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Gasotransmitter delivery via self-assembling peptides: Treating diseases with natural signaling gases☆

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ABSTRACT

Nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) are powerful signaling molecules that play a variety of roles in mammalian biology. Collectively called gasotransmitters, these gases have wide-ranging therapeutic potential, but their clinical use is limited by their gaseous nature, extensive reactivity, short half-life, and systemic toxicity. Strategies for gasotransmitter delivery with control over the duration and location of release are therefore vital for developing effective therapies. An attractive strategy for gasotransmitter delivery is though injectable or implantable gels, which can ideally deliver their payload over a controllable duration and then degrade into benign metabolites. Self-assembling peptide-based gels are well-suited to this purpose due to their tunable mechanical properties, easy chemical modification, and inherent biodegradability. In this review we illustrate the biological roles of NO, CO, and H_2S, discuss their therapeutic potential, and highlight recent efforts toward their controlled delivery with a focus on peptide-based delivery systems.

Contents

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1. Introduction

Nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S) were all discovered in the late 1700s, and all were regarded for centuries solely as toxins and environmental hazards. Joseph Priestly, discoverer of many gases including oxygen, was the first to isolate both NO and CO, while Carl Wilhelm Scheele is credited with discovering H_2S. Although Scheele was the first to isolate H_2S, mixing iron sulfide with mineral acid to generate "stinking sulfurous air" [1], its toxic effects had been described even earlier [2]. H_2S toxicity is now understood to be a result of cytochrome *c* oxidase inhibition and subsequent mitochondrial poisoning [3]. The toxicity of NO and CO were confirmed later—NO through its ability to form reactive oxygen species, such as nitrogen dioxide and peroxynitrite [4], and CO through its ability to bind hemoglobin [5,6], inhibiting oxygen transport.

Perhaps because of their notorious toxicities and sordid histories, the possibility that NO, CO, and H_2S could play biological roles was dismissed for many years, despite evidence that these gases were produced in mammalian tissues. NO was the first to be recognized as a biological mediator. In late 1970s, Murad showed that NO stimulates soluble guanylate cyclase (sGC) to increase 3′,5′-cyclic guanosine monophosphate (cGMP) production [7]. Later, Furchgott discovered the enigmatic endothelium-derived relaxing factor (EDRF) present in vascular endothelial cells [8], and in 1987 Moncada and Ignarro separately showed that the NO was the primary EDRF [9,10]. Research into NO biology was the basis for the Nobel Prize in Physiology or Medicine in 1998, shared by Ignarro, Furchgott and Murad. Since then, the biological roles of NO have been widely studied, and its therapeutic potential continues to rise [11].

While endogenous production of CO in the mammalian body was first noted in 1950 [12], a physiological role for CO was not discovered until the early 1990s. In 1991, Marks predicted that CO, known then to be produced endogenously by heme catabolism, could mediate physiological functions through binding to the heme of sGC [13]. Two years later, Verma published the first report of CO as a neurotransmitter [14]. Now CO is recognized as a signaling molecule that regulates neurotransmission, relaxes blood vessels, and inhibits platelet aggregation, among other roles [15,16].

The discoveries of NO and CO as signaling molecules suggested a possible physiological role of endogenous H_2S and encouraged its further study. Enzymes that generate H_2S in mammalian cells have been known since the 1950s [17–19], but it was a landmark 1996 paper that established H_2S as a signaling gas [20]. In this work Kimura suggested that H_2S is a neuromodulator based on evidence that H_2S facilitates the induction of long-term potentiation in the hippocampus. In addition, he established that endogenous H_2S relaxes smooth muscle cells in synergy with NO [21]. In 2003 the first molecular target of H_2S in the cardiovascular system was discovered by Wang [22]. Unlike NO, which dilates blood vessels by activating sGC, H_2S specifically targets K_{ATP} channels to relax smooth muscle cells. Further studies have shown that H_2S works both alone and in synergy with NO and CO in cell signaling and regulation in many organs and systems throughout the body.

The physiological roles of NO, CO, and H_2S have been studied for a relatively short period compared to other signaling agents, but vital biological processes mediated by these gases continue to be discovered. Establishing their physiological roles, determining their mechanisms of action, and developing therapeutic strategies that rely on these gases all require chemical tools to generate and study them. The physiological roles and therapeutic benefits of NO have been studied for much longer than those of CO and H_2S, but these last two gasotransmitters are catching up quickly. Efforts to develop new donors of all three gases are primarily focused on small molecules, but endeavors to develop materials for gasotransmitter delivery continue to gain strength. These include polymers, hydrogels, inorganic/organic hybrids, and other materials that release NO, CO, or H_2S. Advantages of gasotransmitter delivery from materials, discussed in depth below, include controllable duration of release, the capacity for targeting specific organs, and the ability to localize release by injection or implantation of the material. Substantial progress has been made in this area in just the past 5–10 years, particularly in peptide-based materials for gasotransmitter delivery. These materials, which are gels based on self-assembled short peptides, are particularly attractive for drug and gas delivery due to their biodegradability and lack of toxicity, making them broadly useful in bioengineering, regenerative medicine, and tissue engineering [23,24]. We review here the basics of gasotransmitter biology, highlight several methods for gasotransmitter delivery, and finally discuss peptide-based materials and their use as delivery systems for NO, CO and H_2S.

We begin in Section 2 with a discussion of the main physiological roles and therapeutic potential of NO, CO, and H_2S. Section 3 deals with the main methods for delivery of these gases, including inhalation therapy, small molecule donors, and materials. A detailed discussion of all of the delivery methods is beyond the scope of this review, but we cover the advantages and disadvantages of each method. In Section 4 we introduce peptide-based materials and discuss their uses and general features. Section 5 covers peptide-based materials for gasotransmitter delivery as well as materials that respond to gasotransmitters.

2. Gasotransmitters: NO, CO and H_2S

Once solid evidence for the signaling capacity of H_2S had been established, it became clear that a term was needed to collectively define the three known signaling gases. The term gasotransmitter was coined in 2002 to describe NO, CO, H_2S, and any other gases that have similar characteristics [25]. A gasotransmitter is defined as a gas that:

- Is endogenously produced
- Is freely permeable to membranes
- Has specific cellular and molecular targets
- Has defined physiological functions

In addition, exogenous administration of these gases must mimic the natural functions of the gas for a substance to be defined as a gasotransmitter. Other gases may also be classified as gasotransmitters in the future [26], but only NO, CO, and H_2S are currently known to meet all of these requirements.

2.1. Biological and therapeutic roles of NO

NO is a free radical with a short half-life (seconds) in biological systems. As a result, it must be produced quickly as needed in the body. NO is produced endogenously from L-arginine and oxygen via a family of enzymes called nitric oxide synthases (NOSs) (Fig. 1). Three isoforms of the NOSs have been identified: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). Each enzyme acts to generate NO under different conditions. Consistent with early discoveries, the main actions of NO are mediated through its binding and activating sGC. NO diffuses through lipid bilayers and activates sGC by binding to its heme unit. The best-characterized isoform of sGC, $\alpha1/\beta1$, is activated by nM concentrations of NO, and the resulting complex catalyzes the conversion of guanosine-5′-triphosphate (GTP) into cGMP. The signaling cascade follows with cGMP activating target proteins including various protein kinases. Finally, cGMP is degraded by phosphodiesterases. The cascade (NO → cGMP → kinase activation) provides a large amplification of NO signaling, leading to various downstream outputs [27]. For example, endothelial cells (ECs) that line the entire surface of the circulatory system produce NO by the action of eNOS. Through the signaling cascade described above, the released NO leads to relaxation of smooth muscle cells in the media layer of the blood vessel, with the ultimate effect of blood vessel dilation [11].