European Journal of Pharmacology 834 (2018) 92–102

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Gasotransmitters and the immune system: Mode of action and novel therapeutic targets

Paolo Fagone[a], Emanuela Mazzon[b], Placido Bramanti[b], Klaus Bendtzen[c], Ferdinando Nicoletti[a,*]

[a] Department of Biomedical and Biotechnological Sciences, University of Catania, Catania, Italy
[b] IRCCS Centro Neurolesi Bonino Pulejo, Stada Statale 113, C.da Casazza, 98124 Messina, Italy
[c] Institute for Inflammation Research, Rigshospitalet, Copenhagen, Denmark

ARTICLE INFO

Keywords:
Gasotransmitter
Nitric oxide
Hydrogen sulfide
Carbon monoxide
Immune system
EAE
Neuroinflammation
Multiple sclerosis

ABSTRACT

Gasotransmitters are a group of gaseous molecules, with pleiotropic biological functions. These molecules include nitric oxide (NO), hydrogen sulfide (H_2S), and carbon monoxide (CO). Abnormal production and metabolism of these molecules have been observed in several pathological conditions. The understanding of the role of gasotransmitters in the immune system has grown significantly in the past years, and independent studies have shed light on the effect of exogenous and endogenous gasotransmitters on immune responses. Moreover, encouraging results come from the efficacy of NO-, CO- and H_2S -donors in preclinical animal models of autoimmune, acute and chronic inflammatory diseases. To date, data on the influence of gasotransmitters in immunity and immunopathology are often scattered and partial, and the scarcity of clinical trials using NO-, CO- and H_2S -donors, reveals that more effort is warranted. This review focuses on the role of gasotransmitters in the immune system and covers the evidences on the possible use of gasotransmitters for the treatment of inflammatory conditions.

1. Introduction

Increasing evidence accumulated during the last decades have shed light into the physiological mechanisms that contribute to down-regulate and turn off immune responses. The endogenous immunomodulators that control responses of both the innate and acquired immune system comprise several and different families of molecules that range from hormones (sex hormones, corticosteroids, D3 vitamin) (Cain and Cidlowski, 2017; Rolf et al., 2016), to endogenous antagonists of cytokines, such as soluble receptors and naturally occurring autoantibodies, and antiinflammatory cytokines of the Th2 and Th3 subtype, including interleukin (IL)-10, IL-13 transforming growth-factor (TGF)-beta and IL-35, proteins such as indoleamine-pyrrole 2,3-dioxygenase 1 (IDO1) and heme oxygenase 1 (HO1) and classical immunosuppressive checkpoints, such as cytotoxic T-lymphocyte-associated antigen (CTLA)4 and programmed cell-death protein (PD)1 and PD2 (Gérard et al., 1993; Nicoletti et al., 1997; Bendtzen et al., 2000; Dujmovic et al., 2009; Mackern-Oberti et al., 2014; Meager and Wadhwa, 2014; Raphael et al., 2015; Mbongue et al., 2015; Sakkas et al., 2018; Tocheva and Mor, 2017).

It has been shown that deregulated production of these naturally occurring immunosuppressive and anti-inflammatory mediators, for example due to genetic polymorphisms, may contribute to the development of immunoinflammatory and autoimmune diseases (Barcellini et al., 1996; Arend, 2002).

More recent evidence has shown that additional regulation of immune responses is mediated by a family of gases normally produced in the body such as nitric oxide (NO), hydrogen sulfide (H_2S), and carbon monoxide (CO) reviewed in (Motterlini and Otterbein, 2010; Wallace et al., 2015; Wallace and Wang, 2015).

These gases are collectively termed as gasotransmitters and are characterized by high lipid solubility and ability of diffusing across cell membranes without the requirement of specific receptors or transporters (Wang, 2014). Gasotransmitters are produced endogenously by specific enzymes and mediate several physiological functions through specific molecular targets (Fig. 1). NO was the first identified gasotransmitter. Using L-arginine as substrate, four isoforms of NO synthases catalyze NO production (Wang, 2014). NO is known as an endothelium-derived relaxing factor (Wang, 2014). As a product of heme metabolism, CO represents another gasotransmitter whose function is partly similar to NO (Wang, 2014). Among several other effects, CO relaxes vascular vessels lowers blood pressure, and protects from ischemia/reperfusion damage (Wang, 2014). Hydrogen sulfide (H_2S) is the third gasotransmitter discovered and plays an important role in regulating

* Corresponding author.
E-mail address: ferdinic@unict.it (F. Nicoletti).

https://doi.org/10.1016/j.ejphar.2018.07.026
Received 10 May 2018; Received in revised form 29 June 2018; Accepted 13 July 2018

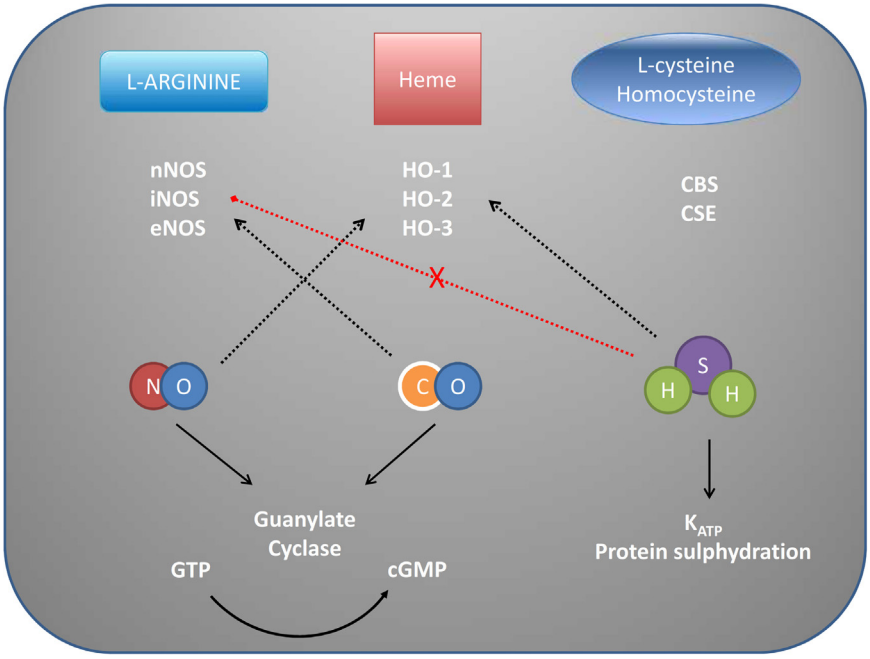

Fig. 1. Carbon monoxide (CO), nitric oxide (NO) and hydrogen sulfide (H_2S) are produced in the mammalian tissues by endogenous enzymes. The figure depicts the substrates, enzymes and downstream targets of the gasotransmitters and their complex relationships. CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; HO, heme oxygenase; NOS, NO synthase; GTP, guanosine-5'-triphosphate; cGMP, cyclic guanosine monophosphate.

neurotransmission and neuromodulation (Wang, 2014). Production and metabolism of these molecules are involved in the regulation of diverse biological processes, including vascular tone, immune function, cell survival, metabolism, and stress response (Wang, 2014). Considerable progress has been made in recent years in the pharmacology of NO, CO and H_2S with the development of several NO-, CO- and H_2S -donors, that have successfully been used in the preclinical setting for the treatment of diverse immunoinflammatory and autoimmune diseases. In recent years, the understanding of the role of gasotransmitters in the immune system has grown significantly, and independent studies have shed light on the effect of exogenous and endogenous gasotransmitters on immune cell types, as well as on preclinical models of immunoinflammatory and autoimmune diseases. However, the information are often scattered and partial. This review, therefore, aims at integrating the knowledge in this field emphasizing current evidence supporting the use of gasotransmitters in the clinical setting.

2. Carbon monoxide

2.1. Physiology of carbon monoxide

In mammals, CO is physiologically produced during heme metabolism in the phagocytic system of the spleen and liver. The process is catalyzed by HO enzymes, encoded by the *HMOX* genes. In the presence of HO enzymes, the porphyrin ring of heme is oxidized and equimolar amounts of CO, ferrous iron and biliverdin are produced. Three isoforms of HO, HO-1, HO-2 and HO-3 are currently characterized. HO-1 is the inducible isoform and its transcriptional levels increase following cellular stress. On the other hand, the HO-2 and HO-3 isoforms are constitutively expressed in many mammalian cells (Foresti et al., 2005). In the promoter region of the human *HMOX1* gene, consensus binding sites for transcription factors have been found. These include nuclear factor-kB (NF-kB), activator protein-1 (AP-1), c-myc and IL-6 response elements. Several factors can induce HO-1, e.g. heat shock, oxidized lipids, nitric oxide, radiation, hydrogen peroxide, hypoxia, lipopolysaccharides (LPS), cytokines - including IL-1, IL-6, IL-10, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and by exogenous CO (Chauveau et al., 2005).

CO is freely diffusible across cellular membranes, and therefore can rapidly bind intracellular targets such as soluble guanylyl cyclase (sGC), heme-containing potassium channels, NO synthase (NOS) and nicotinamide adenine dinucleotide phosphate (NADP)/NADPH oxidase. The binding of CO to heme-containing proteins modifies their conformational status and positively controls their activity, such as with sGC or NOS, that increase cyclic GMP and NO, therefore regulating vasomotor tone and neurotransmission. On the other hand, CO can inhibit the activity of other hemoproteins, such as NADPH oxidase, thus modulating the production of superoxide and mitochondrial cytochrome c. Given its ability to bind metal-containing proteins, CO has been reported to exert several physiological effects, including inhibition of platelet aggregation, anti-proliferative action on smooth muscle, neurotransmission and vasodilation (Foresti et al., 2005).

2.2. Carbon monoxide in immunity

It has been shown that human and rat immature DC express HO-1, and that HO-1 expression drastically decreases upon DC maturation (Chauveau et al., 2005). Also, independent studies have demonstrated that overexpression of HO-1 in DC inhibits LPS-induced maturation and pro-inflammatory functions (Chauveau et al., 2005; Rémy et al., 2009), in turn modulating the suppressive capacity of regulatory T-cells (George et al., 2008). Later, it was ascertained that CO mediates the effects of HO-1 in DC, since biliverdin, bilirubin, and deferoxamine treatment of DC was not able to affect DC maturation, whereas CO significantly decreased the levels of the LPS-induced pro-inflammatory cytokines IL-12p70, IL-12p40, and IL-23. Accordingly, CO-treated DC functioned as weaker stimulators of allogeneic T-cells and the expression of the anti-inflammatory cytokine IL-10 was maintained (Rémy et al., 2009). In animal models, pretreatment of DC with CO is sufficient to prevent adoptive transfer of diabetes (Rémy et al., 2009). Furthermore, exposure of CD11c+ DC to CO downregulates of class II major histocompatibility complex transactivator (*CIITA*) gene expression (Chora et al., 2007), and decreases major histocompatibility complex (MHCII) levels in activated bone marrow-derived DC, with subsequent prevention of the development of intimal hyperplasia and graft failure (Cheng et al., 2010).

In macrophages, physiological levels of CO selectively inhibit LPS-induced increase of TNF-α, IL-1β and MIP-1b in the Raw264.7 cell line (Choi et al., 2000). Concomitantly, CO increases the production of the anti-inflammatory cytokine, IL-10. Otterbein and collegues