An overview of the biological significance of endogenous gases: new roles for old molecules

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Abstract

Biologically active gases that occur naturally in the body include nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H_2S). Each of these molecules is synthesized by enzymes which have been characterized biochemically and pharmacologically, and each acts, via well-established molecular targets, to effect physiological and/or pathophysiological functions within the body. Major biological roles that appear to be common to all three gases include the regulation of vascular homoeostasis and central nervous system function. It is becoming increasingly clear that both the synthesis and the biological activity of each gas are, to some extent, regulated by the presence of the others, and as such it is necessary to consider these molecules not in isolation but acting together to control cell function. Additional, more speculative candidates for gaseous cell signalling molecules include ammonia, acetaldehyde, sulfur dioxide and nitrous oxide. Whether such molecules also play a role in regulating body function remains to be determined.

Introduction

The last two decades or so have seen a marked escalation in interest in the biology of endogenous, biologically active gases. Although the most significant of these is probably still O_2, the role of this particular gas (plus related chemical species such as superoxide/hydroxyl radicals and H_2O_2) as a signalling molecule has been extensively reviewed elsewhere [1,2] and will not be discussed here. Instead, we intend to concentrate in the present review on the more recent discovery of gases which are both synthesized naturally in the body and which produce an array of disparate biological effects. While few would argue that the advent of the modern day era of 'gas biology' commenced with nitric oxide (NO) in the 1980s, it has become increasingly clear in recent years that NO is not the only such biologically active gas and that other molecules such as carbon monoxide (CO) and, more recently, hydrogen sulfide (H_2S) are also important. Indeed, cells are constantly enveloped in and thus exposed to a large number of different gases and, consequently, it can be no great surprise that many of those gases will have roles in the regulation of cell function (Figure 1). With this in mind therefore, the aims of this review are to provide readers with (i) a brief outline of the biology of NO, CO and H_2S, (ii) a discussion of the evidence that suggests that these three gases work together to regulate biological function and (iii) some necessarily speculative comments on the existence of additional biologically active gases in the body.

Key words: acetaldehyde, ammonia, carbon monoxide, endogenous gas, hydrogen sulfide, nitric oxide.

Abbreviations used: CBS, cystathione β-synthetase; CSE, cystathionine γ-lyase; HO, haem oxygenase; K_{ATP}, ATP-gated potassium channel; LPS, lipopolysaccharide; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; iNOS, inducible NOS; sGC, soluble guanylate cyclase.

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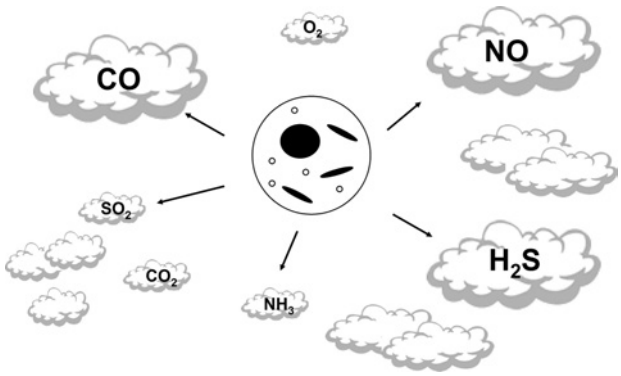
NO, CO and H_2S: similarities and differences

NO, CO and H_2S have many features in common. Apart from being gases at atmospheric pressure, they all are soluble in water to a greater or lesser extent and all are able to freely penetrate cell membranes. Of the three, NO is the only free radical, and as such the most chemically reactive, interacting, for example, with accessible thiol groups in amino acids and proteins to form RSNOs (relatively stable nitrosothiol compounds) [3]. At one time or another, all three of these gases were considered solely as environmental toxicants and crude metabolic poisons, which indeed they are. H_2S, for example, is more toxic than hydrogen cyanide and exposure to as little as 300 p.p.m. in air for just 30 min can be fatal in humans. It is therefore not surprising that little attention was paid to the human biology of these gases prior to the 1980s.

The turning point in 'gas biology' came with the realization that such gases are generated naturally within mammalian cells as illustrated in Figure 2. Thus NO and H_2S are synthesized from L-arginine (by a two-step oxygen-requiring reaction involving L-N^G-hydroxyarginine as an intermediate and with concomitant citrulline production) and from L-cysteine respectively [4,5]. In turn, CO is generated by ring opening of protohaem IX [6]. In each case, multiple enzyme isoforms have evolved to generate these signalling molecules. Thus NO is synthesized by calmodulin-dependent NOS (nitric oxide synthase) (types 1, 2 and 3) [7], while H_2S generation is achieved by several pathways, mainly the pyridoxal phosphate-dependent enzymes, CSE (cystathionine γ-lyase) and CBS (cystathione β-synthetase) [8]. In turn, CO is formed by a specific HO (haem oxygenase) enzyme, which exists as three isoforms, i.e. HO-1, HO-2 and HO-3 [9]. These synthesizing enzymes can be subclassified into either constitutive (NOS1, NOS3 and HO-2) or inducible by inflammatory or other, often 'disease-based' influences (NOS2

Figure 1 | The gaseous environment of mammalian cells



and HO-1). For example, HO-1 is induced by many factors (e.g. hypoxia, heat shock and endotoxin) all of which promote oxidative stress [10]. As such, it seems likely that HO-1 expression in cells/tissues provides an antioxidant mechanism aimed at countering this stress. The exact role of HO-3 is yet to be defined, but so far this enzyme has been identified in the brain and produces relatively meagre amounts of CO.

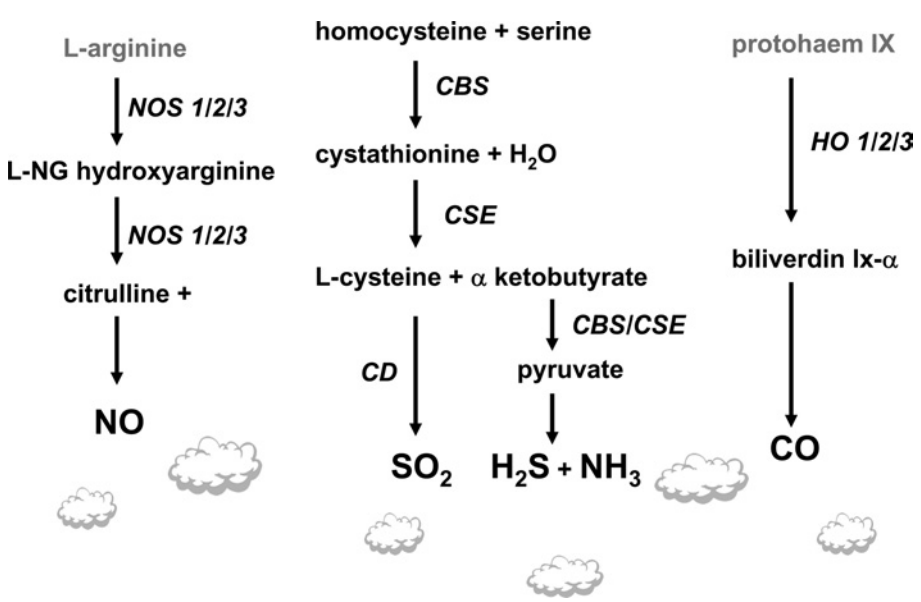
The study of H_2S-synthesizing enzymes is at a somewhat earlier stage than either NOS or HO and thus a more detailed consideration is warranted. Both CSE/CBS occur naturally in many tissues with CSE largely concentrated in the vasculature and CBS chiefly localized in the brain. It is now clear that brain CBS (like NOS) activity is both calcium- and calmodulin-dependent [11], suggesting that 'short-term' control of neuronal H_2S (and indeed NO) production might be achieved by influx of Ca^{2+} into neurons following depolarization. Possible regulatory control of CSE expression has also been suggested. Thus CSE is induced following exposure to LPS (lipopolysaccharide) [12] or in animal models of diseases such as Type 1 diabetes mellitus [13] and pancreatitis [14]. HO enzymes and NOS3 are membrane-bound, while the other enzymes referred to above are all cytoplasmic.

Within the cell, NO, CO and H_2S share a number of molecular targets. All of these gases potently interact with metals/metalloproteins, notably the haem moiety. For example, all are potent inhibitors of mitochondrial cytochrome *c* oxidase and this effect may partly underlie their pronounced toxic effects [15,16]. From the pharmacological viewpoint, the major molecular target for both NO and CO is sGC (soluble guanylate cyclase) [17]; sGC is activated by these gases leading to an increase in intracellular cGMP concentration, which in turn regulates a number of downstream transduction pathways. H_2S does not affect sGC or cGMP concentrations intracellularly although there have been reports that it increases cAMP, especially in neurons [18]. However, the major molecular target for H_2S is likely to be the K_{ATP} (ATP-gated potassium) channel, which when activated leads to relaxation of smooth muscle [19].

The principal biological effects of NO include, but are not restricted to, dilation of blood vessels, inhibition of platelet and leucocyte adhesion to the vessel wall, neurotransmitter roles in the peripheral nervous system [as a NANC (non-adrenergic, non-cholinergic) neurotransmitter in various smooth muscles] and the central nervous system [mediating/moderating particularly the effects of glutamate on NMDA (*N*-methyl-D-aspartate) receptors with concomitant effects on synaptic plasticity including learning and memory]. Numerous detailed reviews on the physiological and pathophysiological significance of NO are available and readers are referred to these for more comprehensive information [20,21].

Figure 2 | Pathways for the biosynthesis of gaseous mediators

CD, cysteine dioxygenase.



Like NO, CO is also an important signalling molecule. Many of the biological effects of CO are similar to NO. These include effects on platelet aggregation and as a neurotransmitter. CO also inhibits proliferation of vascular smooth muscle cells and has anti-apoptotic activity. Once again the reader is referred to the many excellent reviews detailing the biological significance of CO for further information [22,23].

H_2S is the latest molecule that has been touted as the third 'signalling gas'. Less is known of the biology of H_2S but it does relax vascular smooth muscle *in vitro* and *in vivo*, most probably by opening vascular smooth muscle K_{ATP} channels as stated above. Unlike NO, it is not clear whether H_2S plays any role in the control of blood pressure or vascular perfusion in health or disease. However, reduced expression of CSE was observed in lungs along with a decrease in plasma H_2S in rats with experimentally induced hypoxic pulmonary hypertension [24] and in spontaneously hypertensive rats [25], suggesting that H_2S deficiency can predispose to vasoconstriction and perhaps hypertension. Using similar lines of reasoning, CSE induction and excessive H_2S generation may contribute to the hypotension associated with both septic (caecal ligation and puncture) [26] and LPS-induced (endotoxic) shock [12]. Thus, while the physiological role of H_2S in cardiovascular homoeostasis is yet to be defined, there is growing evidence that deranged metabolism of this gas may contribute to vascular disease.

The presence of CBS, coupled with the identification of physiologically relevant amounts of H_2S in the brain, has also suggested a role for this gas in central nervous system function. H_2S increases cAMP levels in neuronal and glial cell lines and primary neuron cultures and also hyperpolarizes CA1 and dorsal raphe neurons, most probably by activating K_{ATP} channels. In addition, H_2S interferes with glutamatergic neurotransmission. For example, both direct electrical stimulation and glutamate application increased H_2S production from mouse cerebral cells, and sodium hydrosulfide (NaHS; H_2S donor) facilitated hippocampal LTP (long-term potentiation) by increasing the sensitivity of NMDA receptors following a rise in intracellular cAMP [18]. Although more work needs to be done, these results raise the possibility that, like NO and CO, H_2S may fulfil some type of transmitter/modulator role in the central nervous system.

Interaction between NO, CO and H_2S: one gas alone or a gaseous syncytium?

It is becoming clearer that NO, CO and H_2S interact with each other at many levels. One important point of interaction is the iron (Fe) protoporphyrin haem component of sGC. NO binds to Fe of the prosthetic haem to form a 5-co-ordinated nitrosyl haem complex leading to conformational changes in the enzyme and a 100-fold or more increase in cGMP generation [27]. In contrast, CO forms a 6-co-ordinated haem complex, presumably triggering lesser conformational changes in the enzyme and much reduced cGMP accumulation. The net effect of this particular molecular interplay is that CO activates sGC only when the NO concentration is relatively low but, at higher ambient NO concentrations, CO blocks the effect of NO on sGC activation.

The effect of H_2S on the response to NO is complicated. Some authors report that H_2S enhances the vasorelaxant effect of NO [28], while others have shown that H_2S reduces such activity [29] perhaps by chemically reacting with NO to form an, as yet, unidentified nitrosothiol moiety [30]. To make matters worse, H_2S can also augment NO release from incubated S-nitrosothiols.

Recent work has concentrated more on the way in which each of these gases affect the expression of their synthesizing enzymes. Several years ago, it was noted that NO donor drugs enhanced H_2S production and increased CSE expression in cultured smooth muscle cells [31]. Conversely, H_2S inhibits NO generation and iNOS (inducible NOS) expression in LPS-exposed macrophages by a mechanism that appears also to involve up-regulating HO-1 [32]. Intriguingly, H_2S administered to rats with experimentally induced hypoxic pulmonary hypertension led to an increase in plasma CO concentration and an increase in pulmonary artery expression of HO-1 protein and mRNA [33].

As yet we do not have a good grasp of how NO, CO and H_2S interact and work together in the body. However, it is abundantly clear, even from this brief description, that any interaction between these gases is going to be multifaceted and complex. Since many cells have the ability to make all three gases (and maybe even more, see below), the key to a full understanding of gas biology probably lies in the synergisms and antagonisms between these molecules.

Additional gaseous mediators: new roles for old gases in health and disease?

The possibility that other gaseous mediators, in addition to NO, CO and H_2S, may occur naturally in the body is speculative but certainly worthy of discussion. Potential candidate molecules include ammonia (NH_3), acetaldehyde, sulfur dioxide (SO_2) and perhaps even nitrous oxide (N_2O).

Ammonia is formed naturally by de-amination of a number of endogenous molecules and occurs in micromolar concentrations in human blood. Pharmacologically, high concentrations of ammonia dilate cerebrovascular smooth muscle [34]. The mechanism of this effect is unclear but intracellular alkalinization is likely to play a role [35]. Hyperammonaemia has also been suggested to trigger expression of iNOS in astroglial cells [36] and, like NO and H_2S, ammonia may be a contributing factor in Alzheimer's disease [37]. Intriguingly, supplemental glutamine (a precursor for ammonia) prevents the intestinal hyperpermeability and bacterial translocation associated with sepsis and the development of MODS (multiple organ dysfunction). Indeed, glutamine is of value in patients suffering from trauma and after surgery. Whether any of the biological effects of glutamine reflects increased biosynthesis of ammonia is certainly very speculative but worthy of further investigation.

Acetaldehyde is generated by alcohol dehydrogenase. It occurs naturally in blood (low concentrations) and relaxes

isolated blood vessels [38] possibly by an action on calcium channels. Interestingly, acetaldehyde is reportedly broken down by vascular endothelial cells [39]. Again, whether acetaldehyde is synthesized in sufficient amounts in the body to exert biological functions remains to be seen.

Less is known about sulfur dioxide (SO_2). This toxic gas can be generated from sulfite and is a vasodepressor in the rat [40] with the ability to increase neutrophil adhesion to cultured pulmonary epithelial cells [41]. The big question about SO_2 is whether this molecule can be synthesized naturally in the body. In this context, SO_2 (along with an analogous compound, carbonyl sulfide) was recently detected in porcine coronary arteries by GC/MS [42].

N_2O is formed in the test tube (at least) by the interaction between NO and thiols. This reaction may occur biologically in that cytosol from rat hepatocytes forms N_2O when provided with NO [43]. It is not known whether N_2O production occurs naturally in the body under physiological or pathophysiological conditions. N_2O has been used as a general anaesthetic for well over a hundred years and, as such, it is not surprising that it affects neuronal function. Thus N_2O inhibits glutamatergic transmission possibly by acting as an NMDA receptor antagonist. Interestingly, N_2O also increases endogenous homocysteine concentrations (a substrate for H_2S) synthesis in lymphocyte cell cultures, which may perhaps suggest some effect on endogenous H_2S metabolism.

This review seeks to shed some light on the increasingly complex role played by 'gases' in the body. It is becoming clearer that mammalian cells synthesize a range of what were previously considered to be 'mere' environmental toxins but which now must be considered biologically important molecules. Bearing in mind the clear interaction between these gases, we propose that further studies should be directed towards a better understanding of how these gases interact with each other.

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