Signal Transduction and the Gasotransmitters

SIGNAL TRANSDUCTION AND THE GASOTRANSMITTERS

NO, CO, and H_2S in Biology and Medicine

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DEDICATION

To Lily, Jennifer, Jessica, and Clover:

You are my source of inspiration.

Rui Wang, *MD, PhD, FAHA*

FOREWORD

The current era of biological investigation is among the most transformational in history. The genuine emergence of genomic strategies and the rapid follow-on of proteomic and information technologies have provided scientists with unprecedented opportunities for discovery. The frontiers of knowledge are simply falling back. With this amazing revolution in understanding of the molecular underpinnings of cellular, tissue, and organismic homeostasis, a greater appreciation for the complexity of signals, networks, and linkages has crystallized. Regulation of biological functions rests not only with transcriptional, translational, and post-translational modifications of proteins, but also in the orchestral harmony of ligands and receptors, cell adhesive systems, cytoskeletal organization, ion channel function, membrane dynamics, and a range of transmitters.

Typically, transmitters have been categorized as those participating in neural functions or as humoral amines. In *Signal Transduction and the Gasotransmitters: NO, CO, and H_2S in Biology and Medicine*, Dr. Rui Wang and the sterling group of contributors he has assembled provide a paradigm-shifting assessment of the new category of transmitters, the gasotransmitters. Although nitric oxide was discovered approximately 20 years ago, it has only recently been appreciated that this famous molecule is among a whole group of substances that play critical roles in cell signaling and regulation, arising either environmentally or endogenously. These diverse molecules include, but are not limited to, nitric oxide, carbon monoxide, and hydrogen sulfide. Considering the now-identified roles of these three gasotransmitters in physiology and toxicology, it is understandable that the contributions are accordingly organized in sections corresponding to each. The origin, quantities, and interactions among these transmitters determine their impact on ionic fluxes, the excitability of muscle and nerve, and metabolism. There is an interesting and perhaps not surprising range of availability of any given gasotransmitter that conveys either physiological benefit or toxicological adversity, even when the gases arise endogenously. Individual chapters clearly frame the spectrum of their disease-related and physiological roles.

Like all nascent fields of study, it is often difficult to predict the full magnitude of importance of certain discoveries. Although the discovery of the role of nitric oxide in biological function deserved the Nobel Prize, and nitric oxide is now known to be a pivotal molecule in many organ systems, it is tempting to speculate that knowledge of the role of endogenous gases in a broader scale, especially as it relates to the homeostatic balancing act or that of other species, is barely coming into its own. Dr. Wang is to be congratulated on bringing the subject of gasotransmitters into coherence. I believe that biologists from many fields will welcome the knowledge that is captured here.

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PREFACE

The endogenous production and physiological function of many gaseous molecules including, but not limited to, nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S), have been increasingly recognized in recent years. These gaseous molecules, defined as gasotransmitters, share common chemical features and biological action modes, but distinguish themselves from classical neurotransmitters and humoral factors. The concept of gasotransmitters has found its application across a wide spectrum of biological systems. Recent advances in the novel and challenging field of gasotransmitter biology and medicine—encompassing biomedical and clinical issues, health services, and population health studies—are dazzling. Gasotransmitters are important endogenous signaling molecules. Among many cellular and molecular targets of gasotransmitters, membrane ion channels are the key signal transduction link regulated by gasotransmitters. The regulation of ion channels by gasotransmitters can result from the activation of different second messengers or the direct interactions between gasotransmitters and ion channel proteins. The latter is a novel mechanism and has attracted great attention from researchers in every field of biomedical studies.

Many books have been published that focus on neurotransmitters and other classical signal transduction pathways. *Signal Transduction and the Gasotransmitters: NO, CO, and H_2S in Biology and Medicine* reviews the biology and medicine of gasotransmitters with an emphasis on signaling transduction mechanisms in general, and ion channel regulation in particular. Following an account of the historical evolution of the gasotransmitter concept, the endogenous metabolisms of gasotransmitters and their regulation, the comparison of the toxicological profiles and biological actions, and interactions among gasotransmitters in terms of their production and effects are discussed. The physiological roles of NO, CO, and H_2S in the regulation of cardiovascular, neuronal, and gastrointestinal systems, as well as of cell metabolism are reviewed. The interaction of gasotransmitters with K_{Ca} channels, KATP channels, voltage-gated Ca^{2+} channels, voltage-gated Na^+ channels, and cyclic nucleotide-gated ion channels are presented. Included in the array of different mechanisms for the interaction of NO, CO, and H_2S are channel phosphorylation, *S*-nitrosylation, carboxylation, sulfuration, and altered cellular redox status. Guidance and suggestions can be found for exploring and characterizing lesser known gasotransmitters.

Signal Transduction and the Gasotransmitters: NO, CO, and H_2S in Biology and Medicine should serve as a summary and a standard reference source concerning signal transduction mechanisms underlying the physiological functions of gasotransmitters. Clinical scientists and physicians as well as other professional health workers should be excited by the advances in gasotransmitter research described in this book. The authors hope that scientists from both basic biology and health science disciplines find this book useful, interesting, and inspiring.

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I GASOTRANSMITTERS: PAST, PRESENT, AND FUTURE

1 The Evolution of Gasotransmitter Biology and Medicine

From Atmospheric Toxic Gases to Endogenous Gaseous Signaling Molecules

Rui Wang

CONTENTS

SUMMARY

Overproduction of many atmospheric gases, from natural resources and anthropogenic activities, impose a serious environmental concern with adverse health effects. Among pollutant gases are nitric oxide (NO), carbon monoxide (CO), and hydrogen sulfide (H_2S). Over several decades, studies from numerous laboratories have demonstrated that gases such as NO, CO, and H_2S not only are generated in the human body but also play important physiological roles. These particular gases share many common features in their production and function but carry on their tasks in unique ways, which differ from classic signaling molecules, in the human body. Collectively, these endogenous molecules of gases or gaseous signaling molecules compose a family of "gasotransmitters." The regulation of ion channels by gasotransmitters, either directly via chemical modification of ion channel proteins or indirectly via second messengers, exerts significant influence on cellular functions. *S*-nitrosylation, carboxylation, and sulfuration may represent mechanisms of direct interaction of NO, CO, and H_2S with ion channel proteins, respectively.

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This chapter summarizes the history and evolution of the concept of the gasotransmitter and outlines the criteria used to identify novel gasotransmitters. Gasotransmitter research is accelerating into the next phase. Many new gasotransmitter candidates are being investigated. Alterations in the metabolism and functions of gasotransmitters under different pathological conditions are being explored, which may shed light on the pathogenesis and management of many diseases. Thus, research on gasotransmitters is certainly as important to clinical practice and community health as it is to basic research, if not more so.

Key Words: Gasotransmitter; nitric oxide; carbon monoxide; hydrogen sulfide; signal transduction.

"Air is a physical substance; it embraces us so intimately that it is hard to say where we leave off and air begins. Inside as well as outside we are minutely designed for the central activity of our existence—drawing the atmosphere into the centre of our being, deep into the moist, delicate membranous labyrinth within our chests, and putting it to use."—David Suzuki, The Sacred Balance

1. INTRODUCTION

Humans tend to treat atmospheric gases, such as oxygen, carbon dioxide (CO_2), nitrogen, carbon monoxide (CO), and hydrogen sulfide (H_2S), like sunshine and water—nature's gifts to us. Accompanying the arrival of the Industrial Revolution, the Third Wave is a high tide of natural gas production from industrial sources. In the public eye, most natural gases are nothing but toxicants, wastes, and pollutants, with oxygen as possibly the only exception. By definition, environmental toxicants are "agents released into the general environment that can produce adverse health effects among large numbers of people" *(1)*. Gas pollutants as environmental toxicants can induce both acute and chronic health hazards at societal as well as individual levels. The health hazards of these toxic gases become magnified in our public life. When this is coupled with public concern about the production of natural gases, it then becomes a health issue impacting both environmental and occupational health.

Scientists have worked with two schools of thought searching for the biological production and the physiological function of natural gases, be it detrimental or beneficial. One ancient frontier is the study of the biological production of gases. Archaea and microbes produce great amounts of gas, not only for their own use, but also for the necessity of life in their environment. Interestingly enough, these studies consistently demonstrate the production of numerous natural gases by microorganisms. For example, many bacterial types, such as Proteus vulgars, produce CO *(2)*. The biological production and utilization of H_2S have been best known for particular bacteria and archaea *(3)*. Human beings sit on top of the genomic life tree. Do we inherit or share any of these abilities from low forms of life to produce gases in our body? Plant life generates oxygen from light, a process of photosynthesis through the use of chlorophyll. Humans are not equipped in this way. However, our bodies do produce CO_2, ammonium, and other gases. The human body is often in this way treated as a pollutant when an analogy is drawn to the automobile or even a restaurant kitchen, which also generates useless gases, toxicants, or other types of harmful byproducts. The records of endogenous production of CO and H_2S in human tissues can be traced back hundreds of years. The human body can generate a myriad of gases with unknown functions—*the truth is still out there.* This body of knowledge, unfortunately, has not been completely used to facilitate the understanding of human physiology.

Scientists working in the second frontier—the physiological function of biological gases, a natural extension of the first frontier—brought about this revolution. In this regard, nitric oxide (NO), a pioneer gas, is doubtless the molecule of a new era. Over the last several decades, studies from thousands of worldwide laboratories have demonstrated that gases such as NO, CO, and H_2S are not only generated in humans but also have important physiological properties.

These gases share many common features in their production and function while carrying out their tasks in unique ways that differ from classic signaling molecules in the human body. Collectively, these endogenous molecules of gases, or gaseous signaling molecules, compose a family of "gasotransmitters," a nomenclature composed of "gas" and "transmitters."

This introductory chapter is devoted to discussing the conceptual transition of biological gases from toxic wastes and pollutants to important physiological gasotransmitters.

2. PRODUCTION AND HEALTH HAZARDS OF ATMOSPHERIC GASES

2.1. Nitric Oxide

Natural causes—lightening, forest fires, and organic decay—lead to the generation of oxides and nitrogen (NOx). Soil microorganisms also produce NOx. NO and N_2O are emitted from anaerobic soils by denitrifiers such as *Pseudomonas* spp. or *Alcaligenes* spp. and from aerobic soil by autotrophic nitrifiers such as *Nitrosomonas europaea (4)*. Motorized vehicles are the major mobile combustion source of NOx production. In 1994, one study showed that in a long, 7.5-km Norwegian road tunnel, with traffic flowing in both directions, the atmospheric NO_2 concentration exceeded the Norwegian air quality limits for road tunnels 17% of the time. When traffic was reduced through the tunnel, the mean NO_2 concentration was significantly lowered *(5)*. Stationary combustion sources of NOx include heat power plants and industrial factories *(6)*. Cigarette smoking generates a considerable amount of NO and NO_2 *(7)*. The biological treatment of nitrogen-rich wastewater with a high concentration of ammonium likewise yields NO and NO_2, although this might not contribute significantly to general environmental pollution with NOx *(8)*.

As the initial product of NOx from a reaction between nitrogen and atmospheric oxygen, NO quickly transforms to NO_2 either through simple oxidation involving molecular oxygen or through a photochemical reaction involving irradiation by sunlight. As a result, health hazards of atmospheric NO must be considered in conjunction with NO_2. Mercer et al. *(9)* found that after adult rats were exposed to 0.5–1 ppm of NO for 9 wk, the fenestration numbers in the alveolar septa of the lung increased more than 30-fold in the control rats, and 3-fold of NO_2 in the exposure group. The number of interstitial cells in the NO group was significantly reduced by 29%. Likewise, a significant reduction in the thickness of interstitial space was observed in the NO-treated rats, but not in the NO_2-treated rats, compared with the control rats *(9)*. Their study demonstrated that a low level of atmospheric NO exposure is more potent than NO_2 in producing interstitial lung damage. It is believed that most NO toxicity is mediated by the interaction of NO with superoxide producing peroxynitrite. This leads to oxidative damage to targeted cells and tissues. Epidemiological data often show controversial results on the adverse health effects of NO_2 *(6)*, partially because of the difficulty in determining the actual atmospheric NO_2 levels to which a specific portion of the population was exposed. Controlled animal and human studies provide evidence that high NO_2 levels weaken pulmonary

defense mechanisms and change human airway responsiveness. Lipid peroxidation *(10)* and protein oxidation *(11)* have been described as part of the cellular mechanisms of NO_2-induced health hazards. The most important and consistent conclusion is that exposure to high NO_2 concentrations may exemplify a specific health risk for a subpopulation of people with respiratory diseases, such as asthma and chronic obstructive pulmonary disease.

Over a 1-yr period, Giroux et al. *(12)* examined the correlation of acute myocardial infarction, atmospheric levels of NOx, temperature, and relative humidity. Among 282 patients with acute myocardial infarction, it was determined that the infarction area was reduced when the daily NO level in the atmosphere was higher than 13 $\mu g/m^3$ and the average daily temperature was lower than 13°C.

NO and NO_2 act as phytotoxic agents, damaging plant health as well. The growth of plants becomes poorer and productivity lower when exposed to high NOx levels *(13)*.

2.2. Carbon Monoxide

The toxicology profile of CO has been portrayed for hundreds of years. CO is among the most abundant air pollutants in North America. Because it is colorless, odorless, and noncorrosive, intoxication by CO is hard to detect, which earns CO the reputation of the "silent killer." A report in 1982 by the US Centers for Disease Control revealed that approx 4000 deaths and 10,000 cases of individuals requiring medical attention occur annually because of acute CO intoxication *(14)*.

All types of incomplete combustion of carbon-containing fuels yield CO. Natural processes such as metabolism and production of CO by plants and oceans release CO into the atmosphere. Oxidation of methane and nonmethane hydrocarbons by hydroxyl radicals and ozone, either natural or anthropogenic, is also a significant mode of CO production in the atmosphere. The most notable ways that humans contribute to the production of CO are the operation of internal combustion engines; the fueling of appliances with gas, oil, wood, or coal; and the disposal of solid waste. Cigarette smoking also produces a substantial amount of CO.

Whether an elevated environment of CO levels leads to human intoxication is influenced by the exposure and duration of pulmonary ventilation function, as well as the endogenous buffering capacity (i.e., the level of carbonmonoxy-hemoglobin A [HbCO A]), and the partial pressures of CO and oxygen. Acute ambient CO poisoning occurs as suddenly elevated CO concentration accelerates the binding of CO to normal adult hemoglobin (Hb) (Hb A), forming HbCO A. The formation of HbCO A impairs two functions of Hb. The oxygen storage function of Hb A is significantly reduced because the affinity of CO to Hb A is approx 250 times greater than that of oxygen *(15)*. The affinity of myoglobin to CO is approx 25-fold that of oxygen. The oxygen transportation function of Hb A is also reduced, because the release of oxygen from HbCO A to the recipient tissue becomes more difficult. CO binds to one of the four oxygen-binding sites of Hb A via the formation of a hydrogen bond between CO and the distal histidine residues of Hb A *(16)*. This binding, in turn, increases the affinity of oxygen to HbCO A. With tissue hypoxia being the major toxicological consequence of CO poisoning, the combination of CO with other heme-proteins, such as cytochrome P450, cytochrome-*C* oxidase, catalase, and myoglobin, may also in part account for the toxic effects of CO *(2,17)*. Because of their high demand for oxygen, the brain and heart are the most vulnerable organs, to the CO-induced acute hypoxia. Neurological and myocardial injuries associated with acute CO intoxication can be fatal unless medical treatment is provided immediately. The

normal background of the HbCO A level in a healthy nonsmoker is about 0.5–1% *(18)*. Early neurological symptoms such as headaches, dizziness, nausea, vomiting, disorientation, and visual confusion occur when the HbCO A level reaches 10–30%. Depending on the CO exposure level, duration, and treatments, the prognosis in patients with acute CO poisoning varies *(17)*.

Chronic environmental CO exposure may constitute one risk factor for cardiovascular diseases. A retrospective study of 5529 New York City bridge and tunnel officers unmasked the relationship between occupational exposure to CO and mortality from heart disease *(19)*. The CO exposure level of the tunnel officers was much higher than that of the bridge officers. There were 61 deaths from arteriosclerotic heart diseases in tunnel officers, which was higher than the expected 45 deaths based on the New York City population. Once the exposure was eliminated, the high risk of arteriosclerotic heart disease in the tunnel officers dissipated.

There has been a long-lasting debate on whether chronic CO inhalation as intrinsically linked to cigarette smoking acts either alone or with other environmental stressors to induce hypertension *(20,21)*. Increases, decreases, or no change in blood pressure after CO exposure has been reported. What should be remembered is that the adverse health effect of cigarette smoking is not a simple mirror image of CO inhalation. Immediately following cigarette smoking, an acute but transient increase in the smoker's blood pressure occurs, which has been largely ascribed to the nicotine in smoking. This hypertensive effect of nicotine is overcompensated by CO in the end. The blood pressure of these long-term smokers is decreased, or at the very least not increased, without other cardiovascular complications *(22)*. This notion was further supported in an animal study in which borderline hypertensive rats were exposed to chronic CO. This treatment actually led to hypotension, not hypertension, in these animals *(20,22)*. Chronic CO inhalation leads to many diseases, chiefly those linked to hemodynamic responses to CO and hypoxia-adaptive changes *(23)*. Cardiac hypertrophy exemplifies the cardiovascular complications of chronic CO exposure. Continuous exposure in adult male rats to 700 ppm of CO for 27 d *(24)* or 500 ppm CO for 30 d *(25)* induced volume-overload cardiac hypertrophy. Hypertrophy of both the left ventricle (22%) and right ventricle (37%) developed with hematocrit increased nearly 50%. Chronic CO exposure also alters normal development of the cardiovascular and other systems. In one experiment, 1-d-old rat pups were exposed to 500 ppm of CO for 30 d, and cardiac histology analysis was performed at 61 and 110 d of age *(26)*. One notable alteration was the significant increase in small arteries across all heart regions. The diameter of the large arteries in the entire heart region was also greater than that in the control rats. The architectural impact of coronary vessel changes following chronic neonatal CO exposure would be considerable on cardiovascular functions, especially those at different developmental stages and in adulthood.

2.3. Hydrogen Sulfide

The presence of H_2S in our environment is easily recognizable for its peculiar rotten-egg smell *(27,28)*. Atmospheric H_2S has both natural and anthropogenic sources. Volcanic gases, marshes, swamps, sulfur springs, and decaying matter such as from mushrooms all release H_2S into the environment. Emissions from oil and gas refineries, paper mills, and sewer networks also result in odor, health, and corrosion problems. Acute intoxication of H_2S can be lethal *(29)* and is one of the leading causes of sudden death in the workplace *(30)*. At least 5563 cases of intoxication and 29 deaths resulting from H_2S exposure occurred in the United States between 1983 and 1992 *(31)*. Loss of the central

respiratory drive is one of the major mechanisms for acute H_2S death *(27,28,32,33)*. The interaction of H_2S with many enzymes and macromolecules, including Hb, myoglobin, and cytochrome oxidase, exerts a profound effect on the vitality of cells *(34–36)*. Disorders of the central nervous, cardiovascular, respiratory, and gastrointestinal systems have been reported with acute H_2S intoxication *(34,37)*.

The health hazard of chronic H_2S exposure has also been observed *(36)*. Bates et al. *(38–40)* carried out a series of studies in the city of Rotorua, New Zealand, which is located over an active geothermal field. Approximately one-quarter of the population had been exposed regularly to high concentrations of H_2S from 143 to 1000 ppb. During 1981–1990, a higher mortality risk for respiratory diseases and a higher morbidity risk for neuronal diseases (both peripheral and central nervous systems) were observed in the Rotorua population compared with the rest of the population of New Zealand *(38,39)*. Another improved survey based on 1993–1996 morbidity data linked adverse health outcomes of Rotorua to other regions within Rotorua with high, medium, or low H_2S exposure levels *(40)*. This recent study again demonstrated an H_2S exposure-response tendency for disorders of the nervous system and sense organs as well as circulatory and respiratory diseases. Furthermore, a retrospective epidemiological study examined 2853 married, adult, nonsmoking women in a petrochemical complex in Beijing, China *(41)*. During their first trimester of pregnancy, about 57% of the surveyed woman had been exposed to petrochemicals. The results showed a significantly increased risk of spontaneous abortion when exposed to H_2S (odds ratio [OR]: 2.3; 95% confidence interval [CI] 1.2–4.4), benzene (OR: 2.5; 95% CI: 1.7–3.7), and gasoline (OR: 1.8; 95% CI: 1.1–2.9).

The average odor threshold for H_2S is about 0.5 ppb *(42)*. A low level of H_2S exposure does not appear to have had any adverse long-term health effect *(42)*. According to the Agency for Toxic Substances and Disease Registry, the acute minimal risk level for H_2S currently is set at 70 ppb, i.e., 24-h daily exposure to 70 ppb of H_2S over a period of 14 d or less *(42)*. An investigation was conducted in a Pennsylvania elementary school that complained of H_2S odors putatively related to the nearby mushroom-composting operations *(43)*. During the spring of 1998, 1-h averages of atmospheric H_2S levels were found to be consistently below 10 ppb at a control school, but between 11 and 59 ppb for 7 d for the outside air, and 5 d for the inside air at the exposed school. During the autumn of 1998, 1-h averages of atmospheric H_2S levels were consistently below 10 ppb at the control school, but between 11 and 129 ppb for 9 d for the outside air, and 7 d for the inside air at the exposed school. The investigators stated: "No consistent association was found between exposure to low levels of hydrogen sulfide and any adverse health effects. It was concluded that the students attending the elementary school near the mushroom-composting operations were not exposed to any significant public health hazard" *(43)*.

More details about the chemical and physical properties and toxicology profile of H_2S are discussed in Chapter 17.

3. PRODUCTION AND PHYSIOLOGICAL EFFECTS OF ENDOGENOUS GASES

Decades of environmental and occupational health studies describe NO, CO, and H_2S as vicious toxicants that exert a detrimental influence only on human health. This conventional thinking has gradually lost ground. First is the evidence that NO is actually endogenously generated with profound biological and physiological effects. The endog-

enous production of CO, on the other hand, has been known for a long time. The re-evaluation and realization of the physiological importance of CO to the homeostatic control of the human body have been achieved only in the past 10 yr or so *(44)*. Like NO and CO, H_2S at physiologically relevant levels affects structures and functions of the human body at the molecular, cellular, tissue, and system levels.

3.1. Nitric Oxide

Application of nitrate-containing compounds, starting with nitroglycerin, for medicinal purposes can be traced back more than 150 yr. Less than two decades ago, the discovery that a simple gas, NO, was critical for endothelium-dependent vasorelaxation led to a revision of the doctrine about cell signal transduction *(45)*. The enzymatic synthesis of NO from L-arginine occurs in almost every type of cell, catalized by NO synthases. Many endogenous substances modulate the activities of NO synthases. The first discovered was a neurotransmitter, acetylcholine. Decomposition and biotransformation of NO in vivo have also been clearly demonstrated *(46)*. To capitalize on the discovery of endogenous NO, on October 12, 1998, Robert Furchgott, Louis Ignarro, and Ferid Murad were awarded a Nobel Prize in Medicine and Physiology for their discoveries concerning NO as a signaling molecule in the cardiovascular system. Today, the physiological importance of NO has been extended far beyond the cardiovascular system. NO has critical regulatory roles in physiological functions of many different types of cells, tissues, organs, and systems. Abnormal metabolism and/or functions of NO have also been described for pathogenic processes of many diseases. On the incomplete list of diseases involving NO are hypertension, diabetes, ischemia/reperfusion heart damage, cardiac attack, inflammation, stroke, erectile dysfunction, aging, menopause, hyperlipidemias, atherosclerosis, cancer, drug addiction, intestinal motility, memory and learning disorders, neuronal degenerating diseases, septic shock, sunburn, anorexia, tuberculosis, and obesity.

3.2. Carbon Monoxide

In 1898, Saint-Martin and Nicloux gave the first indication of endogenous CO. In 1950, Sjöstrand provided experimental evidence for the endogenous production of CO *(47)*. The biological and physiological function of endogenous CO had been either unknown or ignored for the ensuing half-century. Although lipid peroxidation yields endogenous CO, breakdown of the α-methane bridge of heme is the major route for the endogenous production of CO. Three isoforms of microsomal heme oxygenases (HOs) are involved in the enzymatic CO production in vivo. For more details about endogenous CO production and regulation, refer to Chapter 10.

Endogenous CO plays an important role in long-term potentiation (LTP) as a retrograde messenger in the brain *(48,49)*. This role of CO is similar to that of NO but may be mediated by different mechanisms. One hypothesis is that NO induces LTP by stimulating NMDA receptors, whereas it induces CO by stimulating metabotropic glutamate receptors. The involvement of 5-HT(3) receptors in the induction of ganglionic LTP by CO has also been suggested.

CO released from the vascular wall modulates proliferation and apoptosis of smooth muscle cells as well as endothelial cells. Relaxation of various types of smooth muscles by CO has also been consistently shown. Endogenous cellular levels of CO vary under different pathophysiological conditions, contributing to different disorders. Readers are referred to two recently published books for more detailed descriptions of the different biological effects of CO under physiological and pathophysiological conditions *(50,51)*.

Regarding regulation of heme metabolism, the physiological importance of HO has long been recognized. In addition to the degradation of heme, HO catalyzes the production of CO as well as biliverdine and ferrous iron. However, CO had not been taken into account for its beneficial effects of HO until little more than a decade ago. The breakthrough discovery of NO opened the way to further research on membrane/receptor-independent signaling by gas molecules. In 1991, Marks and colleagues *(52)* hypothesized that CO might be another important endogenous gaseous molecule. This pioneering thinking stirred up the resurgence of CO as a physiological signaling molecule *(44)*.

As CO biology has bloomed in recent years, more and more enthusiasm has been injected into HO biology. Research on CO and HO is now closely interacted and coevolved. This HO/CO field is experiencing phenomenal growth, spurred on by scientists and health workers, from the laboratory bench to the hospital bedside and by trainees from graduate students to postdoctoral fellows.

3.3. Hydrogen Sulfide

A significant amount of H_2S is produced by mammalian cells, and this substance has been measured in both circulatory blood and in isolated tissues and cells *(53)*. Two pyridoxal-5'-phosphate-dependent enzymes, cystathionine β-synthase [CBS] (EC 4.2.1.22) and cystathionine γ-lyase [CSE] (EC 4.4.1.1), are responsible for the majority of the endogenous production of H_2S in mammalian tissues, which use L-cysteine as the main substrate *(53)*. Ammonium and pyruvate are two other end products, in addition to H_2S, of CBS- and/or CSE-catalyzed cysteine metabolism. H_2S is also produced endogenously through the nonenzymatic reduction of elemental sulfur using reducing equivalents obtained from the oxidation of glucose *(53)*.

The elimination of H_2S from the body takes place mainly in the kidney. Mechanisms for biotransformation and scavenging of H_2S in vivo include oxidation in mitochondria, methylation in cytosol, and scavenging by methemoglobin or metallo- or disulfide-containing molecules such as oxidized glutathione. The appendix to this chapter gives detailed descriptions of the metabolism of H_2S *(53)*.

Similar to the story of CO, in which HO captured all of the glories initially, H_2S has lived for a long time in the shadow of H_2S-generating enzymes. These enzymes initially were characterized in the liver and kidney *(54,55)*. The physiological processes modulated by these enzymes were also elucidated in the liver and kidney, but the role played by H_2S was not studied further. Even homocysteine, a precursor of H_2S that is catabolized by the same H_2S-generating enzymes, received more attention from the perspective of atherosclerosis.

Recent studies have contributed significantly to our understanding of the physiological roles of H_2S in the nervous and cardiovascular systems. At physiologically relevant concentrations, H_2S reduced KCl-stimulated releases of the corticotropin-releasing hormone *(56)*. NaHS, a donor of H_2S, induced a concentration-dependent (27–200 μ*M*) hyperpolarization and reduced input resistance of CA1 neurons or dorsal raphe neurons *(34)*. This concentration range is physiologically relevant in the brain *(57)*. Changes in K^+ conductance were identified to be the main ionic basis for these effects of NaHS, and K_{ATP} channels in neurons were speculated as the specific targets.

N-methyl-D-aspartate (NMDA) receptors are another target of H_2S. In the presence of a weak tetanic stimulation, NaHS at 10–130 μ*M* facilitated the induction of hippocampal long-term potentiation in rat hippocampal slices by enhancing the NMDA-induced inward current *(57)*. Activation of the cyclic adenosine monophosphate-dependent protein kinase pathway likely mediates the interaction of H_2S and NMDA receptors *(58)*.

In the cardiovascular system, H_2S has been demonstrated at physiologically relevant concentrations to relax vascular tissues by opening K_{ATP} channels in vascular smooth muscle cells (VSMCs) *(59,60)*. In this case, NO serves as a trigger to increase H_2S production and release *(59)*. Evidence has also been presented for the relaxant effects of NaHS on rabbit isolated ileum, rat vas deferens, and guinea pig isolated ileum at physiologically relevant concentrations *(61)*. Inhibition of the H_2S-generating enzyme CSE caused a slowly developing increase in the contraction of the guinea pig ileum as a result of field stimulation *(61)*.

4. GASOTRANSMITTERS IN EVOLUTION

Table 1 lists organized activities for promoting research on and advancing our understanding of gasotransmitters. A 2-yr span saw the birth of a scientific society, a scientific journal, and the first scientific conference specifically devoted to NO (1996–1998). Since then, NO biology and chemistry have been the subject of many international meetings. Following the first world Internet meeting on cardiovascular effects of CO in 1998, two HO/CO conferences were held in 2000 and 2002 and another HO conference in 2003. **Table 2** lists selective monographs and books on the different types of gasotransmitters. Most of these books are on NO, and two are related to endogenous CO.

While this book was being edited, the *Antioxidants and Redox Signaling* journal published a special forum issue entitled "Gaseous Signal Transducers," discussing the biological roles of NO, CO, and H_2S. Another cheering development was the creation of the first strategic training program for gasotransmitter research in 2003, entitled "Gasotransmitter REsearch And Training" (GREAT). More than 15 researchers from four Canadian universities participated in this 6-yr program, supported by the Canadian Institutes of Health Research. The GREAT program will provide trail-breaking interdisciplinary and transdisciplinary training for local and international students, postdoctoral fellows, and researchers on sabbatical. The training program will be delivered through an array of courses; a trainee exchange program; laboratory, clinical, and community health research; and training-mentoring initiatives. A compulsory component of the GREAT program is a three-credit course, "Gasotransmitter Biology and Medicine." Another course offered through this program is "Career Development Essentials for Gasotransmitter Trainees."

Determination of endogenous levels of NO, CO, and H_2S; identification of the enzymes responsible for the production of these gases; and, most important, elucidation of the physiological functions of these gaseous molecules pave the way for the development of a general concept to envelop all these gases into one family. As can be seen from the aforementioned organized activities, one can only conclude that the era of gasotransmitters is coming and "the medium is the message" (Marshall McLuhan).

5. GASOTRANSMITTERS: DEFINITION OF THE CONCEPT

Vehicles for intercellular communication are either electrical signals via gap junction or chemical substances. The latter category is composed of hormones, autocoids, and transmitters. Hormones are released from endocrine cells into the bloodstream. The concentration of hormones is diluted to a relatively stable level when they reach distant multiple organs and cells. This endocrine mode of action is distinctive from the paracrine action of transmitters, in which transmitters, once released, usually act on adjacent postsynaptic cells. A definition of autocoids is not strictly precise. In general, autocoids (such as prostaglandins, adenosine, and platelet-activating factor) act on the same cells from which they are produced. Similar to the effects of hormones and transmitters,