Amino Acids (2009) 37:65–78
DOI 10.1007/s00726-009-0264-5

REVIEW ARTICLE

Amino acids and gaseous signaling

Xilong Li · Fuller W. Bazer · Haijun Gao · Wenjuan Jobgen · Gregory A. Johnson · Peng Li · Jason R. McKnight · M. Carey Satterfield · Thomas E. Spencer · Guoyao Wu

Received: 1 February 2009 / Accepted: 12 February 2009 / Published online: 6 March 2009

Abstract Gases, such as nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H_2S), and sulfur dioxide (SO_2) are known toxic pollutants in the air. However, they are now recognized as important signaling molecules synthesized in animals and humans from arginine, glycine (heme), and cysteine, respectively. At physiological levels, NO, CO, and SO_2 activate guanylyl cyclase to generate cGMP which elicits a variety of responses (including relaxation of vascular smooth muscle cells, hemodynamics, neurotransmission, and cell metabolism) via cGMP-dependent protein kinases. H_2S is also a crucial regulator of both neurological function and endothelium-dependent relaxation through cGMP-independent mechanisms involving stimulation of membrane K_{ATP} channels and intracellular cAMP signaling. Additionally, NO, CO, and H_2S confer cytoprotective and immunomodulatory effects. Moreover, NH_3 is a major product of amino acid catabolism and profoundly affects the function of neurons and the vasculature through glutamine-dependent inhibition of NO synthesis. Emerging evidence shows that amino acids are not only precursors for these endogenous gases, but are also regulators of their production in a cell-specific manner. Thus, recent advances on gaseous signaling have greatly expanded our basic knowledge of amino acid biochemistry and nutrition. These exciting discoveries will aid in the design of new nutritional and pharmacological means to prevent and treat major health problems related to developmental biology and nutrient metabolism, including intrauterine growth restriction, preterm birth, aging, neurological disorders, cancer, obesity, diabetes, and cardiovascular disease.

Keywords Gas · Metabolism · Nutrients · Signaling

Abbreviations

AA	Amino acid
CBS	Cystathionine β-synthase
CSE	Cystathionine γ-lyase
EC	Endothelial cells
GABA	γ-Aminobutyrate
HO	Heme oxygenase (HMOX)
LPS	Lipopolysaccharide
MPST	β-Mercaptopyruvate sulfurtransferase
NFκB	Nuclear factor kappa B
NAC	*N*-Acetyl-cysteine
NMDA	*N*-Methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
VSMC	Vascular smooth muscle cells

Introduction

Nitric oxide (NO), a toxic gas produced by automobile engines and power plants, was discovered in 1987 as the endothelium-derived relaxing factor synthesized from arginine in animals and humans (Ignarro et al. 1987). This

Unless otherwise indicated, amino acids mentioned in this paper are L-isomers.

X. Li · F. W. Bazer · H. Gao · W. Jobgen · P. Li · J. R. McKnight · M. C. Satterfield · T. E. Spencer · G. Wu (✉)
Department of Animal Science, Texas A&M University, College Station, TX 77843, USA
e-mail: g-wu@tamu.edu

G. A. Johnson
Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX 77843, USA

seminal finding has fundamentally changed classical views about the role of endogenous gases in cell physiology. Subsequent work has shown that other environmental gaseous pollutants, such as carbon monoxide (CO), hydrogen sulfide (H_2S), and sulfur dioxide (SO_2) also affect important biological functions (Maines 1997; Orlando et al. 2008; Yang et al. 2008).

NO, CO, H_2S, and SO_2 are water-soluble colorless molecules that easily penetrate biological membranes and exert their effects independent of membrane receptors (Barañano et al. 2001). NO and CO are odorless, but H_2S, SO_2 and NH_3 each have a characteristic strong, pungent odor (e.g., the smell of rotten eggs for H_2S). Notably, NO is a highly reactive free radical, whereas CO, H_2S, and SO_2 are strong reducing agents. None of these gases are stable in physiological solutions. It is now established that amino acids (AA) are the sources of these gases in animals and humans (Qu et al. 2007; Yang et al. 2008).

A growing body of evidence indicates that AA not only act as signaling molecules themselves (Deldicque et al. 2008; Liao et al. 2008; Rhoads and Wu 2008), but also regulate the production of NO, CO, H_2S, SO_2, and NH_3 (Chang et al. 2008; Wu and Meininger 2002). The objectives of this review are to provide the basis for integration of multiple pathways whereby these gases are biosynthesized and to highlight their physiological roles, with particular attention to the cardiovascular, nervous, immune, and anti-oxidative systems. This knowledge will aid in the development of new nutritional and pharmacological means to prevent and/or treat a variety of chronic diseases.

Endogenous synthesis of gaseous molecules

Synthesis of NO from arginine

NO is synthesized by the oxidation of a guanidino nitrogen of L-arginine, with citrulline as the co-product (Bredt and Snyder 1994). This process is catalyzed by one of the three isoforms of NO synthase (NOS): neuronal NOS (nNOS; also known as NOS1), inducible NOS (iNOS; also known as NOS2), and endothelial NOS (eNOS; also known as NOS3). nNOS, eNOS, and iNOS are localized primarily in cytoplasm and mitochondria, plasma membrane and cytoplasm, and cytoplasm, respectively (Jobgen et al. 2006). Both nNOS and eNOS were named after the tissue and cell type from which they were originally isolated. nNOS was purified from the cerebellum of rat brain, eNOS from bovine aortic endothelial cells (EC), and iNOS from a mouse macrophage cell line activated by interferon γ and bacterial lipopolysaccharide (LPS). It is now known that nNOS and eNOS are constitutively expressed in a cell-specific manner and produce low levels of NO. In contrast, iNOS is induced by certain immunological stimuli (including LPS and inflammatory cytokines) and generates a large amount of NO.

All isoforms of NOS depend on NADPH, calmodulin, FAD, FMN, and tetrahydrobiopterin as essential cofactors for their enzymatic activities (Fig. 1). In addition, nNOS and eNOS, but not iNOS, require calcium for generation of NO (Bredt and Snyder 1994). Nearly all cell types can recycle citrulline into arginine via argininosuccinate synthase and argininosuccinate lyase, and this intracellular arginine-citrulline cycle helps sustain sufficient concentrations of arginine to support NO production (Wu and Brosnan 1992).

In cells and blood, NO is rapidly oxidized via many nonenzymatic reactions to nitrite and nitrate, with nitrate being the major product. For example, NO is readily oxidized to nitrite via autoxidation or reacts with superoxide anion to yield peroxynitrite (an oxidant). NO and nitrite can also be oxidized by oxyhemoglobin or oxymyoglobin to form nitrate. Nitrite and nitrate are excreted by the kidneys. The half-life of NO in physiological solutions is extremely short (<5 s), but it can be transported as a glutathione adduct to conserve its biological activity (Wu et al. 2004). Determination of nitrite and nitrate provides a valid indicator of NO synthesis by cells.

Synthesis of CO from glycine-derived heme

Although CO can be formed by lipid peroxidation, over 90% of it is produced from the degradation of heme by membrane-bound heme oxygenase (HO or HMOX) in the body (Durante et al. 2006). The HO system depends on NADPH-cytochrome 450 for its catalytic activity. Equimolar amounts of biliverdin, iron, and CO are produced by cleavage of the heme methene bridge (Fig. 2). In cells, heme is synthesized from glycine and succinyl CoA via a

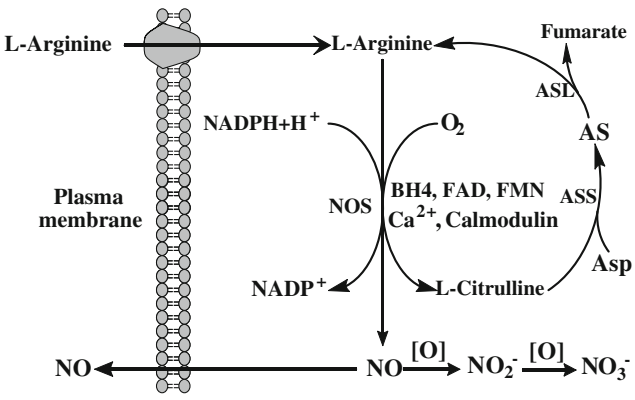

Fig. 1 Synthesis of NO from L-arginine in animal cells. *AS* argininosuccinate, *BH4* tetrahydrobiopterin, *ASL* argininosuccinate lyase, *ASS* argininosuccinate synthase, *Asp* L-aspartate

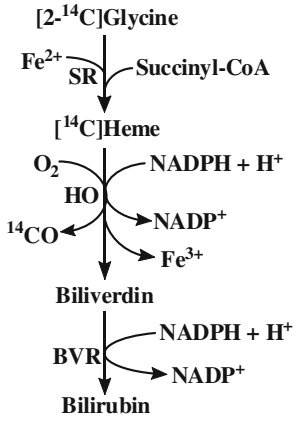

Fig. 2 Synthesis of CO from glycine-derived heme in animal cells. *BVR* biliverdin reductase, *SR* a sequential series of reactions involving δ-aminolevulinate synthase (a mitochondrial enzyme), δ-aminolevulinate dehydratase (a cytosolic enzyme), uroporphyrinogen I synthase (also known as porphobilinogen deaminase), uroporphyrinogen I/uroporphyrinogen III cosynthase, uroporphyrinogen decarboxylase (a cytosolic enzyme), coproporphyrinogen oxidase, protoporphyrinogen oxidase, and heme synthase (also known as ferrochelatase). The last three named enzymes are located in mitochondria

series of reactions involving both cytoplasm and mitochondria, and the carbon of CO is derived from the methyl carbon (carbon-2) of glycine. There are three isoforms of HO: HO1 (also known as heat shock protein 32), HO2, and HO3. HO1 is a highly inducible protein, and its activity can increase up to 100-fold when cells are treated with oxidants, endotoxins, or inflammatory cytokines. In contrast, HO2 is constitutively expressed and its activity is not altered by these agents. HO1 and HO2 proteins are encoded by two different genes (Cruse and Maines 1988). In the late 1990s, the HO3 cDNA was isolated from rat brain and its protein was purified (McCoubrey et al. 1997). Recent work conducted in our laboratory demonstrated that adipose tissue expresses HO3 which may function to regulate lipolysis and oxidation of energy substrates (Fu et al. 2005; Jobgen et al. 2009b). The predicted amino acid sequence of HO3 differs from that of HO1, but has ~90% identity with HO2 (McCoubrey et al. 1997).

Unlike NO, CO is not an oxidant and, therefore, does not cause oxidative damage to macromolecules. However, like NO, CO reversibly binds heme-containing proteins, including oxyhemoglobin, myoglobin and cytochrome c oxidase, and soluble guanylyl cyclase (sGC) (Maines 1997). It is indeed interesting that a product of HO (CO) binds its physiological substrate (heme), and this reaction may be a mechanism for regulating CO bioavailability. The half-life of CO in the blood circulation has been reported to be 40–75 and 22–120 min, respectively, for pigs (Aberg et al. 2004) and humans (Shimazu et al. 2000). Endogenously generated CO is transpired to the atmosphere primarily by the lungs.

Synthesis of H_2S and SO_2 from cysteine

Desulfuration of cysteine for production of H_2S and SO_2 occurs via multiple pathways (Fig. 3), which include

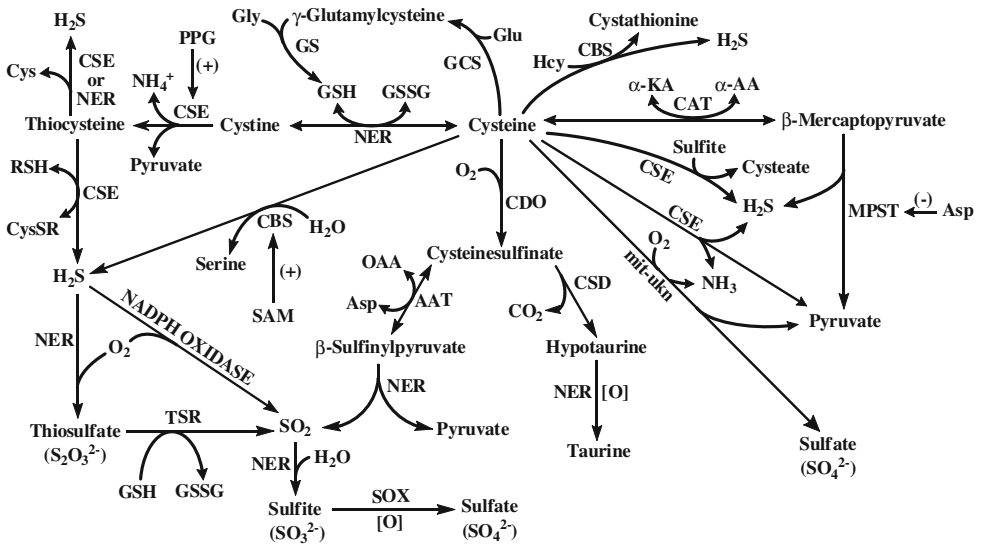

Fig. 3 Synthesis of H_2S and SO_2 from cysteine in animal cells. *AAT* amino acid transaminase, *Asp* L-aspartate, *CAT* cysteine aminotransferase, *CDO* cysteine dioxygenase, *CSD* cysteinesulfinate decarboxylase, *CysSR* cysteine-thiol complex, *GCS* γ-glutamylcysteine synthase, *Glu* L-glutamate, *Gly* glycine, *GS* glutathione synthase, *GSH* reduced glutathione, *GSSG* oxidized glutathione, *α-KA* α-ketoacid, *α-AA* α-amino acid, *Mit-ukn* unidentified mitochondrial enzyme(s), *NER* non-enzyme catalyzed reaction, *OAA* oxaloacetate, *PPG* propargylglycine (an irreversible inhibitor of cystathionine γ-lyase), *RSH* a thiol-containing compound, *SAM* *S*-adenosylmethionine, *SOX* sulfite oxidase, and *TSR* thiosulfate reductase

cystathionine β-synthase (CBS), cystathionine γ-lyase (also known as cystathionase; CSE), and β-mercaptopyruvate sulfurtransferase (MPST) (Griffith 1987). Cysteine is synthesized from methionine in liver via the transsulfuration pathway that also involves CBS and CSE. These two cytosolic enzymes are pyridoxal phosphate-dependent and responsible for production of most H_2S in animals (Stipanuk et al. 2006). CBS is Ca^{2+} and calmodulin-dependent, whereas CSE is inducible by LPS and likely inflammatory cytokines (Kamoun 2004).

Expression and activity of CBS and CSE vary greatly among cell types and tissues (Zhao et al. 2003). For example, brain has a relatively high abundance of CBS, but a very low level of CSE. In contrast, CSE, but not CBS, is expressed in rat vascular tissues (e.g., artery and aorta) and EC (Zhao et al. 2001; Yang et al. 2008). In addition, the liver has high activities of both CBS and CSE (Stipanuk et al. 2006). Thus, H_2S levels are greatly reduced in brains of CBS knock-out mice, but their EC still produce H_2S (Yang et al. 2008). In adult rats, rates of H_2S generation per gram of tissue are greatest in homogenates of liver, followed by kidney, brain and heart, and then aorta, and small intestine (Kamoun 2004; Zhao et al. 2003).

SO_2 can be generated from (1) the spontaneous degradation of β-sulfinylpyruvate (an intermediate of cysteine degradation); (2) oxidation of H_2S by NADPH oxidase; and (3) oxidation of H_2S through a series of reactions involving the non-enzymatic formation of thiosulfate and its subsequent enzyme-catalyzed reaction with reduced glutathione (Qu et al. 2007). Because most cysteine is degraded via the cysteine dioxygenase pathway in animals (Stipanuk et al. 2008), nonenzymatic cleavage of β-sulfinylpyruvate is likely the major source of SO_2 in vivo.

Both H_2S and SO_2 are exhaled by lungs, but sulfite and sulfate are excreted by kidneys. In saline, H_2S exists in equilibrium with HS^-, with the ratio of H_2S to HS^- being approximately 1:2. In physiological solutions, SO_2 spontaneously reacts with water to yield sulfurous acid (H_2SO_3) and then sulfite (SO_3^{2-}) through dissociation of bisulfate (HSO_3^-): $SO_2 + H_2O \leftrightarrow H_2SO_3 \leftrightarrow HSO_3^- + H^+ \leftrightarrow SO_3^{2-} + 2H^+$.

Production of NH_3 from AA, purines, and pyrimidines

Except for developing conceptuses (embryos/fetuses and associated extraembryonic membranes) and growing animals (Wu et al. 2008a, b), AA are not stored in tissues and must be degraded to yield NH_3 in a cell-specific manner (Suryawan et al. 2008). The production of NH_3 from AA is catalyzed by many enzymes, including phosphate-activated glutaminase, glutamate dehydrogenase, amino acid oxidase, glycine synthase, ω-amidase, cysteine desulfhydrase, histidase, and 2-amino *cis–cis* muconate semialdehyde reductase. In addition, degradation of purines and pyrimidines generates NH_3, particularly in exercising skeletal-muscle. For example, adenylate deaminase converts AMP into NH_3 plus IMP, adenosine deaminase oxidizes adenosine to yield NH_3 plus inosine, and guanase deaminates guanine into NH_3 plus xanthine. In aqueous solution, free NH_3 is at equilibrium with NH_4^+. Under physiological conditions (pH 7.4 and 37°C), the ratio of NH_3 to NH_4^+ is approximately 2:98.

Regulation of the synthesis of gaseous molecules by AA

Synthesis of NO

Inadequate intake of protein from the diet reduces systemic NO synthesis by eNOS, nNOS, and iNOS, resulting in cardiovascular abnormalities and compromised immune functions (Wu et al. 1999). The underlying mechanisms involve reduced expression of the enzymes and reduced availability of essential cofactors (Table 1). The Km values of various NOS isoforms for arginine are very low (3–10 μM), compared with its intracellular concentrations (0.5–2 mM) in most cell types (Li et al. 2008a; Wu and Meininger 2002). However, increasing extracellular concentrations of arginine from 0.1 to 10 mM dose-dependently stimulates NO production by EC and macrophages (Wu and Meininger 2002). This arginine paradox is now explained by enhanced expression of (1) GTP cyclohydrolase-I protein for tetrahydrobiopterin synthesis in EC (Kohli et al. 2004; Wu and Meininger 2009) and (2) iNOS protein in cytokine-activated leukocytes and neurons (Lee et al. 2003). Because only enterocytes can synthesize citrulline from glutamine/glutamate or proline (Wu and Morris 1998), extracellular arginine provides the nitrogenous substrate for NO generation in other cell types (Wu and Meininger 2000). Thus, inhibition of arginine transport by lysine reduces arginine concentrations, and therefore, NO synthesis in cells (Closs et al. 2000).

Glutamine is an important substrate for arginine synthesis (Hu et al. 2008; Wu et al. 2007a) and regulates whole-body homeostasis (Lagranha et al. 2008a, b). Increasing extracellular concentrations of glutamine from 0 to 2 mM dose-dependently reduces NO synthesis by bovine and porcine EC (Arnal et al. 1995; Meininger and Wu 1997). In addition, glutamine inhibits NO-dependent cerebral neurogenic vasodilation (Lee et al. 1996) and dietary glutamine supplementation attenuates systemic NO production in rats (Houdijk et al. 1998). It is now known that metabolism of glutamine to glucosamine-6-phosphate (an inhibitor of NADPH synthesis via the pentose cycle) is necessary for glutamine-dependent inhibition of NO synthesis by EC (Wu et al. 2001). In contrast, glutamine is

Table 1 Effects of dietary protein and amino acids on NO synthesis in cells and tissues

Factor	Effector	Effect	Cell or tissue type
Protein deficiency	nNOS and eNOS	↓	Whole body, kidney, brain, muscle
	iNOS	↓	Whole body, lung, kidney, brain, macrophage
Arginine	eNOS and BH_4	↑	Endothelial cells, whole body, heart, VSMC
	iNOS translation	↑	Astrocytes
Citrulline	Arginine availability	↑	Endothelial cells, neurons, VSMC
Glucosamine	NADPH	↓	Endothelial cells
	iNOS expression	↓	Macrophage, spleen, lung, liver
Glutamate	NMDA and nNOS	↑	Brain
	iNOS	↑	Brain
Glutamine	NADPH	↓	Endothelial cells
	iNOS	↑	Macrophage
	iNOS	↓	Gut
Glycine	iNOS expression	↓	Liver
	nNOS	↑	Brain
Lysine	Arginine transport	↓	Many cell type
Taurine	eNOS	↑	Endothelial cells
	iNOS	↓	Many cell type
Homocysteine	eNOS and BH4	↓	Endothelial cells
	iNOS	↑	Kidneys and VSMC
GABA	nNOS and Ca^{2+}	↑	Neurons
	iNOS	↑	Neurons and endothelial cells

BH4 tetrahydrobiopterin, ↑ increase, ↓ decrease

required for production of iNOS protein by macrophages, thereby stimulating NO production under conditions of immunological activation to kill pathogenic microorganisms (Li et al. 2007).

Glutamate and glycine bind the different sites of the excitotoxic NMDA receptor, resulting in Ca^{2+} influx and nNOS activation for NO synthesis (Atlante et al. 2001). Similarly, GABA (a decarboxylation product of glutamate) stimulates NO production by nNOS in hypothalamic magnocellular neurons via divergent G-protein signaling pathways (Di et al. 2009). There is also evidence that GABA and glutamate increase iNOS protein abundance in ischemic brains (Harty et al. 2004), thereby contributing to oxidative stress in neurons. The enhancement of NO synthesis by high concentrations of glutamate and GABA in the brain and other nervous tissues provides a metabolic basis for their neurotoxicity. In contrast, glycine reduces iNOS expression in endotoxin- or cytokine-challenged liver cells (Li et al. 2007), thereby attenuating hepatic injury under inflammatory conditions.

Homocysteine is an independent risk factor for cardiovascular disease. There is direct evidence that increasing extracellular concentrations of homocysteine dose-dependently inhibits NO synthesis by EC (Zhang et al. 2000) and platelets (Mutus et al. 2001), thereby impairing endothelium-dependent relaxation. Similarly, elevated levels of homocysteine in response to dietary methionine loading or hypercysteinemia reduce NO synthesis by eNOS or nNOS and results in endothelial dysfunction (Chao et al. 2000). Importantly, the adverse effects of homocysteine can be alleviated by nutrients (e.g., folic acid and vitamin B12) that facilitate its conversion into methionine (Chao et al. 2000). In contrast to the constitutively expressed NOS isoforms, homocysteine promotes iNOS expression in vascular smooth muscle cells (VSMC) (Welch et al. 1998) and kidneys (Zhang et al. 2004) through NFκB-dependent transcriptional activation of *Nos2*. iNOS-induced oxidative stress may be a major mechanism responsible for atherosclerosis in obese and diabetic subjects with high circulating concentrations of the methionine metabolite.

Taurine is a potent antioxidant and the most abundant free AA in the brain, heart, skeletal muscle, and leukocyte (Mühling et al. 2008; Saransaari and Oja 2008). This β-AA stimulates NO synthesis by eNOS and nNOS (Abebe and Mozaffari 2003), while inhibiting iNOS expression and production of excess NO by cytokine-activated cells (e.g., macrophages, neurons and VSMC) by reducing NFκB activity (Barua et al. 2001). In addition, through the formation of taurine chloramines, taurine inhibits iNOS expression and excess NO synthesis in various cell types, including hepatocytes, macrophages, and glial cells (Wu and Meininger 2002). Thus, taurine protects organisms from oxidant-induced tissue damage.

Synthesis of CO

Arginine is a key physiological regulator of CO production in cells. For example, dietary supplementation with arginine increases HO3 expression in white adipose tissues of the Zucker diabetic fatty rat (a genetically obese type-II diabetic animal model) (Fu et al. 2005) and diet-induced obese rats (Jobgen et al. 2009b). Notably, a common finding in these two animal models in response to arginine supplementation is enhanced expression of the HO3 gene in the fat pad, despite their marked differences in global expression of adipose tissue genes. Additionally, arginine upregulates HO1 expression and CO generation in cells (including VSMC) likely through an NO-dependent mechanism (Chung et al. 2008).

Physiological levels of glutamine induce HO1 expression in animals. Because of an important role for glutamine in gut function (Wang et al. 2008a, 2009), much research has been directed to intestinal epithelial cells. Coeffier et al. (2002) originally reported that enteral provision of glutamine enhanced HO1 expression at both mRNA and proteins levels in human duodenal mucosa. Subsequent work established that pretreatment of LPS-challenged rats with glutamine increased HO1 expression in mucosal epithelial cells and lamina propria cells of the intestine, and prevented intestinal tissue injury (Uehara et al. 2005). Similarly, glutamine increased HO1 protein and prevented hemorrhagic shock-induced intestinal tissue injury (Umeda et al. 2009). Interestingly, tin mesoporphyrin (a specific inhibitor of HO1) completely abolished the protective effect of glutamine on rat intestine (Uehara et al. 2005). Thus, induction of HO1 by glutamine may be necessary for its cytoprotective benefit.

Glutamate and alanine, two products of glutamine catabolism, may stimulate CO synthesis in multiple cell types. For example, glutamate increases CO production in cerebral microvascular EC expressing both HO1 and HO2 (Parfenova et al. 2001). Additionally, through glutamate receptor-mediated NO production, glutamate stimulates HO2 expression in piglet cerebral microvessels (Leffler et al. 2005). Thus, glutamate enhances CO generation in the endothelium, resulting in the relaxation of piglet cerebral arteries (Fiumana et al. 2003). There is also evidence that alanine may exert its antioxidant effect on EC by augmenting HO1 expression (Grosser et al. 2004). However, such a cytoprotective effect of alanine was not observed in intestinal epithelial cells (Haynes et al. 2009) or neutrophils (Mühling et al. 2007).

Taurine chloramine and taurine bromamine, products of taurine metabolism in the myeloperoxidase halide system, have anti-inflammatory properties. They enhance HO1 protein levels in resting and LPS-activated J774.2 macrophages, leading to inhibition of cyclooxygenase-2 expression and prostaglandin E_2 production (Olszanecki et al. 2008). Likewise, taurine chloramine promotes HO-1 expression in human fibroblast-like synoviocytes and suppresses interleukin-1β-induced production of pro-inflammatory cytokines (Muz et al. 2008).

Sulfur AA may also regulate CO synthesis in diverse cell types. Culture of EC with 0.3–10 mM methionine dose-dependently increased HO1 protein expression and CO production (Erdmann et al. 2005). However, because of high concentrations used (3–100 times plasma level), the physiological significance of this finding remains to be established. Interestingly, *N*-acetyl-cysteine (NAC; an effective precursor of cysteine) reduces HO1 expression at both mRNA and protein levels in VSMC (Hartsfield et al. 1997) and injured brains (Yi and Hazell 2005). This may be a mechanism whereby NAC protects cells from oxidative stress. Either cysteine or glutathione may mediate a stimulatory effect of NAC on CO production via alteration of the cellular redox state.

Some dipeptides, which are products of protein degradation in the intestinal lumen and cells, are potent regulators of CO production. For example, preincubation of EC with methionine-tyrosine markedly increased HO1 protein levels and decreased NADPH-mediated radical formation (Erdmann et al. 2005). Notably, effects of the dipeptide

Table 2 Effects of amino acids on CO synthesis in cells and tissues

Factor	Effector	Effect	Cell or tissue type
High protein	HO1, HO2 and HO3	↑	Vascular and nonvascular tissues
Arginine	HO3	↑	Adipose tissue
Glutamine	HO1	↑	Intestine
Glutamate	HO1 and HO2	↑	Endothelial cells and brain
Alanine	HO1	↑	Endothelial cells
Taurine	HO1	↑	Macrophages
Methionine	HO1	↑	Endothelial cells
Met–Tyr	HO1	↑	Endothelial cells
Glycine	Heme	↑	Vascular and nonvascular tissues
N-Acetyl-cysteine	HO1	↓	Injured brain and VSMC

Met–Tyr methionine–tyrosine, ↑ increase, ↓ decrease

were abolished by zinc deuteroporphyrin IX 2,4-bis-ethylene glycol, an HO inhibitor (Erdmann et al. 2005). Recently, Mizuguchi et al. (2008) reported that administration of a tetrapeptide (D-Arg-Dimethyltyrosine-Lys–Phe) to rats promoted HO1 expression in the kidneys, while decreasing renal tubular apoptosis and damage in unilateral urethral obstruction (Table 2).

Synthesis of H_2S and SO_2

High intakes of dietary methionine or cysteine increase urinary excretion of sulfate in rats and humans (Stipanuk et al. 2006), indicating enhanced production of both H_2S and SO_2 in vivo (Table 3). Indeed, the activity of hepatic CSE was markedly increased for CO production in rats fed a high-protein (90%) or high-cysteine (4%) diet (Simpson and Freedland 1976). Similar results have been reported for cats fed a taurine-supplemented diet (Park et al. 1999). Also, *S*-adenosylmethionine (a metabolite of methionine) is an allosteric activator of CSE, whereas aspartate is an inhibitor of MPST (Kamoun 2004). In addition, indirect evidence suggests that intracellular glycine likely modulates H_2S production through the synthesis of glutathione (Wu et al. 2004).

Arginine may play an important role in regulating H_2S and SO_2 synthesis in animals. For example, Yanfei et al. (2006) reported that oral administration of arginine to rats with high blood flow-induced pulmonary hypertension increased (1) CSE expression in smooth muscle cells of the pulmonary arteries; (2) concentrations of H_2S in plasma; and (3) H_2S production by the lungs. It is not known whether arginine itself or its metabolite(s) regulates H_2S production. However, there is evidence that NO upregulates CSE expression and H_2S synthesis in VSMC (Zhao et al. 2001).

Culture of astrocytoma cells with NAC dose-dependently increased production of H_2S and SO_2, and beneficially inhibited proliferation of those cells (Jurkowska and Wrobel 2008). Furthermore, because the cystine/glutamate exchange transporter (system x_c^-) is a rate-controlling step in the provision of intracellular cysteine (Shih et al. 2006), elevated levels of extracellular glutamate (1–3 mM) inhibit the uptake of cystine (0.1 mM) by neuronal cells and thus synthesis of H_2S and SO_2. In contrast, lower concentrations of glutamate (e.g., 0.1 mM) and NMDA (0.1 mM) stimulated H_2S production in brain cell suspensions in the presence of Ca^{2+} and glycine possibly through NMDA activation (Kimura et al. 2005). Thus, H_2S may mediate, in part, the physiological and pathological actions of glutamate (Table 3).

Gaseous molecules in health and disease

Cardiovascular functions

Although NO is known as a major endothelium-dependent relaxing factor (Ignarro et al. 1987), there is growing evidence for important physiological functions for CO, H_2S, and SO_2 in the cardiovascular system (Yang et al. 2008). It has been shown that NO stimulates H_2S production in vascular tissues (Zhao et al. 2003), whereas H_2S inhibits the arginine–NO pathway in aorta and EC (Geng et al. 2007). Additionally, endothelial NO has a permissive role in CO- and perhaps H_2S-induced vascular dilation (Barkoudah et al. 2004). Thus, there may be cross-talk between various gaseous signaling pathways, and physiological levels of NO regulate vascular tone and hemodynamics in synergy with other gaseous vasoactive factors.

NO and CO bind the heme group of sGC, thereby activating this enzyme for generation of cGMP from GTP (Bredt and Snyder 1994). There is also evidence for enhancement of aortic cGMP availability in response to SO_2 treatment (Li and Meng 2009). cGMP triggers cGMP-dependent kinases and the phosphorylation of target

Table 3 Effects of dietary protein and amino acids on H_2S synthesis in cells

Factor	Effector	Effect	Cell or tissue type
High protein	CSE and CBS	↑	Vascular smooth muscle cells
Methionine	CSE and CBS	↑	Liver and possibly intestine
Cysteine	CSE and CBS	↑	Vascular and nonvascular tissues
Arginine	CSE	↑	Vascular smooth muscle cells
SAM	CSE	↑	Various cell types
Glycine	Glutathione	↑	Various cell types
N-Acetyl-cysteine	Cysteine	↑	Astrocytoma cells
NMDA	Ca^{2+}	↑	Brain
Glutamate (0.1 mM)	NMDA	↑	Brain
Glutamate (1–3 mM)	X_c^- transporter	↓	Brain, liver, and other tissues
Aspartate	MPST	↓	Various cell types

SAM *S*-ademosylmethionine, ↑ increase, ↓ decrease

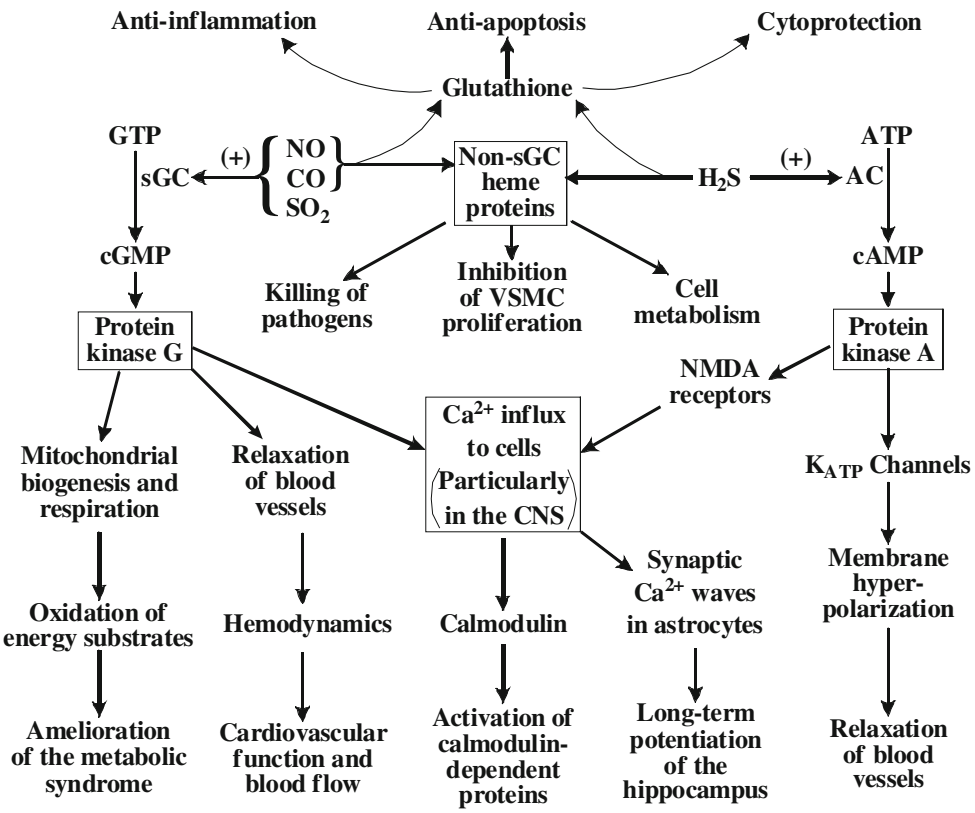

Fig. 4 Gaseous signaling in cells via cGMP and cAMP-dependent and independent pathways in animal cells. *AC* adenylate cyclase, *CNS* central nervous system, and *sGC* soluble guanylate cyclase

proteins that elicit a series of physiological responses (e.g., relaxation of VSMC, vasodilation, and mitochondrial biogenesis) (Fig. 4). In addition, NO exerts some of its physiological effects (e.g., inhibition of VSMC proliferation) via cGMP-independent mechanisms (e.g., inhibition of ornithine decarboxylase) (Ignarro et al. 2001; Maines 1997; Wei et al. 2001). Interestingly, H_2S has a Ca^{2+}-dependent effect on vasodilation via cGPM-independent mechanisms that may involve (1) direct stimulation of ATP-sensitive K^+ channels and membrane hyperpolarization (Tang et al. 2005); (2) a reduction in production of oxidants (Kiss et al. 2008); and (3) Ca^{2+}/calmodulin signaling (Yang et al. 2008) in VSMC. Emerging evidence shows that physiological levels of both NO and H_2S inhibit myocardial injury induced by oxidants (e.g., homocysteine) (Chang et al. 2008). Of particular note, these two gases play important roles in cardiovascular function, as impaired endothelium-dependent vasorelaxation and pronounced hypertension occur in mutant mice lacking either eNOS (Huang et al. 1995) or CSE (Yang et al. 2008).

Neurological functions

As novel neural messengers, NO, CO, and H_2S differ markedly from classical neurotransmitters (e.g., glutamate, acetylcholine, and noradrenalin) in their biosynthesis, chemical nature, cellular localization, and mechanisms of action (Barañano et al. 2001). There is distinctive localization for nNOS and HO2 in brains and the male reproductive tract, but these two proteins are co-localized in the myenteric plexus of the small intestine (Burnett et al. 1998). In neurons, NO and CO, either alone or in combination, enhance the synthesis of cGMP from GTP (Qu et al. 2007). These two gases act downstream from the *N*-methyl-D-aspartate (NMDA) receptor as retrograde messengers at synapses and enhance long-term potentiation (a type of synaptic plasticity as a mechanism of learning) in the hippocampus (Zhuo et al. 1993). Thus, nNOS deficiency results in testosterone-dependent behavioral abnormalities in male mice (Nelson et al. 1995). Endogenous CO may also play an important role in memory processing (Cutajar and Edwards 2007). In contrast, H_2S released from astrocytes or glia surrounding synapses facilitates induction of hippocampal long-term potentiation via activation of NMDA receptors (Kimura et al. 2005). This gas also increases Ca^{2+} concentrations in glial cells and induces Ca^{2+} waves in astrocytes to mediate glial signal transduction (Qu et al. 2007).

While high concentrations of NO, CO, and H_2S are deleterious to all cells, physiological levels of these gases have potent cytoprotective effects, particularly on neurons (Chang et al. 2008; Voss and Grune 2007). For example, inhibition of endogenous NO synthesis blocks NMDA-mediated neurotrophic and neuroprotective effects in

neurons (Pantazis et al. 1998). Conversely, nNOS-derived NO plays an essential role in promoting the survival of granule cells in the cerebellum and protecting those cells from ethanol-induced neurotoxicity (Bonthius et al. 2008). Similarly, overexpression of eNOS confers cytoprotection from reactive oxygen species, endoplasmic stress, and DNA damage in neuronal cells (De Palma et al. 2008). Notably, an appropriate dose of inhaled NO has a neuroprotective effect in preterm infants (Marks and Schreiber 2008) possibly through (1) increased production of cGMP by cGS; and (2) stimulation of ATP-sensitive K^+ channels via activation of the Ras/AMPK pathway (Lin et al. 2004).

Findings from recent studies indicate that CO and bilirubin, produced by HO1 and HO2, are neuroprotective. For example, overexpression of HO1 protects dopaminergic neurons against neurotoxicity induced by 1-methyl-4-phenylpyridinium (Hung et al. 2008). In addition, HO1 knock-out mice exhibit a profound lesion in the brain following the administration of NMDA into the striatum (Ahmad et al. 2006). Moreover, overexpression of HO2 protects olfactory receptor neurons from glutathione depletion-induced apoptosis (Chen et al. 2005).

H_2S has an anti-inflammatory effect by augmenting glutathione availability in neurons. This conclusion is based on the facts that (1) H_2S increases GCS activity either by direct activation of the enzyme or through a post-transcriptional mechanism (e.g., enhancement of mRNA translation and/or inhibition of protein degradation) (Qu et al. 2007); (2) H_2S enhances concentrations of cAMP that activate protein kinase A, leading to NMDA phosphorylation and the opening of Ca^{2+} channels (Kimura et al. 2005); and (3) H_2S stimulates ATP-sensitive K^+ channels in neuronal cells, causing the efflux of K^+ and membrane hyperpolarization (Tang et al. 2005). At present, little is known about a role for SO_2 in neurological function.

NH_3 participates in many vital reactions of nitrogen metabolism (e.g., as a substrate for glutamate dehydrogenase, glutamine synthetase and carbamoylphosphate synthetase-I), but hyperammonemia can be lethal to the central nervous system (Wu and Morris 1998). A physiological role for NH_3 in cell signaling via activation of cAMP-dependent signaling pathways has been proposed (Faff et al. 1996). However, the lowest concentration (250 μM) used in the study was fourfold greater than that in plasma of healthy humans. Emerging evidence shows that NH_3 itself is not harmful to the brain, but its conversion into glutamine by glutamine synthetase inhibits endothelial NO synthesis and, therefore, blood flow and oxygen supply to the brain (Lee et al. 1996). In support of this notion, Kawaguchi et al. (2005) reported that rats with hyperammonemia did not exhibit neurotoxicity or impaired endothelium-dependent relaxation when glutamine synthesis was blocked by L-methionine-*S*-sulfoximine.

Immunological functions

Physiological levels of NO have anti-inflammatory effects (Chung et al. 2008), whereas excess NO produced by iNOS mediates cytotoxicity of monocytes and natural killer cells against tumor cells, viruses, bacteria, fungi, malignant cells, intracellular protozoa, and parasites in mammals, birds, and other species of animals (Li et al. 2007). For example, activated macrophages cannot kill target cells when NO synthesis from arginine is inhibited (Hibbs et al. 1987). Additionally, NO plays an important role in both innate and acquired immunity (Bogdan et al. 2000). However, as an oxidant and inhibitor of enzymes containing an iron-sulfur center, high levels of NO rapidly react with H_2O_2 to form peroxynitrite (Fang et al. 2002) which oxidizes biomolecules (e.g., proteins, amino acids, lipids, and DNA) and causes cell injury and death. Thus, large amounts of NO can exert a deleterious effect on mammalian cells and mediate the pathogenesis of many diseases, including the autoimmune destruction of pancreatic β-cells in type-I diabetes mellitus, arthritis, glomerulonephritis, inflammatory bowel disease, and neurological disorders (Li et al. 2007).

High concentrations of CO, H_2S, and SO_2 inhibit microbial growth and kill pathogenic organisms (Nobre et al. 2007; Zhang and Bhatia 2008; Zhi et al. 2007), but also induce apoptosis and tissue injury via the NFκB cell signaling pathway (Kodavanti et al. 2006; Song et al. 2004). Thus, physiological levels of these gases are important mediators of immune responses. This notion is further substantiated by the following lines of evidence. First, CO is a key modulator of NO-mediated anti-apoptotic and anti-inflammatory functions in hepatocytes and macrophages, as these effects are absent in mice lacking HO-1 or receiving HO1 inhibitors (Chung et al. 2008). Similar results were obtained for human enterocytes treated with enterohemorrhagic *Escherichia coli* (Vareille et al. 2008). Second, CO derived from HO1 prevented reactive oxygen species-induced translocation of Toll-like receptors (TLR) 2, 4, 5 and, 9 to lipid rafts in macrophages, thereby inhibiting TLR signaling and conferring potent anti-inflammatory effects (Nakahira et al. 2006). Third, CO reduces NO synthesis by iNOS in hepatocytes (Kim et al. 2008) and intestinal cells (Vareille et al. 2008), decreases the circulating levels of proinflammatory cytokines (e.g., interleukins 1β and 6, and tumor necrosis factor-α) (Brusko et al. 2005; Kim et al. 2008; Ning et al. 2005), and increases production of anti-inflammatory cytokines (e.g., interleukin 10) via the p38 mitogen-activated protein kinase pathway (Dolinay et al. 2004). Also, inhaled CO confers anti-inflammatory effects against ventilator-induced lung injury (Dolinay et al. 2004). Fourth, low doses of H_2S or NaHS (an H_2S donor) attenuate the

synthesis of pro-inflammatory cytokines, reduce leukocyte adherence to the endothelium, inhibit leukocyte activation, and exert anti-inflammatory effects in the gastrointestinal tract and lungs (Esechie et al. 2008; Zhang et al. 2008). Importantly, parenteral administration of H_2S has been reported to prevent acute lung injury and improve survival in mice with burn and smoke inhalation (Esechie et al. 2008).

Metabolism of energy substrates

NO, CO, and H_2S are key regulators of mitochondrial metabolism (Kiss et al. 2008; Nisoli et al. 2003). At high doses, these three gases inhibit cytochrome c oxidase, therefore reducing substrate oxidation and ATP production (Galli 2007; Mannick 2007). Interestingly, nontoxic levels of H_2S also decrease cellular oxidative metabolism, attenuate production of reactive oxygen species, and possibly increase longevity of animals (Kiss et al. 2008; Miller and Roth 2007). In contrast, physiological levels of NO enhance mitochondrial biogenesis, oxidative phosphorylation, as well as activation of specific transcription factors and signaling pathways via a cGMP-dependent mechanism (Nisoli et al. 2003). Thus, NO derived from NOS1 and NOS3 stimulates the oxidation of energy substrates (e.g., glucose and fatty acids) and inhibits the synthesis of glucose, triglycerides, and low-density-lipoprotein (Jobgen et al. 2006). Similarly, we found that addition of a CO donor to incubation medium dose-dependently increased oxidation of glucose and oleic acid in adipose tissue and skeletal muscle of rats (Table 4). Thus, upregulation of NO and CO synthesis, within physiological ranges, may reduce adiposity. In support of this notion, oral administration of arginine via drinking water increased NOS1 and HO3 expression in adipose tissue of both Zucker diabetic fatty rats (Fu et al. 2005; Wu et al. 2007b) and diet-induced obese rats (Jobgen et al. 2009a), while reducing fat gain and improving whole-body insulin sensitivity in both animal models. Similarly, partly through regulation of nutrient metabolism, dietary supplementation with arginine reduced fat mass (He et al. 2008; Tan et al. 2008) and enhanced anti-oxidative capacity (Ma et al. 2008) in growing-finishing pigs. Furthermore, arginine supplementation increased embryonic/fetal survival and growth in gilts (Mateo et al. 2007) and rats (Zeng et al. 2008). Interestingly, Li et al. (2008b) reported that weekly intraperitoneal administration of an HO1 inducer (cobalt protoporphyrin, 3 mg/kg body weight) to obese diabetic mice for 6 weeks reduced visceral and subcutaneous fat, concentrations of proinflammatory cytokines (e.g., interleukins 6 and 1β, and TNFα) and glucose in plasma, and also improved whole-body insulin sensitivity. These findings indicate that NO and CO may ameliorate adverse effects of obesity and metabolic syndrome in humans and animals.

Conclusion and perspectives

There is a growing body of evidence that gases that are potentially very toxic (e.g., NO, CO, H_2S and SO_2) can function as signaling molecules to impact both physiological and pathological processes in animals and humans. This

Table 4 Effect of CO donor on glucose and fatty acid oxidation in rat retroperitoneal adipose tissue and gastrocnemius muscle

Tissue	CO donor (μM)					
	Glucose oxidation			Oleic acid oxidation		
	0	5	30	0	5	30
Adipose	954 ± 37^{c}	1,086 ± 50^{b}	1,226 ± 54^{a}	1.62 ± 0.05^{c}	1.90 ± 0.07^{b}	2.18 ± 0.08^{a}
Muscle	30.5 ± 1.2^{c}	35.7 ± 1.4^{b}	40.4 ± 1.7^{a}	2.57 ± 0.08^{c}	3.02 ± 0.09^{b}	3.40 ± 0.11^{a}

Values, expressed as nmol substrate/2 h per g tissue, are mean ± SEM, $n = 14$. Means with different superscript letters (a–c) within a row of each substrate differed ($P < 0.05$), as analyzed by one-way analysis of variance and the Tukey multiple comparison test (SAS Institute, Cary, NC). Male Sprague–Dawley rats (325–350 g) were fed a regular nonpurified diet (Product Cat #8604, Harlan Teklad). At 6 h after the last feeding, rats were euthanized with CO_2 and gastrocnemius muscle and retroperitoneal adipose tissue were obtained for metabolic studies, as described by Jobgen et al. (2009a). The tissues (~100 mg) were incubated in duplicate at 37°C for 2 h in 1 ml of oxygenated (95% O_2/5% CO_2) Krebs bicarbonate buffer (Wu et al. 1994) containing 5 mM D-glucose, 0.2 mM oleic acid and either D-[U–^{14}C]glucose (300 dpm/nmol) or [1-^{14}C]oleic acid (250 dpm/nmol). The medium also contained 0, 5 or 30 μM CO donor ($[Ru(CO)_3Cl_2]_2$) or CO donor control product ($RuCl_2(DMSO)_4$), which were generously provided by Dr. Paul J. Kemp (Williams et al. 2004). The chemical CO donor and CO donor control product were dissolved in dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in all incubation media was 0.1% (vol/vol). $^{14}CO_2$ was collected using NCS-II and its radioactivity was measured in a liquid scintillation counter (Wu et al. 1994). Rates of substrate (glucose or oleic acid) oxidation were calculated on the basis of (1) the specific radioactivity of ^{14}C-labeled substrate in incubation medium and (2) the assumption that tracee carbons were completely oxidized to CO_2. Compared with the absence of CO donor (0 μM), 5 and 30 μM CO donor control product did not affect ($P > 0.05$) glucose or oleic acid oxidation in retroperitoneal adipose tissue or gastrocnemius muscle (e.g., 950 ± 41 and 1.69 ± 0.07 nmol substrate/2 h per g adipose tissue, respectively, for glucose and oleic acid; 30.8 ± 1.2 and 2.60 ± 0.09 nmol substrate/2 h per g muscle tissue, respectively, for glucose and oleic acid in the presence of 30 μM CO donor control product.)

recognition has resulted in a paradigm shift in our thinking and understanding of potential roles for endogenous gases in cell biology and nutrition. Amino acids are not only precursors for biosynthesis of these gases but are also important regulators of both gene expression and activities of key synthetic enzymes in a cell-specific manner. It is likely that NO, CO, H_2S, and SO_2 act in exquisite cooperation through multifaceted and complex interactions to affect cell function and whole body homeostasis.

Much knowledge has been generated about non-genomic mechanisms for actions of NO, involving Ca^{2+}, cGMP, and cAMP signaling (Fig. 4). However, little is known regarding effects of CO, H_2S or SO_2 on gene transcription and translation or intracellular protein synthesis and degradation. With the advance of genomics-based technologies (e.g., genomics, proteomics, and metabolomics) (Capone et al. 2008; Wang et al. 2008b; Yan and He 2008; Zhao et al. 2008), this area of biomedical research will likely be highly significant and productive. Furthermore, because microorganisms in the intestine (particularly the large intestine) produce relatively large amounts of H_2S, SO_2 and NH_3, and, to a lesser extent, NO and CO (Blachier et al. 2007), important roles for these gases in health and disease of the gut warrants investigation.

Disappointingly, there is a paucity of information regarding changes in the synthesis of CO, H_2S, and SO_2 by vascular or nonvascular tissues under a variety of nutritional conditions (e.g., underfeeding, obesity and high-protein intake) in both males and females (either pregnant or non-pregnant). Nonetheless, recent advances on gaseous signaling have greatly expanded our basic knowledge of AA biochemistry and nutrition. We expect these new discoveries to aid in the development of novel nutritional and pharmacological paradigms to prevent, ameliorate, and treat major health problems related to developmental biology and nutrient metabolism, which include intrauterine growth restriction, preterm birth, aging, neurological disorders, cancer, obesity, diabetes, and cardiovascular disease.

Acknowledgments This work was supported, in part, by grants from National Institutes of Health (1R21 HD049449), National Research Initiative Competitive Grants (2008-35206-18764, 2008-35203-19120 and 2009-35203-05211) from the USDA Cooperative State Research, Education, and Extension Service, American Heart Association (0755024Y), and Texas AgriLife Research (H-8200). We thank Dr. Nick Flynn and Dr. Yanan Tian for helpful comments, as well as Mr. Merrick Gearing and Ms. Frances Mutscher for assistance in manuscript preparation.

References

Abebe W, Mozaffari MS (2003) Taurine depletion alters vascular reactivity in rats. Can J Physiol Pharmacol 81:903–909

Aberg AM, Hultin M, Abrahamsson P, Larsson JE (2004) Circulatory effects and kinetics following acute administration of carbon monoxide in a porcine model. Life Sci 75:1029–1039

Ahmad AS, Zhuang H, Dore S (2006) Heme oxygenase-1 protects brain from acute excitotoxicity. Neuroscience 141:1703–1708

Arnal JF, Munzel T, Venema RC et al (1995) Interactions between L-arginine and L-glutamine change endothelial NO production. J Clin Invest 95:2565–2572

Atlante A, Calissano P, Bobba A et al (2001) Glutamate neurotoxicity, oxidative stress and mitochondria. FEBS Lett 497:1–5

Barañano DE, Ferris CD, Snyder SH (2001) Atypical neural messengers. Trends Neurosci 24:99–106

Barkoudah E, Jaggar JH, Leffler CW (2004) The permissive role of endothelial NO in CO-induced cerebrovascular dilation. Am J Physiol Heart Circ Physiol 287:H1459–H1465

Barua M, Liu Y, Quinn MR (2001) Taurine chloramine inhibits inducible nitric oxide synthase and TNF-α gene expression in activated alveolar macrophages: decreased NFκB activation and I*k*B kinase activity. J Immunol 167:2275–2281

Blachier F, Mariotti F, Huneau JF, Tomé D (2007) Effects of amino acid-derived luminal metabolites on the colonic epithelium and physiopathological consequences. Amino Acids 33:547–562

Bogdan CT, Rollinghoff M, Diefenbach A (2000) The role of nitric oxide in innate immunity. Immunol Rev 173:17–26

Bonthius DJ, Bonthiu NE, Li SL et al (2008) The protective effect of neuronal nitric oxide synthase (nNOS) against alcohol toxicity depends upon the NO-cGMP-PKG pathway and NFκB. Neurotoxicology 29:1080–1091

Bredt DS, Snyder SH (1994) Nitric oxide: a physiologic messenger molecule. Annu Rev Biochem 63:175–195

Brusko TM, Wasserfall CH, Agarwal A et al (2005) An integral role for heme oxygenase-1 and carbon monoxide in maintaining peripheral tolerance by CD4(+) CD25(+) regulatory T cells. J Immunol 174:5181–5186

Burnett AL, Johns DG, Kriegsfeld LJ et al (1998) Ejaculatory abnormalities in mice with targeted disruption of the gene for heme oxygenase-2. Nat Med 4:84–87

Capone G, De Marinis A, Simone S et al (2008) Mapping the human proteome for non-redundant peptide islands. Amino Acids 35:209–216

Chang L, Geng B, Yu F et al (2008) Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats. Amino Acids 34:573–585

Chao CL, Kuo TL, Lee YT (2000) Effects of methionine-induced hyperhomocysteinemia on endothelium-dependent vasodilation and oxidative status in healthy adults. Circulation 101:485–490

Chen J, Tu Y, Connolly EC, Ronnett GV (2005) Heme oxygenase-2 protects against glutathione depletion-induced neuronal apoptosis mediated by bilirubin and cyclic GMP. Curr Neurovasc Res 2:121–131

Chung HT, Choi BM, Kwon YG et al (2008) Interactive relationship between nitric oxide (NO) and carbon monoxide (CO): heme oxygenase-1/CO pathway is a key modulator in NO-mediated antiapoptosis and anti-inflammation. Methods Enzymol 441:329–338

Closs EI, Scheld JS, Sharafi M, Forstermann U (2000) Substrate supply for nitric oxide synthase in macrophages and endothelial cells: role of cationic amino acid transporters. Mol Pharmacol 57:68–74

Coeffier M, Le Pessot F, Leplingard A et al (2002) Acute enteral glutamine infusion enhances heme oxygenase-1 expression in human duodenal mucosa. J Nutr 132:2570–2573

Cruse I, Maines MD (1988) Evidence suggesting that the two forms of heme oxygenase are products of different genes. J Biol Chem 263:3348–3353

Cutajar MC, Edwards TM (2007) Evidence for the role of endogenous carbon monoxide in memory processing. J Cogn Neurosci 19:557–562

De Palma C, Falcone S, Panzeri C et al (2008) Endothelial nitric oxide synthase overexpression by neuronal cells in neurodegeneration: a link between inflammation and neuroprotection. J Neurochem 106:193–204

Deldicque L, Sanchez CC, Horman S et al (2008) Antagonistic effects of leucine and glutamine on the mTOR pathway in myogenic C_2C_{12} cells. Amino Acids 35:147–155

Di S, Maxson MM, Franco A, Tasker JG (2009) Glucocorticoids regulate glutamate and GABA synapse-specific retrograde transmission via divergent nongemonic signaling pathways. J Neurosci 29:393–401

Dolinay T, Szilasi M, Liu MY, Choi AMK (2004) Inhaled carbon monoxide confers anti-inflammatory effects against ventilator-induced lung injury. Am J Respir Crit Care Med 170:613–620

Durante W, Johnson FK, Johnson RA (2006) Role of carbon monoxide in cardiovascular function. J Cell Mol Med 10:672–686

Erdmann K, Grosser N, Schroder H (2005) L-methionine reduces oxidant stress in endothelial cells: role of heme oxygenase-1, ferritin, and nitric oxide. AAPS J 29:E195–E200

Esechie A, Kiss L, Olah G et al (2008) Protective effect of hydrogen sulfide in amurine model of acute lung injury induced by combined burn and smoke inhalation. Clin Sci 115:91–97

Faff L, Reichenbach A, Albrecht J (1996) Ammonia-induced taurine release from cultured rabbit Muller cells is an osmoresistant process mediated by intracellular accumulation of cyclic AMP. J Neurosci Res 46:231–238

Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18:872–879

Fiumana E, Parfenova H, Jaggar JH, Leffler CW (2003) Carbon monoxide mediates vasodilator effects of glutamate in isolated pressured cerebral arteries of newborn pigs. Am J Physiol Heart Circ Physiol 284:H1073–H1079

Fu WJ, Haynes TE, Kohli R et al (2005) Dietary L-arginine supplementation reduces fat mass in Zucker diabetic fatty rats. J Nutr 135:714–721

Galli F (2007) Amino acid and protein modification by oxygen and nitrogen species. Amino Acids 32:497–499

Geng B, Cui Y, Zhao J et al (2007) Hydrogen sulfide downregulates the aortic L-arginine/nitric oxide pathway in rats. Am J Physiol Regul Integr Comp Physiol 293:R1608–R1618

Griffith OW (1987) Mammalian sulfur amino acid metabolism: an overview. Methods Enzymol 143:366–376

Grosser N, Oberle S, Berndt G et al (2004) Antioxidant action of L-alanine: heme oxygenase-1 and ferritin as possible mediators. Biochem Biophys Res Commun 314:351–355

Hartsfield CL, Alam J, Cook JL, Choi AMK (1997) Regulation of heme oxygenase-1 gene expression in vascular smooth muscle cells by nitric oxide. Am J Physiol Lung Cell Mol Physiol 273:L980–L988

Harty RF, Ancha HR, Xia Y et al (2004) GABAergic mechanisms of gastroprotection in the rat: roles of sensory neurons, prostaglandins, and nitric oxide. Digest Dis Sci 49:1875–1881

Haynes TE, Li P, Li XL et al (2009) L-Glutamine or L-alanyl-L-glutamine prevents oxidant- or endotoxin-induced death of neonatal enterocytes. Amino Acids. doi:10.1007/s00726-009-0243-x

He QH, Kong XF, Wu G et al. (2008) Metabolomic analysis of the response of growing pigs to dietary L-arginine supplementation. Amino Acids. doi:10.1007/s00726-008-0192-9

Hibbs HB, Taintor RR, Vavrin Z (1987) Macrophage cytotoxicity: role for L-arginine deiminase and imino nitrogen oxidation to nitrite. Science 235:473–476

Houdijk APJ, Visser JJ, Rijinsburger ER et al (1998) Dietary glutamine supplementation reduces plasma nitrate levels in rats. Clin Nutr 17:11–14

Hu CA, Khalil S, Zhaorigetu S et al (2008) Human Δ^1-pyrroline-5-carboxylate synthase: function and regulation. Amino Acids 35:665–672

Huang PL, Huang ZH, Mashimo H et al (1995) Hypertension in mice lacking the gene for endothelial nitric-oxide synthase. Nature 377:239–242

Hung SY, Liou HC, Kang KH et al (2008) Overexpression of heme oxygenase-1 protects dopaminergic neurons against 1-methyl-4-phenylpyridinium-induced neurotoxicity. Mol Pharmacol 74:1564–1575

Ignarro LJ, Buga GM, Wood KS et al (1987) Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 84:9265–9269

Ignarro LJ, Buga GM, Wei LH et al (2001) Role of the arginine-nitric oxide pathway in the regulation of vascular smooth muscle cell proliferation. Proc Natl Acad Sci USA 98:4202–4208

Jobgen WS, Fried SK, Fu WJ et al (2006) Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. J Nutr Biochem 17:571–588

Jobgen W, Meininger CJ, Jobgen SC et al (2009a) Dietary L-arginine supplementation reduces white-fat gain and enhances skeletal muscle and brown fat masses in diet-induced obese rats. J Nutr 139:230–237

Jobgen W, Fu WJ, Gao H et al (2009b) High fat feeding and dietary L-arginine supplementation differentially regulate gene expression in rat white adipose tissue. Amino Acids. doi:10.1007/s00726-009-0246-7

Jurkowska H, Wrobel M (2008) N-acetyl-cysteine as a source of sulfane sulfur in astrocytoma and astrocyte cultures: correlation with cell proliferation. Amino Acids 34:231–237

Kamoun P (2004) Endogenous production of hydrogen sulfide in mammals. Amino Acids 26:243–254

Kawaguchi T, Brusilow SW, Traystman RJ, Koehler RC (2005) Glutamine-dependent inhibition of pial arteriolar dilation to acetylcholine with and without hyperammonemia in the rat. Am J Physiol Regul Integr Comp Physiol 288:R1612–R1619

Kim HS, Loughran PA, Billiar TR (2008) Carbon monoxide decreases the level of iNOS protein and active dimmer in IL-1β-stimulated hepatocytes. Nitric Oxide 18:256–265

Kimura H, Nagai Y, Umemura K, Kimura Y (2005) Physiological roles of hydrogen sulfide: synaptic modulation, neuroprotection, and smooth muscle relaxation. Antioxid Redox Signal 7:795–803

Kiss L, Deitch EA, Szabo C (2008) Hydrogen sulfide decreases adenosine triphosphate levels in aortic rings and leads to vasorelaxation via metabolic inhibition. Life Sci 83:589–594

Kodavanti UP, Schladweiler MC, Ledbetter AD et al (2006) The spontaneously hypertensive rat: an experimental model of sulfur dioxide-induced airways disease. Toxicol Sci 94:193–205

Kohli R, Meininger CJ, Haynes TE et al (2004) Dietary L-arginine supplementation enhances endothelial nitric oxide synthesis in streptozotocin-induced diabetic rats. J Nutr 134:600–608

Lagranha CJ, Doi SQ, Pithon-Curi SQ et al (2008a) Glutamine enhances glucose-induced mesangial cell proliferation. Amino Acids 34:683–685

Lagranha CJ, Levada-Pires AC, Sellitti DF et al (2008b) The effect of glutamine supplementation and physical exercise on neutrophil function. Amino Acids 34:337–346

Lee TJF, Sarwinski S, Ishine T et al (1996) Inhibition of cerebral neurogenic vasodilation by L-glutamine and nitric oxide synthase inhibitors and its reversal by L-citrulline. J Pharmacol Exp Ther 276:353–358

Lee J, Ryu H, Ferrante RJ et al (2003) Translational control of inducible nitric oxide synthase expression by arginine can explain the arginine paradox. Proc Natl Acad Sci USA 100:4843–4848

Leffler CW, Balabanova L, Fedinec AL et al (2005) Nitric oxide increases carbon monoxide production by piglet cerebral microvessels. Am J Physiol Heart Circ Physiol 289:H1442–H1447

Li J, Meng Z (2009) The role of sulfur dioxide as an endogenous gaseous vasoactive factor in synergy with nitric oxide. Nitric Oxide. doi:10.1016/j.niox.2008.12.003

Li P, Yin YL, Li D et al (2007) Amino acids and immune function. Br J Nutr 98:237–252

Li P, Kim SW, Li XL et al (2008a) Dietary supplementation with cholesterol and docosahexaenoic acid increases the activity of the arginine-nitric oxide pathway in tissues of young pigs. Nitric Oxide 19:259–265

Li M, Kim DH, Tsenovoy PL et al (2008b) Treatment of obese diabetic mice with heme oxygenase reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance. Diabetes 57:1526–1535

Liao XH, Majithia A, Huang XL, Kimmel AR (2008) Growth control via TOR kinase signaling, an intracellular sensor of amino acids and energy availability, with crosstalk potential to proline metabolism. Amino Acids 35:761–770

Lin YF, Raab-Graham K, Jan YN, Jan LY (2004) NO stimulation of ATP-sensitive potassium channels: involvement of Ras/mitogen-activated protein kinase pathway and contribution to neuroprotection. Proc Natl Acad Sci USA 101:7799–7804

Ma XY, Lin YC, Jiang ZY et al (2008) Dietary arginine supplementation enhances antioxidative capacity and improves meat quality of finishing pigs. Amino Acids. doi:10.1007/s00726-008-0213-8

Maines MD (1997) The heme oxygenase system: a regulator of second messenger gases. Annu Rev Pharmacol Toxicol 37:517–554

Mannick JB (2007) Regulation of apoptosis by protein *S*-nitrosylation. Amino Acids 32:523–526

Marks JD, Schreiber MD (2008) Inhaled nitric oxide and neuroprotection in preterm infants. Clin Perinatol 35:793–807

Mateo RD, Wu G, Bazer FW et al (2007) Dietary L-arginine supplementation enhances the reproductive performance of gilts. J Nutr 137:652–656

McCoubrey WK Jr, Huang TJ, Maines MD (1997) Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur J Biochem 247:725–732

Meininger CJ, Wu G (1997) L-Glutamine inhibits nitric oxide synthesis in bovine venular endothelial cells. J Pharmacol Exp Ther 281:448–453

Miller DL, Roth MB (2007) Hydrogen sulfide increases thermotolerance and lifespan in Caenorhabditis elegans. Proc Natl Acad Sci USA 104:20618–20622

Mizuguchi Y, Chen J, Seshan SV et al (2008) A novel cell-permeable antioxidant pepide decreases renal tubular apoptosis and damage in unilateral ureteral obstruction. Am J Physiol Renal Physiol 295:F1545–F1553

Mühling J, Burchert D, Langefeld TW et al (2007) Pathways involved in alanyl-glutamine-induced changes in neutrophil amino- and α-keto acid homeostasis or immunocompetence. Amino Acids 33:511–524

Mühling J, Nickolaus KA, Matejec R et al (2008) Which mechanisms are involved in taurine-dependent granulocytic immune response or amino- and α-keto acid homeostasis. Amino Acids 34:257–270

Mutus B, Rabini RA, Staffolani R et al (2001) Homocysteine-induced inhibition of nitric oxide production in platelets: a study on healthy and diabetic subjects. Diabetologia 44:979–982

Muz B, Kontny E, Marcinkiewicz J et al (2008) Heme oxygenase-1 participates in the anti-inflammatory activity of taurine chloramine. Amino Acids 35:397–402

Nakahira K, Kim HP, Geng XH et al (2006) Carbon monoxide differentially inhibits TLR signaling pathways by regulating ROS-induced trafficking of TLRs to lipid rafts. J Exp Med 203:2377–2389

Nelson RJ, Demas GE, Huang PL et al (1995) Behavioural abnormalities in male mice lacking neuronal nitric oxide synthase. Nature 378:383–386

Ning W, Choi AMK, Li CJ (2005) Carbon monoxide inhibits IL-17-induced IL-6 production through the MAPK pathway in human pulmonary epithelial cells. Am J Physiol Lung Cell Mol Physiol 289:L268–L273

Nisoli E, Clementi E, Paolucci C et al (2003) Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science 299:896–899

Nobre LS, Seixas JD, Romao CC et al (2007) Antimicrobial action of carbon monoxide-releasing compounds. Antimicrob Agents Chemother 51:4303–4307

Olszanecki R, Kurnyta M, Biedron R et al (2008) The role of heme oxygenase-1 in down regulation of PGE_2 production by taurine chloramines and taurine bromamine in J774.2 macrophages. Amino Acids 35:359–364

Orlando GF, Wolf G, Engelmann M (2008) Role of neuronal nitric oxide synthase in the regulation of the neuroendocrine stress response in rodents: insights from mutant mice. Amino Acids 35:17–27

Pantazis NJ, West JR, Dai D (1998) The nitric-oxide-cyclic AMP pathway plays an essential role in both promoting cell survival of cerebellar granule cells in culture and protecting the cells against ethanol neurotoxicity. J Neurochem 70:1826–1838

Parfenova H, Neff RA, Alonso JS et al (2001) Cerebral vascular endothelial heme oxygenase: expression, localization, and activation by glutamate. Am J Physiol Cell Physiol 281:C1954–C1963

Park T, Rogers QR, Morris JG (1999) High dietary protein and taurine increase cysteine desulfhydration in kittens. J Nutr 129:2225–2230

Qu K, Lee SW, Bian JS et al (2007) Hydrogen sulfide: neurochemistry and neurobiology. Neurochem Int 52:155–165

Rhoads JM, Wu G (2008) Glutamine, arginine, and leucine signaling in the intestine. Amino Acids. doi:10.1007/s00726-008-0225-4

Saransaari P, Oja SS (2008) Nitric oxide is involved in taurine release in the mouse brain stem under normal and ischemic conditions. Amino Acids 34:429–436

Shih AY, Erb H, Sun X et al (2006) Cystine/glutamate exchange modulates glutathione supply for neuroprotection from oxidative stress and cell proliferation. J Neurosci 26:10514–10523

Shimazu T, Ikeuchi H, Sugimoto H et al (2000) Half-life of blood carboxyhemoglobin after short-term and long-term exposure to carbon monoxide. J Trauma 49:126–131

Simpson RC, Freedland RA (1976) Factors affecting the rate of gluconeogenesis from L-cysteine in the perfused rat liver. J Nutr 106:1272–1278

Song RP, Zhou Z, Kim PKM et al (2004) Carbon monoxide promotes Fas/CD95-induced apoptosis in Jurkat cells. J Biol Chem 279:44327–44334

Stipanuk MH, Dominy JE, Lee JI, Coloso RM (2006) Mammalian cysteine metabolism: new insights into regulation of cysteine metabolism. J Nutr 136:1652S–1659S

Stipanuk MH, Ueki I, Dominy JE et al (2008) Cysteine dioxygenase: a robust system for regulation of cellular cysteine levels. Amino Acids. doi:10.1007/s00726-008-0202-y

Suryawan A, O'Connor PMJ, Bush JA et al (2008) Differential regulation of protein synthesis by amino acids and insulin in

peripheral and visceral tissues of neonatal pigs. Amino Acids. doi:10.1007/s00726-008-0149-z
Tan BE, Yin YL, Liu ZQ et al (2008) Dietary L-arginine supplementation increases muscle gain and reduces body fat mass in growing-finishing pigs. Amino Acids. doi:10.1007/s00726-008-0148-0
Tang GH, Wu LY, Liang WB, Wang R (2005) Direct stimulation of K-ATP channels by exogenous and endogenous hydrogen sulfide in vascular smooth muscle cells. Mol Pharmacol 68:1757–1764
Uehara K, Takahashi T, Fujii H et al (2005) The lower intestinal tract-specific induction of heme oxygenase-1 by glutamine protects against endotoxemic intestinal injury. Crit Care Med 33:381–390
Umeda K, Takahashi T, Inoue K et al (2009) Prevention of hemorrhagic shock-induced intestinal tissue injury by glutamine via heme oxygenase-1 induction. Shock 31:40–49
Vareille M, Rannou F, Thelier N et al (2008) Heme oxygenase-1 is a critical regulator of nitric oxide production in enterohemorrhagic Escherichia coli-infected human enterocytes. J Immunol 180:5720–5726
Voss P, Grune T (2007) The nuclear proteasome and the degradation of oxidatively damaged proteins. Amino Acids 32:527–534
Wang W, Qiao S, Li D (2008a) Amino acids and gut function. Amino Acids. doi:10.1007/s00726-008-0152-4
Wang JJ, Wu G, Zhou HJ, Wang FL (2008b) Emerging technologies for amino acid nutrition research in the post-genome era. Amino Acids. doi:10.1007/s00726-008-0193-8
Wang XQ, Ou DY, Yin JD et al (2009) Proteomic analysis reveals altered expression of proteins related to glutathione metabolism and apoptosis in the small intestine of zinc oxide-supplemented piglets. Amino Acids. doi:10.1007/s00726-009-0242-y
Wei LH, Wu G, Morris SM Jr, Ignarro LJ (2001) Elevated arginase I expression in rat aortic smooth muscle cells increases cell proliferation. Proc Natl Acad Sci USA 98:9260–9264
Welch GN, Upchurch GR, Farivar RS et al (1998) Homocysteine-induced nitric oxide production in vascular smooth muscle cells by NF-kappa B-dependent transcriptional activation of *Nos2*. Proc Natl Acad Sci USA 110:22–31
Williams SEJ, Wootton P, Mason HS et al (2004) Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel. Science 306:2093–2097
Wu G, Brosnan JT (1992) Macrophages can convert citrulline into arginine. Biochem J 281:45–48
Wu G, Meininger CJ (2000) Arginine nutrition and cardiovascular function. J Nutr 130:2626–2629
Wu G, Meininger CJ (2002) Regulation of nitric oxide synthesis by dietary factors. Annu Rev Nutr 22:61–86
Wu G, Meininger CJ (2009) Nitric oxide and vascular insulin resistance. BioFactors. dio:10.1002/BIOF.00004
Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336:1–17
Wu G, Knabe DA, Flynn NE (1994) Synthesis of citrulline from glutamine in pig enterocytes. Biochem J 299:115–121
Wu G, Flynn NE, Flynn SP et al (1999) Dietary protein or arginine deficiency impairs constitutive and inducible nitric oxide synthesis by young rats. J Nutr 129:1347–1354
Wu G, Haynes TE, Li H et al (2001) Glutamine metabolism to glucosamine is necessary for glutamine inhibition of endothelial nitric oxide synthesis. Biochem J 353:245–252
Wu G, Fang YZ, Yang S et al (2004) Glutathione metabolism and its implications for health. J Nutr 134:489–492
Wu G, Bazer FW, Davis TA et al (2007a) Important roles for the arginine family of amino acids in swine nutrition and production. Livest Sci 112:8–22
Wu G, Collins JK, Perkins-Veazie P et al (2007b) Dietary supplementation with watermelon pomace juice enhances arginine availability and ameliorates the metabolic syndrome in Zucker diabetic fatty rats. J Nutr 137:2680–2685
Wu G, Bazer FW, Datta S et al (2008a) Proline metabolism in the conceptus: Implications for fetal growth and development. Amino Acids 35:691–702
Wu G, Bazer FW, Davis TA et al (2008b) Arginine metabolism and nutrition in growth, health and disease. Amino Acids. doi:10.1007/s00726-008-0210-y
Yan GR, He QY (2008) Functional proteomics to identify critical proteins in signal transduction pathways. Amino Acids 35:267–274
Yanfei W, Lin S, Junbao D, Chaoshu T (2006) Impact of L-arginine on hydrogen sulfide/cystathionine-γ-lyase pathway in rats with blood flow-induced pulmonary hypertension. Biochem Biophys Res Commun 345:851–857
Yang G, Wu L, Jiang B et al (2008) H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine γ-lyase. Science 322:587–590
Yi JH, Hazell AS (2005) *N*-Acetylcysteine attenuates early induction of heme oxygenase-1 following traumatic brain injury. Brain Res 1033:13–19
Zeng XF, Wang FL, Fan X et al (2008) Dietary arginine supplementation during early pregnancy enhances embryonic survival in rats. J Nutr 138:1421–1425
Zhang HL, Bhatia M (2008) Hydrogen sulfide: a novel mediator of leukocyte activation. Immunopharmacol Immunotoxicol 30:631–645
Zhang XH, Li H, Jin HL et al (2000) Effects of homocysteine on endothelial nitric oxide production. Am J Physiol Renal Physiol 279:F671–F678
Zhang F, Siow YL, Karmin O (2004) Hyperhomocysteinemia activates NF-kB and inducible nitric oxide synthase in the kidney. Kidney Int 65:1327–1338
Zhang HL, Moochlaha SM, Bhatia M (2008) Endogenous hydrogen sulfide regulates inflammatory response by activating the ERK pathway in polymicrobial sepsis. J Immunol 181:4320–4331
Zhao W, Zhang J, Lu Y, Wang R (2001) The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. EMBO J 20:6008–6016
Zhao W, Ndisang JF, Wang R (2003) Modulation of endogenous production of H_2S in rat tissues. Can J Physiol Pharmacol 81:848–853
Zhao XM, Chen LN, Aihara K (2008) Protein function prediction with high-throughput data. Amino Acids 35:517–530
Zhi L, Ang AD, Zhang HL et al (2007) Hydrogen sulfide induces the synthesis of proinflammatory cytokines in human monocyte cell line U937 via the ERK-NF-kB pathway. J Leuk Biol 81:1322–1332
Zhuo M, Small SA, Kandel ER, Hawkins RD (1993) Nitric oxide and carbon monoxide poduce activity-dependent long-term synaptic enhancement in hippocampus. Science 260:1946–1950