fine-regulation of cofactor availabilities etc. Yet, we may be up for a surprise in this area of research, too, as new tools such as slow CO-releasing molecules (comparable to NO-donors and $H_2S$/sulfide donors in the other

two fields) and CO-sensitive probes are becoming available. While there are several ways of inhibiting and upregulating heme oxygenase activity/expression to the best of our knowledge no CO-scavengers are available in our pharmacological tool box. Also, rumour has it that this field is in desperate search for an endogenous biological target now as soluble guanylyl cyclase does not seem to be it.

Small molecule transmitters might be seen as lacking in selectivity as larger molecules (such as hormones) owe their selectivity largely to their three-dimensional structure. However, with NO and CO Nature has chosen two small molecules that, except when required, are curiously unreactive. In particular NO is a radical, yet an unusually unreactive one. By and large, NO only reacts with other radicals and with metals, CO with metals, and $H_2S$ …well, we have to wait and see.

While the NO field has matured over the years, significant challenges remain. Among other things, this relates to the chemistry that governs its biological signalling, and this is where the cross-talk between these three "gasses" comes into play. In order to fully understand how NO elicits its many effects in a targeted fashion we will need to untangle the rich chemical cross-talk between different molecular entities belonging to each of these pathways. To make real progress in this field now we require more detailed insight into how the effects of NO depend on the two other systems (and perhaps further ones to be discovered in the future), how to therapeutically manipulate it for patient benefit, improv stress tolerance of plants, or whatever your particular area of research may be. This is why the Editors of this journal have decided it was time to broaden its scope to include sulfide and CO. As the science evolves, so should we and our journal.

Thus, in addition to sending your best work on NO to this journal, from now on we wish to invite papers from the adjacent $H_2S$ and CO fields to become the premier outlet for publication of research on the chemistry and biology of NO, Sulfide and CO. The biology of these three messengers appears to be so deeply intertwined that it will be difficult, if not impossible, to appreciate how NO works without understanding how the other two operate. Stay tuned, it starts happening in this volume with two reviews by the groups of Jack Lancaster[1] and Chris Kevil[2].

In the first article, Li and Lancaster take us through the perilous and often misunderstood field of sulfide chemistry. This article is a must read for anyone entering (and many of us active in) this field as it provides practical guidelines with which to appreciate synthesis and degradation of $H_2S$ as well as understand possible interactions with other signaling molecules and interactions between $H_2S$ and downstream effector (receptor?) mechanisms. The authors point out that there is clearly much more to be learned about sulfide chemistry and provide a number of interesting avenues for future research. We also learn that the correct IUPAC nomenclature for $H_2S$ is "sulfane", although it is difficult to imagine this in our lexicon in the foreseeable future. In the second article, Kolluru *et al*. discuss the methods for analyzing $H_2S$, and provide an overview of $H_2S$ physiology. They point out that many of the commonly applied methods used to measure $H_2S$ in blood and tissues are associated with substantial artifact and result in unrealistically elevated concentrations. Although this has been pointed out before on numerous occasions, this fact is inexplicably disregarded by many active investigators and certainly cannot be overstated. The authors also provide a good summary of many of the proposed physiological systems in which $H_2S$ has been implicated and an overview of sulfide's interactions with other molecular species. These two papers provide a practical

framework for investigators entering this field as well as a timely reminder to those of us who are already in it. Enjoy.

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