Current Topics

Cellular Physiology of Channels and Transporters in Gastrointestinal Tracts

Regulation of Colonic Ion Transport by Gasotransmitters

Ervice Pouokam, Julia Steidle, and Martin Diener*

Institute for Veterinary Physiology, University of Giessen; Frankfurter Str. 100, D-35392 Giessen, Germany.

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Gaseous molecules such as nitric oxide (NO), hydrogen sulfide (H₂S), or carbon monoxide (CO) are involved in the regulation of colonic water and salt transport, which can be switched between absorption and secretion. Nitric oxide is produced from the amino acid l-arginine by different isoforms of the enzyme NO synthase, which are expressed both by enteric neurons and by the colonic epithelium. NO donors evoke a transepithelial Cl⁻ secretion in vitro. Most actions of NO are mediated by a stimulation of guanosine 5’ cyclic monophosphate (cGMP) synthesis via activation of the soluble guanylate cyclase. In rat colon, NO possesses several main action sites: a stimulation of apical Cl⁻ channels most probably not related to cGMP-dependent phosphorylation, and an increase in the cytosolic Ca²⁺ concentration, which stimulates a Ca²⁺-dependent K⁺ conductance in the basolateral membrane. Hydrogen sulfide, produced during the metabolism of the amino acid l-cysteine, also evokes a Cl⁻ secretion, either by stimulation of secretomotor submucosal neurons as in guinea-pig colon or by activating Ca²⁺-dependent and ATP-sensitive K⁺ channels as in rat colon. The third gasotransmitter, CO, produced during the degradation of heme, evokes anion secretion carried by Cl⁻ and HCO₃⁻. This response is mainly caused by the activation of apical anion channels and a stimulation of Ca²⁺-dependent K⁺ channels via an increase of the cytosolic Ca²⁺ concentration. Consequently, gaseous molecules produced by enteric neurons, epithelial cells, as well—in the case of H₂S—the microbial flora affect key transport enzymes involved in colonic ion transport.

Key words gasotransmitter; ion channel; ion transport; carbon monoxide; hydrogen sulfide; nitric oxide

1. INTRODUCTION

Colonic ion transport can be switched from absorption into secretion of water and electrolytes. Under healthy conditions there is a net colonic absorption of Na⁺ and Cl⁻, the two quantitatively most important anorganic ions transported in the large intestine. This absorption is physiologically replaced by net secretion, predominantly of Cl⁻, e.g. after mechanical distension of the gut wall in order to produce a fluid film for the protrusion of intestinal content. Under pathophysiologic conditions, this active secretion of Cl⁻ plays a prominent role for the development of secretory diarrhea, e.g. after exposure of the gut wall to certain bacterial products such as cholera toxin or different toxins produced from Escherichia coli, vibrio cholerae or other toxigenic bacteria.

In order to be secreted Cl⁻ ions are accumulated above electrochemical equilibrium by a Na⁺–K⁺–2Cl⁻/HCO₃⁻ cotransporter in the basolateral membrane, the prominent Cl⁻ loading transporter in this membrane beside a Cl⁻–HCO₃⁻ exchanger at the same location. When apical Cl⁻ channels open, which are predominantly of the cystic fibrosis transmembrane regulator (CFTR) channel type, there is an efflux of Cl⁻ into the colonic lumen, followed by a paracellular flux of Na⁺ for reasons of electroneutrality, and a flux of water for osmotic reasons.

The switching between absorption and secretion is controlled by classical neurotransmitters, predominantly released from the submucosal plexus, hormones and paracrine substances, which in general act on membrane-bound receptors in order to modify intracellular second messenger systems regulating ionic transport across the epithelium. However, since several years it has become evident that small gaseous molecules, the so-called gasotransmitters, can act as signaling molecules to affect intestinal transport, too. The first of these gasotransmitters which has been discovered was nitric oxide (NO). More recently, hydrogen sulfide (H₂S) and carbon monoxide (CO) have been described to act as gasotransmitters in the gastrointestinal tract. The aim of this review is to give an overview about actions of these gasotransmitters on colonic ion transport and the mechanisms involved.

2. NITRIC MONOXIDE

Nitric oxide is produced from the amino acid l-arginine by the enzyme nitric oxide synthase (NOS), from which three isoforms are known. NOS-1 (neuronal NOS, n-NOS) is the main form expressed in neurons, including enteric neurons of rat colon. NOS-1 plays a role in cell communication, e.g. between inhibitory motorneurons of the enteric nervous system and smooth muscle cells. The isoform NOS-2 (inducible NOS, i-NOS) is located in parts of the immune and cardiovascular system and is upregulated during inflammation. The third enzyme of the NOS-family, NOS-3 (endothelial NOS, e-NOS), produces NO in blood vessels; it is involved in the regulation of vascular functions.

The gas NO is a free radical which is able to pass the plasma membrane via diffusion. Most of its actions are mediated through activation of its main intracellular receptor, the soluble guanylate cyclase. This cytosolic enzyme produces the second messenger guanosine 5’ cyclic monophosphate (cGMP) from guanosine 5’-triphosphate (GTP). Cyclic GMP exerts most of its actions via stimulation of protein kinase G catalyzing the phosphorylation of target proteins, by direct effects on ion channels, or via modulation of cGMP-regulated phosphodiesterases which control the degradation of...
Nitric oxide liberating drugs evoke Cl\textsuperscript{−} secretion across the colon of different species such as rat,\textsuperscript{13,14} guinea-pig,\textsuperscript{15} man,\textsuperscript{15} or pig.\textsuperscript{16} For example, the NO liberating drug 1,2,3,4-oxatriazolium, 5-amino-3-(3,4-dichlorophenyl)-chloride (GEA 3162) induces a Cl\textsuperscript{−} secretion across rat colon, which can be measured in voltage-clamp experiments in Ussing chambers as increase in short-circuit current.\textsuperscript{17} Several action sites in the secretory mechanism of the epithelial cells have been found. An action site at the apical membrane could be identified in epithelia which had been basolaterally depolarized by use of a buffer with a high K\textsuperscript{+} concentration. This shifts the K\textsuperscript{+} diffusion potential, which dominates the membrane potential of the basolateral membrane with its high K\textsuperscript{+} permeability to values near zero and reduces its resistance due to the high concentration of permeant ions (Fig. 1A). Consequently, the transepithelial potential (and also the measured short-circuit current) are dominated by the apical membrane.\textsuperscript{18,19} Under these conditions, the NO donor stimulated a Cl\textsuperscript{−} current across the apical membrane (driven by a Cl\textsuperscript{−} concentration gradient), which was inhibited by glibenclamide, a typical inhibitor of the CFTR channel.\textsuperscript{6} This inhibition was resistant against inhibitors of protein kinases suggesting a possible direct action of NO on the channel (or a regulator of it) not involving phosphorylation of CFTR, which is the classical pathway to regulate the activity of this anion channel.

Experiments at isolated colonic crypts loaded with the Ca\textsuperscript{2+}-sensitive fluorescent dye fura-2 revealed that GEA 3162 evoked an increase of the cytosolic Ca\textsuperscript{2+} concentration due to an influx of extracellular Ca\textsuperscript{2+}. This influx was mediated by an activation of a nonsel ective cation conductance as shown by whole-cell patch-clamp studies. The increase of the cytosolic Ca\textsuperscript{2+} concentration is most probably the reason for the activation of a basolateral K\textsuperscript{+} conductance. Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels constitute a large fraction of the conductance of this membrane. Their activation leads to a hyperpolarization of the cell membrane thereby increasing the driving force for the efflux of negatively charged Cl\textsuperscript{−} anions across apical anion channels.\textsuperscript{20} This activation can be measured in the Ussing chamber when the apical membrane is permeabilized with the ionophore nystatin in the absence of mucosal Na\textsuperscript{+}. This can e.g. be reached after replacement of Na\textsuperscript{+} with the impermeant cation N-methyl-D-glucamine in order to avoid a contribution of the current generated by the 3Na\textsuperscript{+}/2K\textsuperscript{+}/3H\textsuperscript{+}ATPase, a K\textsuperscript{+} current across basolateral K\textsuperscript{+} channels by a K\textsuperscript{+} concentration gradient between the mucosal and the serosal buffer solution (Fig. 1B). This current across basolateral K\textsuperscript{+} channels was stimulated by the NO donor. Furthermore, also the pump current was enhanced by GEA 3162. This was measured when the permeabilization of the apical membrane was performed in the absence of a K\textsuperscript{+} concentration gradient (thus no current is driven across basolateral K\textsuperscript{+} channels), but in the presence of mucosal Na\textsuperscript{+}, which enters the cell via the nystatin pores and thereby stimulates the 3Na\textsuperscript{+}/2K\textsuperscript{+}/3H\textsuperscript{+}ATPase (Fig. 1C). Consequently, the gasotransmitter NO affects several key transport enzymes involved in colonic Cl\textsuperscript{−} secretion (Fig. 2). Interestingly, in rat small intestine in vivo also a proabsorptive role of NO has been observed.\textsuperscript{21,22} The reasons for this discrepancy between the in vivo and the in vitro measurements are completely unknown.

3. HYDROGEN SULFIDE

A further molecule working as gasotransmitter is H\textsubscript{2}S. Hydrogen sulfide is produced from the amino acid l-cysteine by the action of the enzymes cystathionin-β-synthase (CBS) and cystathionine-γ-lyase (CSE).\textsuperscript{23} The best characterised actions of this gasotransmitter concern circulation as H\textsubscript{2}S induces vasodilatation, decreases cardiac inotropy, and inhibits the proliferation of vascular smooth muscle cells.\textsuperscript{24} Two main intracellular action sites of H\textsubscript{2}S are known from different cell systems. This includes the ability of H\textsubscript{2}S to stimulate ATP-sensitive K\textsuperscript{+} channels, an effect that is well characterized e.g. at smooth muscle cells from rat aorta resulting in a lowering of blood pressure,\textsuperscript{25} or at rat insulinoma cells, where H\textsubscript{2}S modulates insulin secretion.\textsuperscript{26} A further effect of H\textsubscript{2}S consists of an increase in the cytosolic Ca\textsuperscript{2+} concentration, demonstrated e.g. at microglial cells from the rat.\textsuperscript{27} In these cells H\textsubscript{2}S stimulates both the release of stored Ca\textsuperscript{2+} from thapsigargin-sensitive stores, as well as an influx of extracellular Ca\textsuperscript{2+}.

In the gastrointestinal tract H\textsubscript{2}S is known to relax smooth muscle from different species\textsuperscript{28} and to induce anion secretion across the colonic epithelium.\textsuperscript{29} There is evidence for the spontaneous production of H\textsubscript{2}S within the gut wall\textsuperscript{30} and for the expression of the key enzymes for H\textsubscript{2}S formation in enteric neurones\textsuperscript{30} or the intestinal epithelium.\textsuperscript{31} However, the action sites of H\textsubscript{2}S involved in the induction of colonic secretion seem to differ strongly in a species-dependent manner. In guinea-pig colon an exogenous H\textsubscript{2}S donor such as...
NaHS induces a Cl⁻ secretion via stimulation of secretomotor neurones of the submucosal plexus; an action which is probably mediated by capsaicin-sensitive transient receptor potential vanilloid 1 (TRPV 1) ion channels. In contrast, in rat colon the same H₂S donor induces a polyphasic Cl⁻/H⁺ secretion, which leads to an increase in short-circuit current transiently interrupted by a negative current (assumed to represent a transient K⁺ secretion).

Hydrogen sulfide exerts several actions at the basolateral membrane (Fig. 3). Experiments with blockers of different types of K⁺ channels demonstrate that the H₂S donor NaHS has a biphasic effect (an initial inhibition followed by a stimulation) on two types of basolateral K⁺ conductances, i.e. ATP-sensitive K⁺ channels, which are inhibited by glibenclamide, and Ca²⁺-dependent K⁺ channels, which are sensitive to quaternary amines such as tetrapentylammonium. This forces anion secretion by enhancing the driving force for Cl⁻ efflux across the apical membrane. The action of H₂S on basolateral K⁺ channels is supported by a biphasic change in the 3Na⁺/2K⁺-pump current. Experiments at fura 2-loaded colonic crypts demonstrate that the modulation of the basolateral K⁺ conductance is paralleled by a change in the cytosolic Ca²⁺ concentration, which consists in a transient fall followed by a long-lasting increase. Different mechanisms underly these two phases: the initial fall is probably mediated by an efflux of Ca²⁺ from the cytosol into the extracellular space. This response is dependent on the presence of extracellular Na⁺ and is blocked by 2,4-dichlorobenzamil, an inhibitor of the Na⁺–Ca²⁺-exchanger, suggesting a transient stimulation of Ca²⁺ outflow by this transporter, which was directly demonstrated by Mn²⁺ quenching experiments. In contrast, the secondary rise of the cytosolic Ca²⁺ concentration was independent from the presence of extracellular Ca²⁺. The pathway, by which this influx occurs, is not known. Possible effects of H₂S on apical ion conductances are still to be examined.

4. CARBON MONOXIDE

A third gaseous molecule has been recognized to act as gasotransmitter in mammals, i.e. CO. Carbon monoxide is a product generated from the metabolism of heme, contained e.g. in hemoglobin or many other cellular enzymes such as e.g. catalase, by the action of heme oxygenases. This process is catalysed by two enzymes, the inducible isoform heme oxygenase I (HO-I) and the constitutive isoform heme oxygenase II (HO-II). A third isoform, HO-III, has been cloned but is probably of minor functional significance because it catalyzes heme degradation to a much smaller degree than the other two enzymes. Like NO, carbon monoxide can stimulate the soluble guanylate cyclase in cells by binding to the heme iron in the enzyme and thereby enhance the intracellular production of cGMP. Although the stimulation...
of cellular cGMP production by CO has clearly been shown, at the level of the isolated enzyme CO is a quite weak activator of the soluble guanylate cyclase when compared with the action of NO.\(^3\)\(^7\) A further action site of CO, which has been characterized \(e.g\). in porcine arteriolar smooth muscle cells, is a direct, \(i.e\). cGMP-independent activation of \(K^+\) channels leading to a hyperpolarisation of the membrane.\(^3\)\(^9\) Another interaction known with ion channels is the cGMP-independent inhibition of \(Na^+\) channels in alveolar epithelium, which may be caused by a modification of histidine residues in the ion channels (or regulators of them) as concluded from experiments with diethyl pyrocarbonate, a histidine-modifying agent.\(^3\)\(^8\)

In the gut wall, enteric neurones, the key players in the regulation of intestinal functions, express the enzymes for CO production such as heme oxygenase II.\(^3\)\(^9\),\(^4\)\(^0\) Both heme oxygenase I and heme oxygenase II are expressed in the rat colon as observed immunohistochemically and by reverse transcription-polymerase chain reaction (RT-PCR). Immuno-reactivity of heme oxygenase I was found mainly in the muscularis propria whereas heme oxygenase II was localized in addition within the colonic epithelium.\(^4\)\(^1\) A CO-releasing molecule, tricarbonyldichlororuthenium(II) dimer (CORM-2), evokes a concentration-dependent anion secretion carried by changes in the transepithelial transport of \(Cl^-\) and \(HCO_3^-\).\(^4\)\(^2\) A similar induction of \(Cl^-\) secretion by a CO donor or pretreatment with heme to stimulate endogenous CO production is already known from the colonic tumor cell line, Caco-2.\(^4\)\(^3\) In these cells, the CO-induced secretion was reduced by an inhibitor of the soluble guanylate cyclase (ODQ; 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one), which is consistent with the ‘classical’ CO signalling pathway. However, CORM-2 induced anion secretion in rat distal colon was neither affected by ODQ nor reduced after pretreatment with an activator of this pathway (YC1; 3-[5’-hydroxymethyl-2’-furyl]-1-benzylindazole) suggesting an action of CO independent from the stimulation of this enzyme.

Carbon monoxide possesses action sites both at the apical and the basolateral membrane. In basolaterally depolarized epithelia, CORM-2 stimulated a \(Cl^-\) conductance which was sensitive against a typical \(Cl^-\) channel blocker such as 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB). The most simple explanation for this observation would be the assumption of a direct interaction of CO with apical anion channels. In the basolateral membrane, CORM-2 activated a \(Ba^{2+}\)-sensitive \(K^+\) conductance. Again, this was paralleled by an increase in the cytosolic \(Ca^{2+}\) concentration suggesting an activation of \(Ca^{2+}\)-dependent \(K^+\) channels by the CO donor (Fig. 4). The increase in the cytosolic \(Ca^{2+}\) concentration depended on the influx of extracellular \(Ca^{2+}\) but not on a release of \(Ca^{2+}\) from intracellular stores. The pathway, by which this \(Ca^{2+}\) influx occurs, is unknown; putative candidates for colonic epithelial cells are the capacitative \(Ca^{2+}\) influx, which is mediated by nonselective cation channels in these cells,\(^4\)\(^4\) or the \(Na^+-\)\(Ca^{2+}\) exchanger, which can act as a \(Ca^{2+}\)-loading mechanism when working in the reverse mode.\(^4\)\(^5\)

5. OUTLOOK

Gasotransmitters represent a relatively new pathway to modulate physiological functions including regulation of colonic ion transport. They are not only produced in the enteric nervous system to be released during excitation of these neurones, but the enzymes involved in their synthesis are also found within the intestinal epithelium suggesting a role in paracrine regulation of epithelial functions. A further site of production, at least in the case of \(H_2S\), is the microbial flora which can use (as alternative to methane) this gas as a kind of hydrogen sink to bind \(H_2\) produced during fermentation of carbohydrates. Consequently, there seems to be an exciting interaction between the key players in the regulation of intestinal functions \(via\) gasotransmitters, which has still to be elucidated in future experiments.

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