Published in final edited form as:
Surgery. 2008 April ; 143(4): 455–459. doi:10.1016/j.surg.2007.10.017.

Gasotransmitters in the gastrointestinal tract

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The 1990s initiated a new paradigm for cell-to-cell signal transduction via "neurotransmitters" with the discovery that a gas, nitric oxide (NO), could be released specifically from nerves and act to transmit the "neural signal" secondary to nerve stimulation. But, unlike the classic paradigm of a neurotransmitter being released extracellularly from prestored vesicles and binding to a membrane-bound receptor on the effector cell, NO is synthesized on demand from nitric oxide synthase (NOS) (rather than stored in vesicles), then released extracellularly, and diffuses across the cell membrane to act on an intracellular enzyme guanylate cyclase to transduce the primary neural signal. The recognition of carbon monoxide (CO) soon followed as a second gaseous neurotransmitter acting similarly to NO. With the recent acknowledgement of hydrogen sulfide (H_2S) as the third gaseous neurotransmitter, the term "gasotransmitter" was introduced to characterize gases which act as neurally released transmitters[1]. NO, CO, and H_2S share distinct properties, which qualify them as gasotransmitters in that they 1) are small molecules of gas; 2) are freely permeable across membranes and do not act via specific membrane receptors; 3) are synthesized endogenously and enzymatically on demand and their generation is regulated; 4) have well-defined specific functions at physiologically relevant concentrations; and 5) their cellular effects may or may not be mediated by second messengers, but these gasotransmitters have specific cellular and molecular targets. Due to their gaseous nature, NO, CO, and H_2S are not stored within the cell in the classic presynaptic vesicles before they are released, but rather they are synthesized and released on demand, which distinguishes these gasotransmitters from classic neurotransmitters such as acetylcholine, norepinephrine, and even the peptide neurotransmitters. Although storage vesicles for gasotransmitters have not yet been identified, protein adducts might in theory serve as storage pools. Furthermore, presynaptic re-uptake of these released gasotransmitters, as occurs with other neurotransmitters, has not been described. Gasotransmitters are rapidly scavenged or enzymatically degraded after their release to terminate their signaling activity, with biologic half-lives on the order of seconds. An additional property shared by the three gasotransmitters is their potential systemic toxicity at supra-physiologic concentrations, which led to the recognition of these gases as air pollutants and toxins before their important *in vivo* functions were identified or even imagined.

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Our understanding of the wide spectrum of physiologic functions of each gasotransmitter in different organ systems continues to grow. This short review will provide an overview about the role of the three established gasotransmitters, NO, CO, and H_2S, focusing primarily on the control of contractile function of the gastrointestinal (GI) tract. We will also address, albeit briefly, their involvement in other important functions, such as inflammation, ileus, pain perception, and carcinogenesis in which they act, not as neurotransmitters, but more as paracrine or even systemically active substances. The number of gasotransmitters might increase with the addition of such candidate gases as ammonia and acetaldehyde; however, because these gases have not fully met the criteria to classify them as gasotransmitters, we will focus on the three established gasotransmitters NO, CO, and H_2S for the purpose of this review.

Nitric Oxide (NO)[2,3]

The observation that relaxation of blood vessels induced by acetylcholine requires an intact epithelial layer led to the search for and discovery of endothelium-derived relaxing factor (EDRF) in the early 1980s. Years later, a new paradigm emerged when EDRF was found to be the gas NO. The initial studies described NO as being released not only from the endothelium but also from neurons, which qualified NO as a new type of "neurotransmitter." In the GI tract, NO is produced under physiologic conditions, primarily by the constitutively expressed, neuronal isoform of nitric oxide synthase (nNOS) and released from inhibitory nitrergic neurons of the enteric nervous system. Endothelial NOS (eNOS), which is also expressed constitutively in endothelial cells, is involved in the control of vascular perfusion of the gut, while the inducible isoform of NOS (iNOS) is expressed mainly in inflammatory cells like macrophages, and its expression can be induced by cytokines; indeed the production of NO by iNOS can release concentrations of NO two to three log-fold greater than nNOS or eNOS and lead to markedly different physiologic and pathophysiologic effects. While iNOS is constituitively active after it is expressed (induced), the activity of the nNOS and eNOS enzymes are regulated by the concentration of intracellular calcium. NOS catalyzes the conversion of the substrate L-arginine to L-citrulline and NO. In addition, NO can be produced from nitrite through reduction. NO is a soluble and highly unstable gas, that oxidizes to nitrite and nitrate with a half-life of only a few seconds.

On its release from nerves in the gut wall, NO diffuses through the cell membrane of the smooth muscle cell and binds to its cytosolic target, soluble guanylate cyclase, inducing production of the second messenger cyclic guanosine monophosphate (cGMP) which then alters ion channel activity, decreases intracellular Ca^{2+}, and leads to cellular hyperpolarization which causes relaxation of smooth muscle cells.

Although the functional involvement of NO varies between species and anatomic regions, NO appears to be the dominant non-adrenergic, non-cholinergic (NANC) inhibitory neurotransmitter in the enteric nervous system. For example, NO is involved in the vagally-mediated accommodation reflex of the stomach and colon to food ingestion as well as in the coordinated control of contractile function during the peristaltic reflex; in the latter reflex, NO, together with vasoactive intestinal polypeptide (VIP) acting as a neurotransmitter, relaxes the bowel distal to an intraluminal bolus, while the procontractile neurotransmitters acetylcholine and substance P mediate the proximal contraction, thereby ensuring the propagation of the bolus in an aboral (distal) direction. Furthermore, NO plays an important role in the relaxation of sphincters in the GI tract, such as the lower esophageal sphincter, the sphincter of Oddi, and the internal anal sphincter. NO often acts in concert with other inhibitory NANC transmitters like VIP as well as its gaseous colleagues CO and H_2S. Best characterized but still not completely understood is the interaction between NO and VIP, and indeed, nNOS and VIP are often co-expressed in neurons of the enteric nervous system[2]. VIP and NO can act in series with neuronally released VIP stimulating the production and release of NO from neuronal nerve

endings and causing an NO-mediated relaxation. Similarly, VIP and NO can act in parallel by stimulating different second messenger systems; VIP induces the production of cyclic adenosine monophosphate (cAMP), while NO induces production of cGMP, with both second messengers leading to muscle relaxation. The complexity of nitrergic neurotransmission and its importance in the control of GI function assures NO its role in the pathophysiology of many different diseases of the GI tract[3]. Impaired NANC nitrergic innervation of the muscle appears to play a crucial role in several disorders of GI dysmotility, such as achalasia, functional dyspepsia, diabetic gastroparesis, delayed gastric emptying during pregnancy or after vagotomy, infantile hypertrophic pyloric stenosis, Hirschsprung′s disease, and also Chagas′ disease. In contrast, the release of NO produced by iNOS in inflammatory cells inhibits contractile activity of the intestine probably via a paracrine effect and appears to play an important role in the pathogenesis of early postoperative ileus.

The above discussion of NO concentrates on its role as a specific gasotransmitter. The production of NO from iNOS is also involved in many aspects of WBC function and the inflammatory response as well in the development of specific vasculopathies, injury, and circulating changes after liver transplantation as well as acute cellular rejection[4]. These effects differ from the specific gasotransmitters role of NO in the nervous system (nNOS).

Carbon Monoxide (CO)

Carbon monoxide (CO) has been identified as the second gasotransmitter in the GI tract, although the role and importance of CO is less well-studied than NO. CO is synthesized from the catabolism of heme by heme oxygenases (HO) with the other products of this enzymatic reaction being biliverdin and iron. Much of the generated CO is scavenged by hemoglobin in red blood cells as COHb and transported to the lungs where it is exhaled. Similar to NO, three isoforms of HO have been described. The inducible isoform HO-1, which can be upregulated in response to oxidative stress or inflammation, and its constitutively expressed counterpart HO-2 have been found in a variety of tissues and species; the constitutive isoform HO-3 has only been demonstrated in rat tissue, and its existence as an independent isoform remains under debate, because it might be a modification of HO-2. In human stomach and jejunum, HO-2 expression has been demonstrated in neurons of the myenteric and submucous plexuses[5].

Similar to NO, neuronal release of CO also causes smooth muscle relaxation in the gut. Several different mechanisms appear to be involved in the NANC inhibitory effect of CO on contractile activity. Besides the induction of NO, CO induces the production of cGMP by stimulation of soluble guanylate cyclase, CO can also increase cAMP levels, stimulate protein kinase A (PKA), and affect different ion channels directly[6]. These mechanisms all lead to hyperpolarization and relaxation of GI smooth muscle. There appears to be an important interaction between CO and NO. HO-2 and nNOS are co-expressed in about 40% of neurons of the myenteric plexus in human stomach and jejunum[5]. Although CO is only a weak activator of guanylate cyclase, its release is associated with a robust induction of cGMP production and muscle relaxation, which appears to be related to the CO-induced increase of NOS activity, release of NO, and the resultant several-fold higher affinity of the released NO to guanylate cyclase. Furthermore, the inhibitory neuropeptide VIP can also stimulate HO-2 to produce CO. In the canine jejunum, HO-2 is expressed not only in neurons of the enteric nervous system but also in smooth muscle cells, suggesting that CO, like NO, may also function as an autocrine messenger in smooth muscle in addition to its effect as a neuronally released gasotransmitter.

As with NO, CO is also known to be involved in inflammatory and immune responses. In rodent and swine models, inhalation of CO before abdominal operations attenuates the postoperative intramural inflammatory response and thereby is believed to decrease postoperative ileus. In rats not pre-treated with inhalation of CO, postoperative ileus can be

ameliorated in part by the use of inhibitors of HO[7]. Finally, HO-1 is involved in mucosal defense mechanisms, and its expression in colon cancer cells in humans appears to be associated with a better long-term survival compared to neoplasms not expressing HO-1. How far CO is involved in this function of HO-1 is unclear at present.

Hydrogen Sulfide (H_2S)

The most recent candidate to join the family of gasotransmitters is H_2S. H_2S is best known probably from its characteristic smell of rotten eggs. As with NO and CO, H_2S is involved in a multitude of physiologic functions, including immune and inflammatory processes, perception and pain mediation, as well as control of gastric mucosal integrity, vascular tone, and most pertinent to this review, probably the control of GI motility as well, although studies of the latter are in their infancy[8]. H_2S is produced endogenously from L-cysteine by two intracellular enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE), which lead to the production of H_2S, pyruvate, and ammonia (potentially the next gasotransmitter). CBS and CSE are expressed to different extents in neurons in the brain and in the enteric nervous system of the gut, as well as in vascular and non-vascular cells in smooth muscle, liver, and kidney. After its release, H_2S is metabolized by several different mechanisms, including mitochondrial oxidation, cytosolic methylation, and oxidation by glutathione, or it is scavenged by met-hemoglobin before it is excreted by the kidney. As with NO, the role of H_2S was studied initially in vascular smooth muscle, where it exhibits its relaxing effect by direct action on ATP-sensitive K^+-channels. Opening of these membrane channels leads to hyperpolarization of the muscle cells followed by closing of voltage-gated Ca^{2+}-channels and subsequent relaxation[1]. The role and mechanisms of action of H_2S in the gut are far from being understood completely or for that matter, even well-established. CBS and CSE are expressed in neurons of the enteric nervous system with marked differences between both species and anatomic regions. Initial rudimentary work showed that H_2S would inhibit contractile activity in the guinea pig and rat ileum in a fashion independent of ATP-sensitive K^+-channels, but little further work has followed. Preliminary work in our laboratory has shown a prominent inhibitory effect on rat jejunal longitudinal muscle that was independent of ATP-sensitive K^+-channels. As with NO and CO, there appears to be a close interaction between H_2S and NO, with NO amplifying the inhibitory effect of H_2S; additional evidence suggests that NO can increase CSE activity acutely, and that chronic exposure to NO up-regulates CSE expression. Studies of H_2S-induced chloride secretion in guinea pig and human colon[9] imply that the presence of H_2S in the mucosa/submucosa of the colon stimulates primary afferent nerve fibers which leads to increased chloride secretion; along this reasoning, it might also be possible that H_2S affects contractile function, not only by direct effects on the smooth muscle itself, but by an effect on neurons of the enteric nervous system that might, in turn, affect smooth muscle function. Another potential source of H_2S, besides the neuronal endogenous production, might be H_2S produced by bacteria within the bowel lumen; however, a physiologic role of H_2S originating from this source has not yet been described. The pathways that appear to modulate the contractile function of the GI tract by H_2S have not yet been studied and serve as fertile territory for future experiments.

In addition to the effect of H_2S as a gasotransmitter affecting GI contractile activity, there is growing evidence that H_2S plays an important role as a pro-inflammatory mediator in abdominal sepsis, endotoxemia, and pancreatitis[10] in contrast to its anti-inflammatory effects in animal models of gastritis and colitis. Furthermore, H_2S has an anti-nociceptive effect on visceral pain perception, but H_2S has also been demonstrated to activate primary sensory neurons and might even participate in the development of colon cancer. Overall, our incomplete understanding of the role and mechanisms of action of H_2S in many aspects of physiology and pathophysiology of the GI tract provides a field for extensive research in the future.

Summary

Mechanisms of cell-to-cell signaling take many forms: classic receptor-mediated transmission, opening and/or closing of ion channels, and this new concept of gasotransmitters diffusing into the cell to exert their effects after being released either by neural stimulation, inflammatory cells, or even bacteria. NO, CO and H_2S are the three gasotransmitters that have pioneered the latter group of signal molecules. Future work is expected to solidify our understanding of their mechanisms of action, delineate their interaction, and define their roles in normal physiology as well as pathophysiology.

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Table 1

Role of gasotransmitters in the GI tract

	Source	Enzyme	Target	Effect
NO	Neurons Inflammatory cells Endothelium	nNOS iNOS eNOS	GI **smooth** muscle cells, vascular smooth muscle cells	Inhibition of GI contractile activity, **modulation** of gut perfusion
CO	Neurons Smooth muscle	HO-1 HO-2 HO-3	GI muscle cells, mucosa, inflammatory cells	Inhibition of GI contractile activity, mucosal defense, anti-inflammatory effect during postoperative ileus
H_2S	Neurons Smooth muscle Liver Kidney Bowel lumen (?)	CBS CSE	GI **smooth** muscle cells, primary afferent nerve fibers, vascular smooth muscle cells, inflammatory cells	Inhibition of GI contractile activity, stimulation of Cl^- - secretion, **modulation** of gut perfusion, pro-/nti-inflammatory effects (?)