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Gases as Uremic Toxins: Is There Something in the Air?

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Summary: The field of uremic toxicity comprises the study of a large number of different substances, classified in relation to various characteristics, for example, protein-binding, dimensions, and so forth. The endogenous compounds of a gaseous nature have received much attention lately from the scientific community because of their increasingly recognized importance in health and disease. Among these substances, some are uremic toxins per se, others are related to uremic toxins, or can become toxic under some circumstances. We divided them into two broad categories: organic and inorganic compounds. Among the organic compounds are phenols, indols, 2-methoxyresorcinol, p-hydroxy hippuric acid and phenyl acetic acid, trimethylamine, and dimethylamine; among the inorganic solutes are ammonia, nitric oxide, carbon monoxide, and hydrogen sulfide. In this article, these substances are described in relation to the elements that they affect or by which they are affected in uremia, which are the blood, breath, stools, and the gastrointestinal tract. In addition, the effect of the dialysis procedure on exhaled gases are described.
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The endogenous compounds of a gaseous nature relevant to the field of uremia and uremic toxicity can be divided into two broad categories: organic and inorganic compounds (Table 1). However, the distinction between inorganic and organic compounds is not always clear. In general, inorganic compounds are those that lack carbon and hydrogen atoms. Inorganic compounds also may be defined with reference to what they are not—organic. Organic compounds usually contain carbon bonds in which at least one carbon atom is linked covalently to an atom of another type (commonly hydrogen, oxygen, or nitrogen). In general, organic compounds comprise those solutes with planar carbon moieties, including a carbon–carbon or a carbon–oxygen double bond, and saturated organic compounds, whose carbon atoms are tetrahedral.

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ORGANIC VOLATILE UREMIC TOXINS

In the Blood

Volatile organic compounds (VOCs) in human blood may be of endogenous or exogenous origin. The alveolar gradient (chemical species concentration in breath minus its concentration in inspired air) is used frequently to differentiate whether a VOC is of primarily endogenous or exogenous origin. VOCs of exogenous origin in the blood are regarded as one of the best surrogates of environmental exposure. Apart from exposure, however, the concentrations of these compounds depend on extrapulmonary elimination. Figure 1 illustrates the origin, distribution, and elimination of VOCs in the body. Some of the volatile compounds in the blood are excreted via the kidneys. Therefore, they may accumulate in renal failure. Among the uremic toxins that have been identified to date, the following groups constitute volatile compounds: phenols, indols, 2-methoxyresorcinol, p-hydroxy hippuric acid, and phenyl acetic acid (PAA). These substances are characterized by their strong pathophysiological effect and an adequate vapor pressure sufficient for extrapulmonary elimination. Many of these compounds are protein-bound and are discussed more extensively in chapter 3 in this issue.[1]

There is solid evidence that volatile phenols may be retained in uremia. Phenol, 2-methoxyresorcinol, p-hydroxy-hippuric acid, and PAA are well-described compounds in this context. These aromatic substances are representative VOCs in the blood, with a documented association to renal function. The serum concentrations of phenol, also known as *carbolic acid* and *phenic acid*, are increased markedly in uremic patients.[2] Phenol belongs to the protein-bound uremic toxins and cannot be eliminated efficiently by

Table 1. Gases in Uremia

Organic	Inorganic
Phenol	Ammonia
2-Methoxyresorcinol	Nitric oxide
P-hydroxy-hippuric acid	Carbon monoxide
Phenyl acetic acid	Hydrogen sulfide
Indols	
2-Methoxyresorcinol	
P-hydroxy hippuric acid	
Phenyl acetic acid	
Trimethylamine dimethylamine	
3-Hydroxy-2-butanone	
Hydroxyacetone	
Isoprene	
Ethane	
4-heptanone	
Dimethyldisulfide	
Dimethyltrisulfide	

hemodialysis.[2] Phenol is produced primarily in the intestine as a bacterial putrefactive metabolite and is excreted by the kidneys.[2] Colon-derived uremic solutes include indole and phenyl compounds.[3] The structural relation of these indoles and phenols to neurotransmitters led to the idea that they may impair cognitive function. Indeed, it recently was shown that they interfere with efflux transport systems of the blood-brain barrier.[2] 2-Methoxyresorcinol is another phenol retained in uremia.[4] Because of its aromatic structure, it is a protein-bound solute as well.

P-hydroxy-hippuric acid is a nitrogen-containing aromatic compound. It is excreted by the kidney and was identified as an inhibitor of the Ca^{2+}–adenosine triphosphatase (ATPase) in end-stage renal disease (ESRD).[5] Besides hormonal changes, inhibition of cellular Ca^{2+}-ATPase was postulated to contribute to uremic toxicity. The concentration of p-hydroxy-hippuric acid correlated negatively with motor nerve conduction velocities. Several organic-like components analyzed by high-performance liquid chromatography, such as hippuric acid and p-hydroxy-hippuric acid, have been shown to be associated with measures of clinical neuropathy, whereas urea, creatinine, urate, and phosphate were not.[6]

PAA is a degradation product of the amino acid phenylalanine. In healthy subjects, PAA acetylates glutamine in both kidney and liver to form phenylacetylglutamine,[7] which is excreted rapidly in the urine. Because of its capacity to divert nitrogen from urea synthesis, phenylacetate has been used therapeutically in children with defects of the urea cycle.[8] PAA accumulates in patients with ESRD.[9] Several biological activities have been identified to date that may contribute to the pathophysiological role of PAA in uremia. The compound inhibits inducible nitric oxide synthase (iNOS) expression, and, consequently, NO production.[9] This mechanism may contribute to increased atherosclerosis and cardiovascular morbidity in patients with ESRD. Interestingly, an association of PAA and arterial vascular properties in patients with chronic kidney disease (CKD) stage 5 has been documented recently.[10] Furthermore, PAA interacts with the innate immune response: PAA has inhibitory effects on the macrophage-killing function, which are mediated by inhibitory effects on transcriptional iNOS regulation.[11] iNOS inhibition by PAA might affect immunoregulatory processes and may influence the aggravation of immunodeficiency of ESRD patients.

In the Breath

Human breath consists mainly of water, nitrogen, oxygen, carbon dioxide, and inert gases. The remaining small fraction of trace components includes several VOCs that determine the breath odor. More than 500 different VOCs have been identified.[10] These volatile substances may be of endogenous origin or may be absorbed from the environment. Physicians have tried to make diagnostic use of specific odors in the exhaled air of their patients for centuries. Hippocrates was one of the first to use the smell of exhaled air for diagnosis. Renal failure is a prominent example of a disease that indeed is associated with a specific breath odor. The fishy smell frequently is referred to as the *uremic fetor*. Nevertheless, the diagnostic use of exhaled VOCs has been rather modest and only some isolated VOCs of uremic fetor have been identified thus far. In this article, we present the chemistry, metabolism, origin, and removal of those VOCs that have been identified to date.

Uremia is associated with the presence of excessive nitrogenous waste products (eg, urea and ammonia).
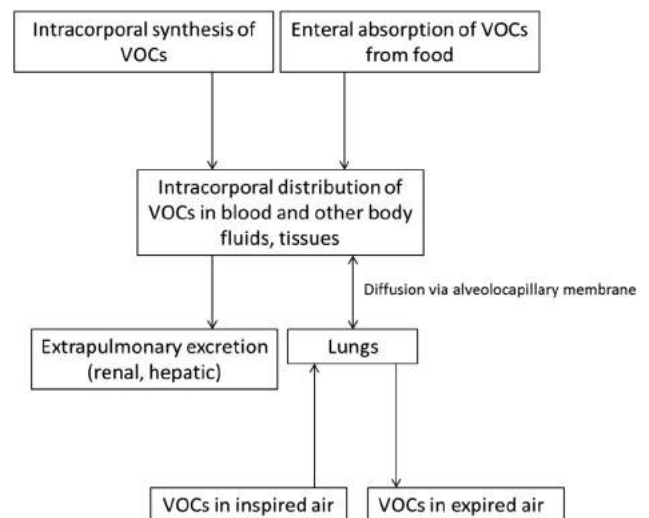
Figure 1. Origin, distribution, and elimination of VOCs in the body.

From a chemical standpoint, ammonia is inorganic, whereas urea is organic. However, ammonia is a key biochemical intermediate because it derives from the amino acid amino groups and, in turn, is converted extensively into urea (ie, the organic end product of this metabolism) in the liver. As explained later, ammonia also is produced by microbial breakdown of urea in the intestine. Therefore, we chose to discuss ammonia together with the organic compounds because it appears more appropriate from a pathophysiological point of view.

The odor of uremic breath frequently has been described as "ammoniacal," "fishy," or "fetid," and has been compared with the smell of stale urine.[10] Ammonia (NH_3) has been detected as one of the compounds underlying this specific odor and assessment of ammonia concentrations in the breath has been postulated to be a possible tool to monitor the efficacy of hemodialysis.[12–14] Moreover, it has been shown that breath ammonia is correlated with blood urea nitrogen and creatinine levels and it was concluded that breath ammonia measurements could be used as a surrogate measure of the retention status of patients with renal failure.[12,15] Ammonia, a colorless gas, is a product of protein catabolism. Ammonia is excreted by healthy kidneys as positively charged ammonium ions or after conversion to urea in the liver. Therefore, both kidney and liver disease may lead to increased concentrations of ammonia in the breath. The bronchial and bronchiolar transfer from the blood to the exhaled air is conducted by Rhesus glycoproteins Rhesus BG and Rhesus CG, whereas the alveolar transport is diffusive or via other transporters.[16] With increased salivary urea concentration, bacterial hydrolyzation leads to a local breakdown of urea to ammonia in the mouth, thus inducing a further increase of the ammonia concentration in uremic breath.[17] Breath ammonia concentrations have been found to be correlated positively to blood urea levels in cyclic automated peritoneal dialysis and hemodialysis patients.[10,14] A recent study showed that ammonia concentrations in the breath also are increased in chronic renal failure not requiring dialysis.[18]

In 1977, Simenhoff et al[17] already found that, apart from ammonia, trimethylamine (TMA) and dimethylamine (DMA) underlie the fishy odor in uremia. TMA and DMA are aliphatic amines, boiling between -6°C and -7°C, thus being highly volatile. DMA and TMA have similar "rotten-fish" smells.[17] TMA is slightly more volatile at room temperature and has an olfactory potency approximately 100 times greater than DMA.[17] TMA is produced in the gut by bacterial metabolism of choline-rich foods, such as eggs, certain vegetables, and organ meat. It is excreted from the body either in an unmetabolized manner in urine, sweat, breath, saliva, and semen, or it is oxidized to trimethylamine

N-oxide (a nonodorous compound) in the liver.[19] ESRD is associated with highly increased concentrations of TMA in the breath that decrease to about half during hemodialysis.[17] This finding has been confirmed repeatedly.[14,20]

Interestingly, DMA is the major metabolite of asymmetric dimethylarginine (ADMA) in the urine. ADMA is produced after the catabolism of proteins containing methylated arginine residues and is metabolized by the enzyme dimethylarginine dimethylaminohydrolase, yielding DMA and citrulline.[21] ADMA is a well-described uremic toxin and an endogenous inhibitor of NOS.[22] It is discussed in more detail in chapter 2 in this issue.[23]

A recent study used a combination of gas chromatography and ion mobility spectrometry for the identification of VOCs retained in uremia. The trial comprised subjects with normal renal function (healthy controls), subjects with CKD corresponding to an estimated glomerular filtration rate of 10 to 59 mL/min/1.73 m^2, and subjects with ESRD before and after a hemodialysis session. It confirmed the findings on ammonia and DMA/TMA as described earlier.[18] Moreover, the study showed that 3-hydroxy-2-butanone (acetoin) and hydroxyacetone accumulated with decreasing renal function and were eliminated by dialysis. 3-Hydroxy-2-butanone is an organic compound generated primarily by bacteria and plants. Acetoin is used for the production of artificial flavors and is a natural ingredient of yogurt, and several fruits and vegetables. Thus, 3-hydroxy-2-butanone in the breath may be of exogenous origin. In pathologic situations, however, it may be generated endogenously as well: an increased concentration of 3-hydroxy-2-butanone in the breath has been found in patients with tumors such as lung tumors or hepatocellular carcinoma.[24–26] According to Phillipps et al,[26] 3-hydroxy-2-butanone may be considered an oxidative product of butane. The production of 3-hydroxy-2-butanone therefore represents increased oxidative activity, which is regarded to be the reason for its predictive value in the detection of lung cancer.[26] It may be speculated that renal failure may lead to the endogenous production of 3-hydroxy-2-butanone as well. Independently from its origin, the data of the earlier-mentioned study suggest a renal elimination.[18]

In contrast to acetone, there are no data on hydroxyacetone in human blood or breath available. Acetone accumulates in the breath in diabetic ketoacidosis and liver disease. In the latter, it contributes to the sweet, musty aroma, called *fetor hepaticus*. Hydroxyacetone belongs to the group of oxygenated atmospheric VOCs.[27] It represents an oxidation product of isoprene (2-methyl-1,3-butadiene), the most abundant hydrocarbon present in exhaled breath.[28] Isoprene is produced by leafy plants.[29] Moreover, it is a by-product of

cholesterol synthesis, during the conversion of mevalonate to mevanolate-5-pyrophosphate and isopentenyl pyrophosphate.[30] Accordingly, breath isoprene concentrations decrease during treatment with statins or during a cholesterol-enriched diet.[31] Increases in isoprene concentrations in the breath have been observed in states of acute tissue injury such as myocardial infarction and acute lung injury.[32,33] A recent study on gas chromatographic breath analysis of uremic rats showed an early increase of isoprene after induction of kidney injury.[34] In human beings, current data indicate an increase of isoprene in the breath during a hemodialysis session.[35,36] Thus, it remains elusive whether the increase of isoprene in the rat model is a consequence of the injury of the ischemic renal tissue or if it indeed reflects the retention of uremic toxins. Accordingly, it remains unclear whether the increased concentration of isoprene may underlie the increase of hydroxyacetone in the exhaled breath of subjects with renal failure.

The concentration of breath ethane in peritoneal dialysis and hemodialysis patients has been compared with healthy controls.[37] The results, however, did not indicate an evident association with renal function because the median concentration of ethane did not differ between peritoneal dialysis patients and healthy controls, and only 50% of the hemodialysis patients showed increased ethane concentrations. Measurement of hydrocarbons such as ethane can reflect oxidative stress because breath hydrocarbons have been associated directly with in vivo breakdown of lipid hydroperoxides.[37] Thus, increased levels of ethane in the breath of hemodialysis patients may be a consequence of increased oxidative stress rather than of decreased elimination of ethane.

The earlier-mentioned study identified 4-heptanone as a VOC that appeared almost exclusively in the breath of participants undergoing hemodialysis.[20] It thereby differs from compounds such as ammonia, TMA, DMA, or acetoin that show increasing concentrations in patients with renal failure both with and without hemodialysis. This indicates that it may be of exogenous rather than of endogenous origin. Indeed, 4-heptanone is a metabolite of diethylhexylphthalate, the most frequently used industrial emollient in synthetic materials. Phthalates are industrial chemicals used as plasticizers, softeners, adhesives, or solvents and are used in hemodialysis tubing systems. 4-Heptanone has been described previously in the plasma of hemodialysis patients as a degradation product of diethylhexylphthalate.[38] In analogy to 4-heptanone, 4-heptanal and 2-heptanone preferentially or exclusively occurred in patients undergoing hemodialysis as well.[18]

The question arises of why the analysis of exhaled VOCs has not been used as a clinical diagnostic tool thus far, although uremic fetor has long been believed to be characteristic of renal failure. There are two obvious reasons. For a long time, the technical complexity of breath sampling, preconcentration, multiple-step separations, and analyses of spectra has impeded attempts such as this. New developments such as the combination of multicolumn gas chromatography and ion mobility spectrometry may be promising candidates for the future in this context because they deliver a result within minutes. Second, the VOCs in exhaled breath may be of endogenous and exogenous origin, as explained earlier. The contribution of environmental factors, however, causes a greater variability of results compared with an exclusively endogenous compound and might necessitate the consideration of a combination of parameters.[39] At the moment, it appears improbable that one VOC in the breath has sufficient sensitivity and specificity to act as a single marker for the detection of impaired renal function.

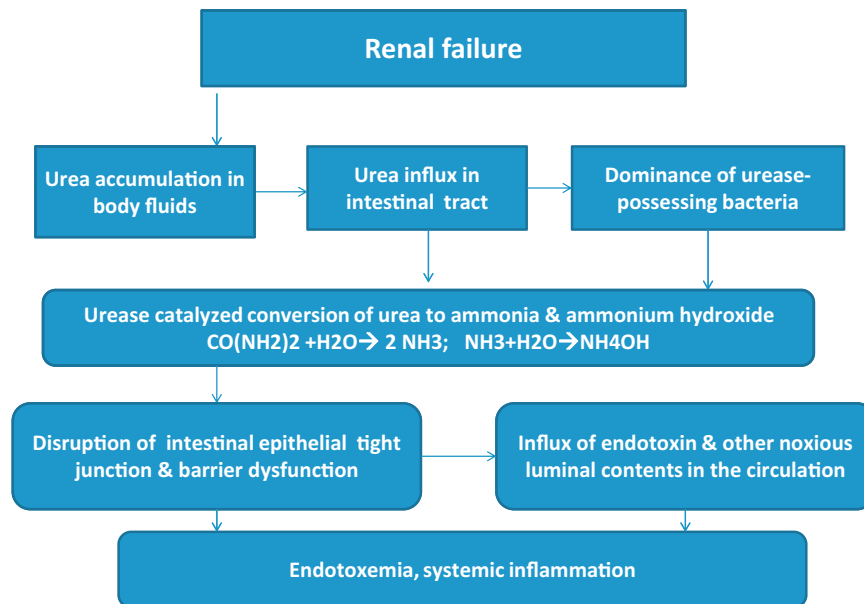## In the Stools and Gastrointestinal Tract

Ammonia

Accumulation of urea in the body fluids as a result of renal failure leads to its influx into the gastrointestinal tract via passive diffusion and incorporation in glandular secretions.[40] Hydrolysis of urea within the gastrointestinal tract leads to formation of large quantities of ammonia ($CO[NH_2]2 + H_2O \rightarrow CO_2 + 2NH_3$). This process is catalyzed by urease, which is present in several microbial species in the intestinal flora. Most of the ammonia generated in the gut is converted to ammonium hydroxide ($NH_3 + H_2O \rightarrow NH_4OH$), which accounts for the increased pH of the intestinal milieu in uremic patients.[41,42] Ammonium hydroxide (the main ingredient of window cleaning liquids) is a detergent, which can cause tissue damage. In recent studies, Vaziri et al[43,44] showed that by damaging the intestinal epithelial barrier structure and function, urea-derived ammonia and ammonium hydroxide in the intestinal tract contribute to the CKD-associated systemic inflammation. Systemic inflammation plays a major role in the progression of CKD and its cardiovascular risk and numerous other complications.[45–47] As described in a recent review, there is mounting evidence pointing to the role of increased intestinal permeability and intestinal barrier dysfunction in the pathogenesis of systemic inflammation in patients and animals with advanced renal disease.[48] However, until recently, the mechanisms by which uremia increased intestinal epithelial permeability were not known. In an earlier study, Vaziri et al[49] found heavy losses of the key protein constituents of colonic epithelial tight junctions (claudin-1, occludin, and zonula occludens-1) in rats with CKD. In a more recent study, similar defects in gastric, jejunal, and ileal epithelial tight junctions in

CKD animals were found.[50] Endotoxemia commonly is present and is a major cause of inflammation in patients with advanced CKD.[51] Because the epithelial tight junction is the main barrier against entry of endotoxin and other noxious products in the circulation, the results of the latter studies elucidated one of the possible sources of CKD-associated endotoxemia. In a follow-up study,[52] the investigators asked whether CKD-induced disruption of the intestinal epithelial barrier is caused by retained uremic toxins or metabolites. To address this issue, they compared the effects of predialysis and posthemodialysis plasma samples from ESRD patients and plasma from healthy controls on the epithelial barrier function and structure in cultured human colonocytes seeded on Transwell plates (Sigma-Aldrich Inc, St. Louis, MO) to form a polarized, impermeable monolayer. They found that compared with control plasma, incubation in media containing ESRD patients' predialysis plasma caused a marked decrease in transepithelial electrical resistance and a significant loss of the tight junction proteins. These findings showed the ability of human uremic plasma to increase epithelial permeability via depletion of tight junction apparatus. The extent of the epithelial barrier damage and dysfunction was significantly less in cells exposed to the postdialysis than the predialysis plasma. These findings pointed to some dialyzable uremic retention product(s) as the cause of uremia-induced intestinal epithelial barrier disruption. In a subsequent study, they tested the hypothesis that the

heavy influx of urea into the gastrointestinal tract and its conversion by microbial urease to ammonia may be responsible for the uremia-induced disruption of the intestinal epithelial barrier structure and function. To this end they incubated the fully polarized human colonocytes in media containing clinically relevant concentrations of urea. To simulate the presence of microbial flora, the experiments were repeated by adding urea plus urease to the culture media. Urea caused a concentration-dependent reduction in the tight junction proteins and in transepithelial electrical resistance. The addition of urease plus urea resulted in cell detachment, dissipation of transepithelial electrical resistance, and massive loss of the tight junction proteins.[43] These experiments documented that uremia-induced disruption of intestinal barrier function is, in part, mediated by urea, which previously had been considered to be a nontoxic retained metabolite (Fig. 2). These findings showed a novel mechanism for the previously documented salutary effect of urea-lowering strategies (eg, low-protein diet and longer and more frequent dialysis regimens in advanced CKD). Clinical studies with an impact on uremic solute removal and on outcomes are reviewed in depth in chapter 7 in this issue.[53]

Activated charcoal commonly is used as a detoxificant to treat acute poisoning and as a degassing agent to reduce flatulence and abdominal bloating. AST-120, a potent activated charcoal preparation has been shown to decrease the plasma concentration of p-cresol sulfate

Figure 2. Renal failure results in accumulation of urea in the body fluids, which leads to massive diffusion of urea in the gastrointestinal tract. The increase in urea concentration in the gastrointestinal tract leads to the dominance of urease-possessing bacteria. Hydrolysis of urea by microbial urease leads to formation of massive amounts of ammonia and ammonium hydroxide, which causes disruption of transcellular and intracellular tight junction proteins. This phenomenon contributes to systemic inflammation by enabling entry of endotoxin and other noxious luminal contents in the intestinal wall and systemic circulation.

and indoxyl sulfate,[54,55] which are among the uremic toxins originating from p-cresol and indole generated by the colonic microbial flora.[3,56,57] In addition, AST-120 administration in animal models of CKD attenuates inflammation and oxidative stress and slows the progression of renal disease.[58–60] The beneficial effects of AST-120 primarily have been attributed to its ability to reduce production and absorption of p-cresol sulfate and indoxyl sulfate.[61,62] Because activated charcoal avidly can adsorb ammonia, a major mediator of the CKD-induced intestinal epithelial barrier destruction and dysfunction, as mentioned earlier, Vaziri et al[43] have unraveled the central role of urea-derived ammonia as a major mediator of the CKD-induced intestinal epithelial barrier destruction and dysfunction. Since activated charcoal avidly can adsorb ammonia, AST-120 is expected to attenuate the uremia-induced intestinal barrier disruption and the resulting systemic inflammation. Consequently, the widely reported salutary effects of AST 120 in reducing inflammation and oxidative stress may be, in part, owing to the preservation of the intestinal epithelial barrier. To address this possibility, the hypothesis that the administration of activated charcoal (AST-120) may attenuate uremia-induced disruption of intestinal epithelial tight junction and thereby reduce endotoxemia and the severity of systemic inflammation in CKD animals was tested. In confirmation of earlier studies, the untreated CKD animals showed depletion of the colonic epithelial tight junction proteins. This was associated with endotoxemia and increased plasma concentration of chemokines, inflammatory cytokines, and adhesion molecules. Treatment with AST-120 attenuated uremia-induced disruption of colonic epithelial tight junction and the significantly reduced endotoxemia and markers of oxidative stress and inflammation.[44] Taken together, these studies have identified ammonia as a gaseous uremic toxin with important systemic effects.

Sulfated organic gases

Meinardi et al[34] found 56 VOCs in the exhaled breath and 36 VOCs generated by the cultured stools of normal and CKD rats. Many of the gases found in the exhaled breath also were present in those emitted from the cultured feces. This observation points to the intestinal flora as the likely source of many VOCs found in exhaled breath. Comparison of data obtained in the CKD and control rats showed marked increases in the abundance of several sulfur-containing gases including dimethyldisulfide and dimethyltrisulfide and two thioesters generated by cultured feces of uremic animals. In fact, butanethioic acid S-methyl ester was absent in control rat feces. The observed production of large quantities of sulfur-containing gases by the colonic microbial flora in the CKD rats is intriguing

and parallels the well-known increase in production of p-cresyl sulfate and indoxyl sulfate, which are gut-derived sulfur-containing uremic toxic solutes in human beings and animals with CKD.[61,62] The mechanism by which CKD heightens generation of sulfur-containing gases by the colonic microbiome is unknown. However, it may be owing to increased sulfur-containing substrates and/or altered microbial metabolism. A possible source of sulfur as a substrate for the microbiome is mucin, which is secreted by intestinal epithelium and consists of a heavily glycosylated protein containing numerous cysteine residues. Increased mucin production in response to the prevailing gastrointestinal inflammation commonly present in advanced CKD[63] or altered gut microbial flora potentially can contribute to increased sulfur-containing gases. In this context, recent studies have shown a profound effect of CKD on the structure and function of the gut microbiome.[64]

During Dialysis

In a recent study, designed to explore the effect of hemodialysis on the composition of exhaled breath, Lee et al[65] found the rapid appearance and dramatic increase in ESRD patients' exhaled breath of 10 hydrocarbon and 4 halocarbon compounds shortly after the onset of the hemodialysis. To determine whether these gases were produced endogenously in response to the dialysis procedure or were released from the hemodialysis circuit, the investigators carefully extracted and analyzed the gases extracted from the dialysis solution, tubing, and dialyzers. None of the target gases were found in either the deionized water supply, dialysate concentrate, or the final dialysate solution. In contrast, all of the target gases were profusely present in the polyvinyl chloride (PVC) tubing and/or the two different dialyzers used in their dialysis facility. The gases emitted from the PVC tubes included n-pentane, n-octane, 3-methylhexane, 3-methylheptane, CH_2CHCl, and $CHCl_3$. In addition, substantial quantities of i-butane, n-butane, n-hexane, n-heptane, 3-methylpentane, and CH_3CH_2Cl were emitted from the dialyzers. These findings identified the hemodialysis tubing and dialyzers as the source of the appearance of these compounds in the patients' exhaled breath shortly after the onset of hemodialysis treatment. Among the VOCs released into the patients' circulation during the hemodialysis procedure, CH_2CHCl, CH_2Cl_2, and $CHCl_3$ are known to be carcinogens and toxic to various organs.[66–68] Besides the 14 compounds that appeared in the patients' exhaled breath during dialysis and were found in the dialyzers and PVC tubing, five other compounds (ie, tetrahydrofuran, chlorobenzene, butanal, 2-butanone [methylene ethylene ketone], and cyclohexanone) were

released from the PVC tubing and dialyzers, but did not appear in the patients' exhaled breath. The reason for the failure of the latter gases to appear in the exhaled breath is unclear. However, it may have been caused by their retention in the patients' bodies via a chemical interaction with various molecules in the blood, lungs, or other tissues. If this is true, then the cumulative retention of these compounds in patients maintained on hemodialysis treatment may have adverse consequences. Taken together, recurrent and chronic exposure to these compounds may contribute to the high morbidity and mortality in the dialysis population. This issue should be considered in the manufacturing of the future generation of dialyzers and dialysis tubing sets.

INORGANIC VOLATILE UREMIC TOXINS

Among the inorganic uremic toxins, we can consider water, sodium, potassium, hydrogen ions, phosphate, magnesium, sulfate, and trace elements. Ammonia was discussed earlier, together with some related organic nitrogenous waste products. Among gaseous compounds, gases identified by standard blood gas analysis are not discussed here. The inorganic volatile compounds of NO, CO, and hydrogen sulfide (H_2S) are gases with special interest in the field of uremia. In fact, this is a "gaseous triumvirate,"[69] with effects on the kidney, renal failure progression, and ESRD. They cannot be considered pure uremic toxins because their concentration in blood and tissues is not increased uniformly. For example, they can be increased or decreased in uremia depending on the variable (dialysis, and so forth). However, they are metabolically or functionally related to uremic toxins, and can be beneficial or toxic according to the model, the concentration, and conditions. In addition, they are able to interact between themselves and influence their respective actions in a complex manner.

All of these gases have the following in common: in the past, they were considered to be just toxic compounds and environmental pollutants, and only recently have their biological functions been appreciated, thus refreshing the quote from Paracelsus: "*Dosis sola facit venenum*" (the dosage makes it either a poison or a remedy). In addition, small size, brief half-life, and the capacity to easily cross membranes are shared features.

Nitric Oxide

In biological systems, NO, a molecular gas, is formed by one of the three isoforms of the enzyme NOS.[70,71] These isoforms were named based on the cell types in which they were first isolated, and are as follows: type I or neuronal cell NOS (nNOS), type II or iNOS, and type III or endothelial cell NOS (eNOS). These enzymes are distinct proteins encoded by genes on different chromosomes, but all three catalyze the adduction of the guanidino nitrogen of the amino acid L-arginine, present as an amino acid residue in proteins, to molecular oxygen, yielding NO and water. Although the three isoforms of NOS catalyze the same reaction, the regulation of their activity varies. Both nNOS and eNOS are expressed constitutively and produce low amounts of NO, in relation with changes in the intracellular calcium concentration. In contrast, iNOS strongly binds to calcium, therefore its function is not influenced by calcium fluxes within the physiologic range. Therefore, it has the capacity to generate large quantities of NO when transcriptionally up-regulated by the inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin 1-β, and interferon-γ.

NO is a paracrine mediator. Consequently, it is produced and released by cells, and readily penetrates the biological membranes of neighboring cells, modulating several signaling cascades. Because it has an extremely short half-life, it exerts its effects locally and transiently.

The most recognized cellular target of NO is heme-containing soluble guanylate cyclase. The stimulation of this compound enhances the synthesis of cyclic guanosine monophosphate (cGMP) from guanosine triphosphate, increasing its cytosolic levels. The effects of NO can be enhanced by inhibiting the breakdown of cGMP, a process catalyzed by a family of phosphodiesterases.

NO also is able to interact with other moieties. First, NO interacts with thiol groups on proteins and small molecules, resulting in the formation of *S*-nitrosothiols. The addition and removal of S-nitrosothiols is a highly regulated process. Second, NO can target Fe/S groups at the catalytic centers of proteins, including hemoglobin.[72] Third, the formation of peroxynitrite from NO and superoxide radical has been implicated in cellular toxicity via the propensity of peroxynitrite to induce posttranslational changes in the tyrosine residues of proteins.[71]

Thus, the biologic effects of NO depend on the concentration of NO produced as well as features specific to the local environment (eg, the presence of thiols and superoxide). In addition, higher concentrations can be toxic, damaging cellular constituents (such as DNA) and inducing hypotension in sepsis.[71]

In the blood vessel wall, basal and calcium-agonist stimulated release of NO can account at least in part for the bioactivity of endothelium-derived relaxing factor.[71] In the kidney, as well as other solid organs, physiologic concentrations of NO function as a tonic vasodilator, working essentially instantaneously.[70]

All three NOS isoforms can be expressed in the kidney.[70] nNOS is expressed principally in the macula

densa and the inner medullary collecting duct. iNOS has been localized in several tubule segments (principally the thick medullary ascending limb but also the distal convoluted tubule and proximal tubule), glomerulus, and the interlobular and arcuate arteries. eNOS is expressed in the endothelium of glomerular capillaries, afferent and efferent arterioles, and intrarenal arteries. The greatest enzymatic activity for NO production in the kidney is found in the inner medullary collecting duct (three- to six-fold higher than that observed in the glomeruli).

The most important actions of NO in the kidney are the regulation of renal hemodynamics, the modulation of fluid and electrolyte transport, and the regulation of damage in response to injury. Endothelial-derived NO contributes to maintaining arteriolar dilation by participating in the paracrine control of renal glomerular vascular resistance and mesangial cell tone. Inhibition of NO synthesis or deletion of eNOS genes in animal models results in hypertension and significant reductions in renal plasma flow and glomerular filtration rate.[73]

In some settings, an increase in eNOS activity may be harmful, such as diabetic nephropathy, in which it induces renal vasodilation and glomerular hyperfiltration.[74]

NO is able to influence solute and water transport.[75] NO inhibits sodium entry in the cortical collecting duct, Na-H exchange in the proximal tubule, the apical Na-K-2Cl cotransporter in the outer medulla, and Na^+/K^+-ATPase activity in several nephronal segments. NO also reduces the responsiveness of the collecting tubule to antidiuretic hormone, facilitating water excretion. Intrarenal NO synthesis is increased when salt intake increases, thereby facilitating renal sodium excretion. The administration of NO donors also augments sodium elimination. Acute or chronic inhibition of NO synthesis impairs urinary sodium excretion, even at concentrations that do not affect renal glomerular or systemic hemodynamics.

NO synthesized by nNOS in the macula densa reduces the tubuloglomerular feedback response (the phenomenon through which the sodium chloride concentration in the fluid arriving to the macula densa is sensed by specialized cells, which through the constriction of the afferent arteriole lead to a decrease in the glomerular filtration rate). Micropuncture experiments using NOS antagonists indicate that NO release from the macula densa is a modulating factor that is augmented during increased sodium chloride delivery, thereby countering the afferent arteriole constriction elicited in the tubuloglomerular feedback response. Thus, changes in macula densa NO production may underlie the resetting of tubuloglomerular feedback that occurs when salt intake is varied; the response is blunted appropriately with a high-salt diet because

maintenance of glomerular filtration promotes excretion of the excess salt.[76]

The role of NO in the response to renal injury depends on the cell type and NOS isoform. The vasodilator function of endothelial-derived NO and its ability to inhibit platelet activation and adhesion reduces injury in glomerulonephritis, whereas NO generated by mesangial and tubular epithelial cells may exacerbate damage, owing in part to the ability of these cells to induce the expression of iNOS in response to inflammatory stimuli. Although mesangial cells do not express significant amounts of any NOS isoforms under basal conditions, they can express iNOS, a feature that has been documented in human glomerulonephritis, animal models of glomerular injury, and in vitro experiments using inflammatory cytokines,[77,78] thus inducing cellular injury.

In CKD, NO deficiency is implicated in disease progression. In fact, in animal models, an increase in NO production mediates the normal compensatory hypertrophy, a phenomenon present, for example, in unilateral nephrectomy, thereby slowing progression, whereas impaired availability, as a result of eNOS inhibition or genetic manipulation, accelerates progression. For example, eNOS gene delivery prevents hypertension and reduces renal failure and injury in rats with reduced renal mass.[79] It recently was shown that after uninephrectomy, the eNOS–NO–protein kinase G pathway mediates compensatory renal hypertrophy through Akt-mTOR pathway. The impairment of NO availability associated with endothelial dysfunction promotes the transition from compensatory hypertrophy to kidney damage.[80]

In uremic and hemodialysis patients, NO levels can be increased, normal, or decreased.[81,82] The presence of several variables, such as inflammation, levels of L-arginine (the NO precursor), and levels of asymmetric dimethyl arginine (ADMA, an endogenous NO synthase inhibitor, increased in renal failure) could explain these differences. In addition, the way NO production is evaluated could be important. In one study, in which a NO-deficient diet was administered, NO_x levels (the NO oxidation products nitrate, NO_2^-, and nitrite, NO_3^-, are together termed NO_x) were increased predialysis, but its production was lower during the dialysis procedure compared with controls. It has been proposed that, although an increase in NO production could be present in these patients, an excess consumption offsets this balance.[81–85] However, in general, the overall evidence is in favor of the idea that NO deficiency occurs in renal failure owing to impaired endothelial and renal NO production, the mechanisms include reduced availability of substrate, in particular L-arginine and increased ADMA, because of reduced dimethylarginine dimethylamino hydrolase (the ADMA metabolizing enzyme) activity.[86] In a recent study, eNOS expression was

reduced in resistance arteries from ESRD patients starting dialysis, whereas ADMA levels correlated with endothelial dysfunction.[87]

Homoarginine is an amino acid derivative mainly synthesized in the kidney. Homoarginine is also a precursor of NO (through the increase in intracellular L-arginine), which directly correlates with kidney function in patients with CKD, and low levels are associated significantly with the progression of kidney disease.[88]

NO is inactivated with superoxide to form peroxynitrite (two reactive oxygen species), which reacts with proteins to form nitrotyrosine and nitrocysteine.[71] In addition, NO reacts with thiol groups in proteins to form S-nitrosothiols and with hemoglobin to form nitrosohemoglobin. The latter compounds act as NO reservoirs. S-nitrosylated–based signaling, a ubiquitous and evolutionarily conserved mechanism of control of cellular function, is involved in the regulation of ion channels, receptors, respiratory proteins, and enzymes that ultimately transduce hypoxic signals into increased alveolar ventilation and perfusion, augmentation of cardiac and skeletal muscle performance, and enhances microcirculatory blood flow.[89] Under the oxidative stress common in renal failure, these compounds have been found to be increased.[90] In addition, plasma S-nitrosothiol levels are associated with pulse pressure and predict cardiovascular outcomes.[91,92]

NO is converted to NO_x, which is excreted in urine. Therefore, these compounds, which also can be reused to form NO in a enterosalivary cycle, could be used to assess NO systemic bioactivity, but as mentioned earlier this approach has conflicting results in the setting of hemodialysis patients. It has been shown that hemodialysis removes nitrite and nitrate from the blood and saliva, thereby reducing systemic NO bioactivity. This may have implications relative to the well-known problem of dialysis hypotension and cardiovascular mortality.[93]

In addition, patients with intradialytic hypertension show imbalances in NO and endothelin-1, indirectly suggesting endothelial cell dysfunction. Intradialytic hypertension is in fact associated with significant impairment in markers of in vivo endothelial cell function, such as brachial artery flow-mediated vasodilation normalized for shear stress.[94]

CO

Recent theories have stated that early life forms were able to use and produce CO, a readily available electron donor.[95] CO is formed in the body from the catabolism of heme by the heme oxygenase (HO) enzymes, as well as from lipid oxidation. The production rate is 384 mmol/d, with an average tissue concentration in the nanomolar range.[96]

CO displays many physiological roles in many systems and organs, which all go in the direction of being anti-apoptotic, anti-inflammatory, anti-oxidant, antiproliferative, and vasodilatory.[97] CO mainly signals through the activation of soluble guanylate cyclase (sGC), which increases cellular cGMP levels. CO also can inhibit cytosolic reduced nicotinamide-adenine dinucleotide phosphate oxidase to limit superoxide formation, while it promotes superoxide formation in mitochondria. CO has both stimulatory as well as inhibitory actions on NO production, and inhibits inflammation and apoptosis via activation of mitogen-activated protein kinase, phosphoinositide 3-kinase/Akt, and peroxisome proliferator-activated receptor γ. In general, the direct binding of CO to metal-containing proteins such as sGC, cytochrome P450 proteins, cytochrome c oxidase, reduced nicotinamide-adenine dinucleotide phosphate oxidase, and NOS, is able to confer a conformational change, which can alter their biological activity.[98]

HO enzymes are expressed in the kidney as HO-2 and HO-1 isoforms. HO-2 is constitutive, whereas HO-1 is inducible. CO can act as a vasodilator or as a vasoconstrictor in renal circulation. The vasoconstrictor effects of CO result from the unusual interaction that it has with NO in the vasculature. Low levels of CO enhance NO production and NO has been reported to induce HO-1. On the other hand, inhibition of NO stimulates CO production and an increase in CO, via overexpression of HO-1 in vascular smooth muscle cells, decreases NO production.[99,100] Recently, it was shown that treatment of rat interlobular arteries with a CO donor, CO releasing molecule-3 (CORM-3), resulted in vasoconstriction owing to increases in cellular superoxide production.[101] Additional studies in angiotensin II hypertensive mice showed that although induction of HO-1 decreased but did not normalize blood pressure, it did not normalize and actually resulted in further impairment of acetylcholine-induced NO relaxation.[102] Therefore, it appears that CO can be both a constrictor and dilator in the renal vasculature depending on the balance between the levels of HO/CO and NO.

In renal tubules, CO inhibits superoxide production to decrease the activity of the sodium-potassium-2 chloride transporter (NKCC2) in the thick ascending loop of Henle. Superoxide also decreases NO levels. NO is an endogenous inhibitor of NKCC2 via cGMP-mediated decreases in apical insertion of the transporter. CO also may have a direct effect on the NKCC2 transporter to decrease its activity, resulting in a decrease in sodium reabsorption in the thick ascending loop of Henle.[96]

In several forms of acute kidney injury, such as cisplatin toxicity, CO inhalation therapy and CO releasing molecules can be beneficial.

In hemodialysis patients, preservation of diffusion capacity of the lung for CO was noted.[103] Recent studies using metabolomics showed that CO is able to inhibit cystathionine β-synthase (CBS), an enzyme pertaining to the transsulfuration pathway, producing cystathionine, or in an alternate reaction, H_2S.[104]

In two models of chronic renal failure, chronic tubule-interstitial nephropathy and focal glomerulo-sclerosis, a down-regulation of several anti-oxidant genes, among which HO-1 was noted, as a result of impaired nuclear factor-erythroid-2–related factor 2 activity.[105,106]

H_2S

H_2S represents the third endogenous gasotransmitter, along with NO and CO.[107]

H_2S is a poisonous gas with a typical odor of rotten eggs. H_2S is liberated in toxic amounts by decaying organic matter, or, in refineries and oil gas fields it represents a major safety hazard. However, the first life forms tolerated and even produced H_2S as a metabolic fuel. H_2S thus represents the third endogenous gaso-transmitter, along with NO and CO.[107]

Three enzymes catalyze the formation of H_2S: CBS (EC 4.2.1.22), cystathionine γ-lyase (CSE) (EC 4.4.1.1), and 3-mercaptopyruvate sulfurtransferase (MST) (EC 2.8.1.2).

In the liver, kidney, enterocytes, vascular smooth muscle cells, and endothelial cells, H_2S is formed by CSE, whereas in the brain its production is attributed mainly to CBS. In addition, MST is operative at cardiac, kidney, and brain levels, and in the vascular endothelium.

Measurement

H_2S detection in blood currently is performed either with the spectrophotometric measurement of methyl-ene blue formation, or with electrochemical methods. However, these assays actually measure free H_2S, plus its protein-bound and acid labile moieties. Recent literature has offered a more sensitive high-performance liquid chromatography method capable of characterizing the different moieties.[108] An excellent review on the topic of H_2S measurement is available,[109] which in addition proposes the concept that the presence of large sulfide reserves, being tightly regu-lated, are likely to play a major role in the regulation of endogenous sulfide-mediated biological functions.

Mechanisms of Action

The main effects of H_2S could be owing to its direct action, or are owing to protein *S*-sulfhydration, medi-ated through HS⁻, which occurs at the level of cysteine residues in proteins, leading to the formation of persulfides (-SSH groups).[110] Several proteins have been found to be affected by this posttranslational modification.

From a chemical point of view, it is impossible that H_2S by itself oxidizes protein thiols, therefore an intermediate species has been postulated to exist. Data by Greiner et al[111] show that at least in vitro and in cell culture, these intermediate species are polysulfides (or more generally sulfane sulfur), which very efficiently oxidize protein thiols. To which extent and by which mechanisms polysulfides are endogenously produced in vivo remains to be clarified further.

It is also possible that, because very high free levels of H_2S are found in mouse aortic tissue compared with the low levels occurring in other tissues, free H_2S mediates its vasoactive functions, while in other tissues a different receptor-like mechanism modulates its activity.[112] It also has been reported that H_2S actions in the cardiovascular and other systems recognize both cGMP and cyclic adenosine monophosphate as second messengers.[113]

H_2S and Oxidation, Atherosclerosis, and Inflammation

H_2S is involved in general as a protective agent in oxidation, inflammation, apoptosis, all events paving the way to acute and chronic diseases such as ischemia-reperfusion injury, pulmonary hypertension, atherosclerosis, CKD progression, and complications.

Yang et al[114] have shown that in mice carrying the deletion of CSE, H_2S consistently is decreased in plasma and tissues, resulting in age-dependent hyper-tension, along with altered endothelium-dependent vasorelaxation.

In addition, decreased endogenous production of H_2S accelerates atherosclerosis in CSE knock-out mice.[115] H_2S exerts anti-atherosclerotic effects through the inhibition of vascular smooth muscle cell prolifer-ation and promotion of the proliferation of endothelial cells.[116] In apolipoprotein E knockout mice,[117] treat-ment with NaHS, a H_2S donor, is able to reduce plaque size, an effect probably mediated by the reduced concentration of intracellular adhesion molecule-1 in circulation and on the endothelial cells.

The H_2S slow-releasing donor GYY4137 shows anti-atherosclerotic activity in high-fat-fed apolipopro-tein E-/- mice, in particular it decreased vascular inflammation, oxidative stress, and atherosclerotic plaque formation, while improving endothelial function.[118]

H_2S is an antioxidant, and in fact as mentioned earlier it directly scavenges superoxide anions, hydro-gen peroxide,[119] and peroxynitrite[120] to suppress oxi-dative stress.

It has been proposed that H_2S may protect against atherosclerosis by preventing H_2O_2-induced injury to endothelial cells, an effect mediated by the preservation of mitochondrial function.[121]

Several reports regarding inflammation have shown proinflammatory or anti-inflammatory actions, which can be explained by differences in H_2S concentrations and models used. We analyzed, in an in vitro model entailing monocyte adhesion to an endothelial monolayer, the changes induced by H_2S on various potential targets, including cytokines, chemokines, and proteases, playing a crucial role in inflammation and cell adhesion. Results show that H_2S prevents the increase in monocyte adhesion induced by TNF-α. Under these conditions, down-regulation of monocyte chemoattractant protein-1 (MCP-1), chemokine C-C motif receptor 2, and an increase of cluster of differentiation 36 could be detected in monocytes. In endothelial cells, H_2S treatment reduces the increase in MCP-1, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and of a disintegrin and metalloproteinase metallopeptidase domain 17 (ADAM17), both at the gene expression and protein levels. H_2S significantly reduces activation of ADAM17 by phorbol myristate acetate (PMA) in endothelial cells, with a consequent reduction of both ADAM17-dependent TNF-α ectodomain shedding and MCP-1 release. In conclusion, H_2S is able to prevent endothelial activation triggered by TNF-α. The mechanism of this protective effect is mediated mainly by down-modulation of ADAM17-dependent

TNF-converting enzyme activity with consequent inhibition of soluble TNF-α shedding and its relevant MCP-1 release in the medium.[122]

Evidence shows that H_2S is involved in aging by inhibiting free-radical reactions, activating sirtuin1 (SIRT1), and probably interacting with the age-related gene Klotho.[123]

H_2S is produced in the kidney by combined actions of CBS, and CSE and MST, present mainly in the proximal tubule. H_2S affects both the renal tubule and vasculature. CSE deficiency in mice leads to reduced renal H_2S production and is associated with increased mortality and severity of damage after renal ischemia/reperfusion injury. H_2S administration rescues mice from mortality and injury, underlining the role of CSE in the regulation of oxidative stress induced after renal ischemia.[124]

CKD Progression and Complications

H_2S concentration is decreased in the plasma of hemodialysis patients.[125] Red cell sulfhemoglobin (a putative marker of chronic H_2S exposure) levels, is low in this patient population, and is accompanied by high plasma homocysteine and cysteine levels, with a significant negative correlation between cysteine and H_2S. Gene expression of CSE in blood mononuclear cells is significantly lower, realizing a condition in which a transcriptional down-regulation of the gene encoding for a key H_2S-producing enzyme is present.[126] These findings are in line with what was
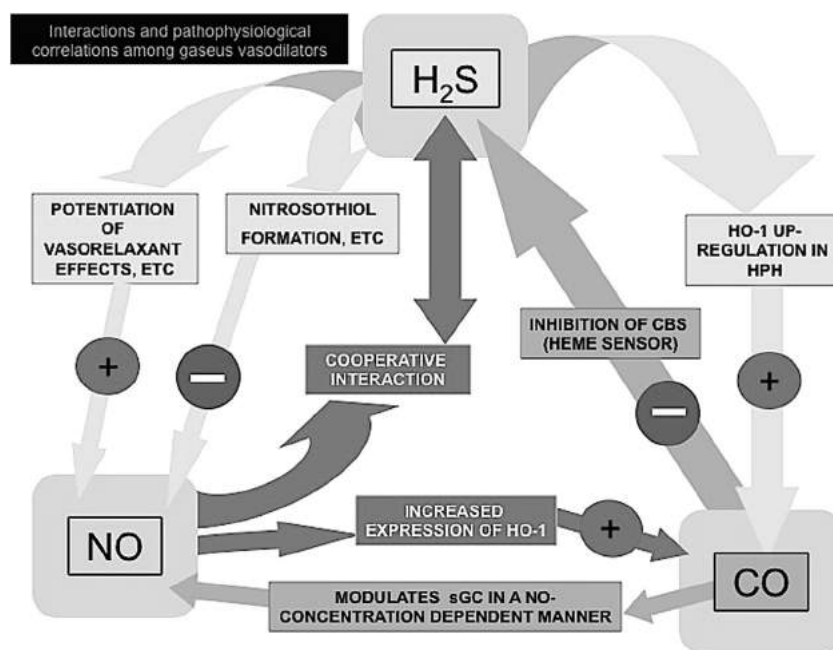
Figure 3. Interactions between NO, CO, and H_2S. These three gaseous vasodilators are shown with their major reciprocal interactions. HPH, hypoxic pulmonary hypertension; HO-1, heme oxygenase-1.

found in a 5/6 nephrectomy rat model of CKD, in which H_2S is low in plasma and tissues such as liver and kidney.[126]

These findings are in accordance with the existence of one or more uremic toxins inhibiting both CBS and CSE, without excluding the presence of post-translational modifications.

After a single dialysis session, plasma H_2S levels increase significantly, a finding that is compatible both with the idea that dialysis eliminates a uremic toxin that inhibits the H_2S-generating enzymes, and with the idea that dialysis hypotension could result from H_2S release.[127]

Interactions

The interactions between these three gases has been proven by many studies (Fig. 3). For example, H_2S is able to determine an up-regulation in the HO-1 gene and protein expression in hypoxic pulmonary hypertension,[128] thereby plasma CO level is increased. CO, on the other hand, inhibits CBS, and therefore decreases H_2S[104]; in this respect it has been proposed that CBS is a specific CO sensor.[129] CBS is a highly regulated enzyme, allosterically activated by S-adenosylmethionine, the active methionine derivative (not shown). CBS also contains a heme group acting as a sensor, modulating enzyme activity in response to redox change and to CO binding.[130]

H_2S exerts a double-faced interaction on NO. H_2S could, through the formation of a putative nitrosothiol, bind to NO and therefore decrease its availability[131] or independently from the formation of this adduct[92]; other investigators have shown that H_2S enhances the vasorelaxant effects of NO.[132] NO donor drugs enhance H_2S production and increase CBS expression in smooth muscle cells.[132] There are many ways through which H_2S interacts with NO aside from nitrosothiol formation.[92] Recent work has shown cooperative interaction of H_2S and NO, which increases and maintains cellular cGMP levels essential for vasorelaxation and angiogenesis and shows the potential for each of these molecules to control the actions and effective concentration of the other.[133]

NO is able to increase the expression of HO-1 in several model systems. sGC acts as the primary NO receptor, containing a heme moiety at the binding site.[134] CO exerts a NO-concentration–dependent modulating action on sGC: it activates sGC when NO is low, whereas at higher NO concentrations, CO blocks the effects of NO on sGC activation.[69]

TREATMENT STRATEGIES

The therapeutic approach with these gases is still in its infancy, with the exception of NO in lung disease of the newborn. First of all, the exact pathologies in which these gases should be used is not clear; the form of the gas donor is being investigated as well, and many other questions are still open. However, this field shows much promise.

For example, strategies aimed at decreasing the urea and VOC burden by moderate reduction in protein intake and longer and more frequent dialysis may attenuate CKD-associated systemic inflammation and improve CKD/ESRD patients' well-being and survival. Use of oral adsorbents such as AST-120 to remove ammonia may prove beneficial. Improvements in manufacturing of dialyzers and components of the hemodialysis circuit to reduce or eliminate release of toxic gaseous, particulate, and soluble compounds can enhance the well-being of ESRD patients maintained on hemodialysis. Use of NO-, CO-, and H_2S donors is under active scrutiny for ameliorating the ischemia-reperfusion injury present in transplantation and other situations, and for reducing the cardiovascular risk of uremia.

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