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BASIC–ALIMENTARY TRACT

Inhibition of Hydrogen Sulfide Generation Contributes to Gastric Injury Caused by Anti-Inflammatory Nonsteroidal Drugs

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Background & Aims: **Hydrogen sulfide (H_2S), an endogenous gaseous mediator that causes vasodilation, is generated in mammalian tissues by cystathionine β-synthase (CBS) and cystathionine-γ-lyase (CSE). Here, we have investigated the role of H_2S in a rodent model of nonsteroidal anti-inflammatory drug (NSAID) gastropathy.** ***Methods:*** **Rats were given acetyl salycilic acid (ASA) or an NSAID alone or in combination with NaHS, an H_2S donor, and killed 3 hours later. Gastric blood flow was measured by laser-Doppler flowmetry, whereas intravital microscopy was used to quantify adhesion of leukocytes to mesenteric postcapillary endothelium.** ***Results:*** **At a dose of 100 μmol/kg, NaHS attenuated by 60%–70% the gastric mucosal injury, and tumor necrosis factor (TNF)-α, intercellular adhesion molecule (ICAM)-1, and lymphocyte function-associated antigen (LFA)-1 mRNA up-regulation induced by NSAIDs ($P < .05$) NaHS administration prevented the associated reduction of gastric mucosal blood flow ($P < .05$) and reduced ASA-induced leukocyte adherence in mesenteric venules. NaHS did not affect suppression of prostaglandin E_2 (PGE_2) synthesis by NSAIDs. Glibenclamide, a K_{ATP} channel inhibitor, and DL-propargylglycine, a CSE inhibitor, exacerbated, whereas pinacidil, a K_{ATP} opener, attenuated gastric injury caused by ASA. Exposure to NSAIDs reduced H_2S formation and CSE expression (mRNA and protein) and activity by 60%–70%. By promoter deletion and mutation analysis, an Sp1 consensus site was identified in the CSE promoter. Exposure to NSAIDs inhibits Sp1 binding to its promoter and abrogates CSE expression in HEK-293 cells transfected with a vector containing the core CSE promoter. Exposure to NSAIDs inhibits Sp1 and ERK phosphorylation.** ***Conclusions:*** **These data establish a physiologic role for H_2S in regulating the gastric microcirculation and identify CSE as a novel target for ASA/NSAIDs.**

Gaseous transmitters are a growing family of regulatory molecules involved in multilevel regulation of physiologic and pathologic functions in mammalian tissues.[1] Although nitric oxide (NO) is the best characterized member of this family, it is increasingly recognized that carbon monoxide (CO) and hydrogen sulfide (H_2S) might also function as gaseous mediators in mammalian cells.[1,2]

H_2S synthesis from cysteine occurs naturally in a range of mammalian tissues principally through the activity of 2 pyridoxal-5′-phosphate-dependent enzymes, cystathionine γ lyase (CSE) and cystathionine β synthetase (CBS), although alternative sources (eg, by activity of cysteine aminotransferase and/or 3- mercaptosulphurtransferase) cannot yet be discounted.[1–4] In some tissues, CBS and CSE are both required for generation of H_2S, whereas, in others, one enzyme suffices. CBS-derived H_2S is a physiologically relevant neurotransmitter in the central nervous system in which exposure to this gaseous transmitter[1] results in activation of adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels. In the cardiovascular system, H_2S, mostly derived from CSE, modulates endothelium-dependent and -independent vasodilation.[1,5,6] Highlighting the functional role of H_2S, CBS deficiency leads to hyper-homocyst(e)inemia, a condition that includes elevated serum levels of homocysteine, homocystine, or homocysteine-mixed disulfides, and is associated with increased blood pressure and endothelial dysfunction.[7] In rodents, CBS/CSE deficiency induced by

Abbreviations used in this paper: CBS, cystathionine β-synthase; CSE, cystathionine-γ-lyase; H_2S, hydrogen sulfide; MPO, myeloperoxidase; NO, nitric oxide; PGE_2, prostaglandin E_2.

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genetic-deletion[8,9] or chronic treatment with DL-propargylglycine, an irreversible and selective inhibitor of CSE,[10] results in reduced nitric oxide (NO) bioactivity. This leads to severe endothelial dysfunction characterized by impaired aortic relaxation to acetylcholine and a paradoxical vasoconstriction of mesenteric microvessels in response to bradykinin.[9]

Gastrointestinal injury is a common complication of nonsteroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid (ASA) therapy.[11] Owing to the inhibition of cyclooxygenase (COX) isoenzymes in the gastrointestinal tract, ASA and NSAIDs reduce the intrinsic ability of the gastric mucosa to resist injury induced by endogenous and exogenous agents.[11,12] Thus, inhibition of generation of COX-1- and COX-2-derived eicosanoids (prostaglandin E_2 [PGE_2] and the lipoxin analogue, aspirin-triggered lipoxin) results in altered gastric mucosal blood flow and increased leukocyte-endothelial adhesive interactions in the gastric microcirculation, an essential step in the process of acute gastric injury caused by ASA/NSAIDs.[13–15] Human and animal studies have highlighted the role of gaseous mediators, particularly NO, in maintaining gastric mucosal integrity.[16,17] Thus, by modulating expression/activity of adhesion molecules at the leukocyte-endothelium interface and by maintaining gastric mucosal blood flow, NO compensates for depressed generation of protective eicosanoids.[18] Whether or not the gastric mucosa has the ability to generate H_2S and the regulatory functions exerted by this gaseous mediator in this tissue is unknown.

Materials and Methods

Materials

Aspirin; indomethacin; ketoprofen; diclofenac; lipopolysaccharide (LPS; *Escherichia coli* 0111:B4 serotype); sodium hydrogen sulfide (NaHS); L-cysteine, glibenclamide, a K_{ATP} channel blocker; pinacidil, a K_{ATP} opener; DL-propargylglycine; antiphosphoserine antibody; and all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). The stock solution of NaHS was freshly prepared by dissolving NaHS immediately before use. All tissue culture reagents, including minimal essential medium (MEM), fetal bovine serum (FBS), penicillin, and streptomycin, were obtained from Gibco (Milan, Italy). Filtered, deionized water was used for buffer preparation. Silver and sulphide ion selective electrodes were from ThermoOrion (Beverly, MA). The anti-human Sp1 antibody was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and anti-ERK and anti-phospho-ERK antibody were obtained from New England Biolabs (Beverly, MA). The anti-CSE antibody was a generous gift of Dr. N. Nishi, Department of Endocrinology, Kagawa Medical School, Kagawa, Japan.

Acute Damage

All studies were approved by the animal study committees of the University of Perugia and the University of Calgary. Male Wistar rats (225–275 g) were obtained from Charles River Breeding Farms (Monza, Italy, or Montreal, Canada) and maintained on standard laboratory rat chow on a 12-hour light/dark cycle. Rats were deprived of food for 24 hours before being given one of the following orally: ASA (30 mg/kg), indomethacin (10 mg/kg), ketoprofen (30 mg/kg), ASA (30 mg/kg) plus celecoxib (100 mg/kg), or vehicle.[13–15] Rats were killed 3 hours later, and gastric mucosal damage was measured. To assess gastric mucosal damage, the lengths (in mm) of all lesions were measured with a digital calliper, and a "gastric damage score" was calculated for each stomach by summing these values.[13] This assessment was performed by an individual blinded to the treatments the rats had received. In addition, samples of the body region of the stomach were excised and processed, as described previously,[15] for measurement of myeloperoxidase (MPO) activity as an index of leukocyte accumulation in the tissue.

To investigate whether H_2S administration could prevent the gastric injury caused by NSAIDs, rats were treated intraperitoneally with NaHS, as the H_2S donor, at doses of 25, 50, 100, or 150 μmol/kg or with vehicle, and 30 minutes later were given ASA orally at a dose of 50 mg/kg.[13–15] The rats were killed 3 hours later, and gastric mucosal damage and MPO activity were evaluated as described above. In these experiments, NaHS was used as the H_2S donor for the following reasons: (1) NaHS dissociates to Na^+ and HS^- in solution, then HS^- associates with H^+ and produces H_2S. At physiologic pH, ≃one third of the H_2S exists as the undissociated form (H_2S), whereas the remaining two thirds exists as HS^- at equilibrium with H_2S; (2) the use of NaHS enables us to define the concentrations of H_2S in solution more accurately and reproducibly than bubbling H_2S gas; (3) the influence of Na^+ ions (less than 1 mmol/L) is negligible; and (4) NaHS at concentrations used in the present study does not change the pH of the medium.

To investigate the role of endogenous H_2S in modulating gastric mucosal resistance to damage induced by ASA and NSAIDs, rats were pretreated with DL-propargylglycine[5,10] at a dose of 10 mg/kg per day for 5 days and then administered ASA (50 mg/kg). The rats were killed 3 hours later, and gastric mucosal injury and MPO were assessed. In another set of experiments, we determined whether or not cysteine or N-acetylcysteine, which are known to release H_2S after enzymatic degradation by CSE and CBS, would influence gastric resistance to ASA-induced damage. Rats were treated with cysteine (15 mg/kg) or N-acetylcysteine (15 mg/kg) prior to oral administraton of ASA (50 mg/kg). Three hours later, the extent of gastric damage was assessed, as described previously.

Finally, to determine whether K_{ATP} channels are involved in the gastric protection afforded by H_2S,[6] rats were pretreated intraperitoneally (IP) with NaHS (100 μmol/kg) alone or in