

GASEOUS MEDIATORS IN THE ENTERIC NERVOUS SYSTEM

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SUMMARY

The enteric nervous system is an independent nervous system that controls vital gut functions including motility and secretion. Enteric neurons use a variety of neurotransmitters to modulate neural as well as muscle and epithelial activity. Among them are three gaseous mediators which are nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H₂S). All three have potent inhibitory effects on smooth muscle. While NO and CO are released from terminals of enteric neurons to directly affect muscle or epithelial cells, H₂S exerts its effect via extrinsic afferent neurons. At least in the guinea pig and human gut H₂S appears to activate transient receptor potentials vanilloid receptor 1 (TRPV1) expressing extrinsic afferent neurones. This mode of action is important for the prosecretory effect of H₂S.

INTRODUCTION

The enteric nervous system (ENS) is considered to be an independent nervous system that controls and coordinates motility, blood flow and secretion. The ENS is thereby crucial to maintain vital functions such as transit of luminal contents, secretion and absorption of ions water and nutrients, microcirculation and barrier functions. One of the hallmarks of the ENS is its similarity to the brain hence its alias “little brain of the gut”. The ability to function as a little brain implies that the ENS, like the CNS, uses a variety of neurotransmitters to coordinate nervous activity as well as activation and inhibition of the various non-neuronal effector cells in the gut (*Schemann and Neunlist, 2004*). Beside the classical neurotransmitters, the ENS uses the three known gaseous mediators nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H₂S)

to modulate neural activity as well as activity of muscle and epithelium. The three gasomediators share some common features. For example the concentration of the three gasomediators has to be tightly controlled, as all of them are potentially toxic and lethal. In addition, NO, CO and H₂S are lipophilic and thereby easily cross the cell membrane. They activate or inhibit signalling cascades inside the cell, as their action does not involve “classical” neurotransmitter receptors. Last but not least all three of them are synthesised by enzymes close to their site of release and action. While their final action may be similar for some effector systems, NO, CO and H₂S have specific mode of actions and their relevant contribution varies according to the physiological and pathophysiological state of the organ or body (see Table 1).

Table 1: Summary of synthesis, functions and putative mode of actions of nitric oxide (NO), carbon monoxide (CO) and hydrogen sulphide (H₂S)

Gasomediator	Synthesis	Functions	Mode of action
NO	By neuronal NOS ¹⁾ : mostly in myenteric and less in submucous neurones	Decrease nerve activity Muscle relaxation Weak secretagogue	Presynaptic inhibition cGMP↑ and muscle hyperpolarisation involves nerves and prostanoids
CO	By HO-2 ²⁾ : mostly in myenteric and less in submucous neurones	Neuronal effect ? Muscle relaxation Effect on secretion ?	cGMP↑ via NO and muscle hyperpolarisation
H ₂ S	By CSE ³⁾ and CBS ⁴⁾ : mostly in submucous and less in myenteric neurones	No direct effect on enteric neurones Relaxation of smooth muscle Strong secretagogue	? TRP ⁵⁾ channel expressing extrinsic afferents

¹⁾nitric oxide synthase; ²⁾haem oxygenase-2; ³⁾cystathionine gamma-lyase; ⁴⁾cystathionine beta-synthase; ⁵⁾transient receptor potential.

NO AS A GASEOUS MEDIATOR IN THE GUT

In the ENS, nitric oxide is mainly synthesised by inhibitory muscle motor neurones of the myenteric plexus. Only a very few interneurones, some of which also synthesise acetylcholine, express nitric oxide synthase (NOS), the enzyme which produces NO. In some species including humans NO is also synthesised by enteric neurones of the submucous plexus. NO relaxes the smooth muscle in the stomach, small and large intestine of all species studied so far (*Salzman, 1995; Sanders, 1996*). Interstitial Cells of Cajal (ICC) are crucial in this relaxation as the nitric oxide released from enteric neurons uses the ICC network to spread the inhibitory signal to the smooth muscle. The inhibitory action of nitric oxide

depends on increase in cGMP, opening of potassium channels and finally hyperpolarisation of smooth muscle. NO also has an inhibitory action on enteric neurones. At least in the guinea pig it consists of a pre-synaptic inhibition of transmitter release (*Tamura et al., 1993*). Interestingly, this affects only synapses involved in slow excitatory synaptic transmission but not those that mediate fast excitatory postsynaptic potentials. The physiological relevance of NO released from submucous neurones is not as clear as that described for the smooth muscle. NO does increase secretion probably involving neural actions and release of prostaglandins, respectively.

CO AS A GASEOUS MEDIATOR IN THE GUT

CO is generated by haem-oxygenase-2 (HO-2), which is constitutively expressed in many inhibitory neurones of

the ENS (*Gibbon and Farrugia, 2004*). The membrane potential gradients along and across the muscle layers of

the gastrointestinal tract require the generation of CO by HO-2. The presence of CO is also necessary for normal inhibitory neurotransmission in circular smooth muscle and appears to permit nitric oxide-mediated inhibitory neurotransmission. Loss of HO-2 activity slows gut transit. It is striking that neurons expressing haem oxy-

genase by far outnumber NOS expressing neurons in the ENS, the functional meaning of which is unknown. There is not report on the effect of CO on the activity of enteric neurons, yet the data from neurochemical coding studies would suggest that CO is present in some enteric interneurons.

H₂S AS A GASEOUS MEDIATOR IN THE GUT

While the role of CO and in particular NO in gut functions is well established, the role of the newest member of the gaseous mediators, namely H₂S in the gut is just emerging. So far it seems clear that H₂S exerts widespread functions on neurons, muscle lamina propria and epithelial cells. Synthesis of H₂S mainly involves two enzymes, cystathionine gamma-lyase (CSE) and cystathionine beta-synthase (CBS). Strikingly, more than 90% of guinea pig and human submucous and myenteric neurons express both CSE and CBS. Myenteric ICC were CSE-immunoreactive. While the role of H₂S in ICC is not understood, its role in the ENS has been thoroughly studied. Thus the exogenous H₂S donor NaHS relaxes guinea-pig gut smooth muscle (*Teague et al., 2002*). This effect does not involve K_{ATP} channels which appear to mediate the H₂S induced relaxation of vascular smooth muscle.

Possible role of hydrogen sulphide as a pre-secretory modulator has been studied in human and guinea-pig gut (*Schicho et al., 2006*). NaHS increased chloride secretion in human and guinea-pig colon. This effect requires intact nerves, as it is not observed in the colonic epithelial cell line T84. The secretory response was reduced significantly by the nerve blocker tetrodotoxin, by capsaicin desensitization, and the TRPV1 antagonist capsazepine.

The endogenous H₂S donor L-cysteine mimicked the effect of NaHS and this secretion was also diminished significantly by capsaicin desensitization, the CBS inhibitor amino-oxyacetic acid, and the CSE inhibitor propargylglycine. NaHS increased spike discharge in 23% of guinea pig and 36% of human submucous neurons, but had no effect on Ca⁺⁺ mobilization in isolated cultured guinea-pig enteric neurons. This excitatory response was reduced significantly by capsaicin desensitization and capsazepine. The presence of H₂S producing enzymes in human and guinea-pig enteric neurons, the excitatory action in the ENS, and the pro-secretory effects of NaHS strongly suggest H₂S as a gut-signalling molecule. Its action mainly involves TRPV1 expressing extrinsic afferent terminals, which in turn activate enteric secretomotorneurons.

H₂S production is also important under pathological conditions (*Attene-Ramos et al., 2006*). Thus persistent sulphate-reducing bacterial colonization and high luminal and faecal H₂S levels have been reported in ulcerative colitis and colorectal cancer. Whether this is primary or secondary to the disease needs to be investigated. The potent anti-inflammatory actions of H₂S in the upper gut open new clinical applications. Initial results suggest that addition of H₂S releasing moiety to

NSAID significantly improves side effects and thereby tolerability without affecting efficacy (*Wallace, 2007*).

LITERATURE

- Attene-Ramos, M.S, Wagner, E.D, Plewa, M.J, and Gaskins, H.R.: Evidence that hydrogen sulfide is a genotoxic agent. *Mol. Cancer Res.* 4, 9-14 (2006).
- Gibbons, S.J., and Farrugia, G.: The role of carbon monoxide in the gastrointestinal tract. *J. Physiol.* 556, 325-336 (2004).
- Schemann, M. and Neunlist, M.: The human enteric nervous system. *Neurogastroenterol. Motil.* 16, 55-59 (2004).
- Sanders, K.M.: A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology* 111, 492-515 (1996).
- Salzman, A.L.: Nitric oxide in the gut. *New. Horiz.* 3, 352-364 (1995).
- Schicho, R., Krueger, D., Zeller, F., Von Weyhern, C.W., Frieling, T., Kimura, H., Ishii, I., De Giorgio, R., Campi, B., and Schemann, M.: Hydrogen sulphide is a novel prosecretory neuromodulator in the Guinea-pig and human colon. *Gastroenterology* 131, 1542-1552 (2006).
- Tamura, K., Schemann, M., and Wood, J.D.: Actions of nitric oxide-generating sodium nitroprusside in myenteric plexus of guinea pig small intestine. *Am. J. Physiol.* 265, G887-G993 (1993).
- Teague, B., Asiedu, S., and Moore, P.K.: The smooth muscle relaxant effect of hydrogen sulphide *in vitro*: Evidence for a physiological role to control intestinal contractility. *Br. J. Pharmacol.* 137, 139-145 (2002).
- Wallace, J.L.: Hydrogen sulfide-releasing anti-inflammatory drugs. *Trends Pharmacol. Sci.* 10, 501-505 (2007).