Vascular Pharmacology 60 (2014) 32–41

Contents lists available at ScienceDirect

Vascular Pharmacology

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

Pharmacological characterization of the vascular effects of aryl isothiocyanates: Is hydrogen sulfide the real player?

Alma Martelli [a], Lara Testai [a], Valentina Citi [a], Alice Marino [a], Francesca G. Bellagambi [b], Silvia Ghimenti [b], Maria C. Breschi [a], Vincenzo Calderone [a,*]

[a] Dipartimento di Farmacia, Università di Pisa, via Bonanno, 6, I-56126 Pisa, Italy
[b] Dipartimento di Chimica e Chimica Industriale, Università di Pisa, via Risorgimento, 35, I-56126 Pisa, Italy

ARTICLE INFO

Article history:
Received 5 July 2013
Received in revised form 12 November 2013
Accepted 16 November 2013

Keywords:
Hydrogen sulfide
H_2S-releasing drugs
Hypertension
Vascular smooth muscle
Isothiocyanate

Chemical compounds studied in this article:
Hydrogen sulfide (PubChem CID: 402)
Phenyl isothiocyanate (PubChem CID: 7673)
4-carboxyphenyl isothiocyanate (PubChem CID: 242946)
GYY4137 (PubMed CID: 53393943)
Diallyl disulfide (PubMed CID: 16590)

ABSTRACT

Hydrogen sulfide (H_2S) is an endogenous gasotransmitter, which mediates important physiological effects in the cardiovascular system. Accordingly, an impaired production of endogenous H_2S contributes to the pathogenesis of important cardiovascular disorders, such as hypertension. Therefore, exogenous compounds, acting as H_2S-releasing agents, are viewed as promising pharmacotherapeutic agents for cardiovascular diseases. Thus, this paper aimed at evaluating the H_2S-releasing properties of some aryl isothiocyanate derivatives and their vascular effects. The release of H_2S was determined by amperometry, spectrophotometry and gas/mass chromatography. Moreover, the vascular activity of selected isothiocyanates were tested in rat conductance (aorta) and coronary arteries. Since H_2S has been recently reported to act as an activator of vascular Kv7 potassium channels, the possible membrane hyperpolarizing effects of isothiocyanates were tested on human vascular smooth muscle (VSM) cells by spectrofluorescent dyes. Among the tested compounds, phenyl isothiocyanate (PhNCS) and 4-carboxyphenyl isothiocyanate (PhNCS–COOH) exhibited slow-H_2S-release, triggered by organic thiols such as L-Cysteine. These compounds were endowed with vasorelaxing effects on conductance and coronary arteries. Moreover, these two isothiocyanates caused membrane hyperpolarization of VSM cells. The vascular effects of isothiocyanates were strongly abolished by the selective Kv7-blocker XE991. In conclusion, the isothiocyanate function can be viewed as a suitable slow H_2S-releasing moiety, endowed with vasorelaxing and hypotensive effects, typical of this gasotransmitter. Thus, such a chemical moiety can be employed for the development of novel chemical tools for basic studies and promising cardiovascular drugs.

1. Introduction

Hydrogen sulfide (H_2S) has been recently discovered as a physiological gasotransmitter endogenously produced in substantial amounts by mammalian tissues. At physiological concentrations, H_2S mediates important physiological effects in many systems. In the cardiovascular system, H_2S production is mainly ensured by the enzyme cystathionine-gamma-lyase (CSE), starting from the aminoacid L-Cysteine [1]. H_2S plays a key role in regulating cardiovascular homeostasis, acting as a direct relaxing agent in the vascular smooth muscle [2].

It is presently accepted that an impaired production of endogenous H_2S contributes to the pathogenesis of important cardiovascular disorders, such as hypertension. In fact, the H_2S pathway is pivotally involved in the regulation of blood pressure and exogenous H_2S-donors effectively prevent the progression of hypertension [3] and decrease the blood pressure in experimental models of hypertension [4]. Consistently, the genetic deletion of CSE in mice is associated with blunted levels of H_2S in blood, heart and aorta, with increased blood pressure values and decreased endothelium-mediated vasorelaxant effects [5]. Impaired H_2S biosynthesis has been also observed in the setting of cardiovascular complications associated with experimental models of diabetes mellitus [6].

The patho-physiological roles of endogenous H_2S in the cardiovascular system highlight the great usefulness of its pharmacological modulation for pharmacotherapeutic purposes [7,8]. Thus, exogenous compounds, which exhibit the pharmacodynamic profile of H_2S-releasing agents, are

Abbreviations: AB, assay buffer; ACh, acetylcholine; AngII, angiotensin II; CF, coronary flow; CSE, cystathionine-gamma-lyase; DADS, diallyl disulfide; DiBac4(3), bisoxonol dye bis-(1,3-dibutylbarbituric acid); DMSO, dimethylsulfoxide; GYY4137, morpholin-4-ium-4-methoxyphenyl-morpholino-phosphinodithioate; H_2S, hydrogen sulfide; HASMC, human aortic smooth muscle cell; HR, heart rate; L-NAME, L-nitroarginine methyl ester; LVDP, left ventricular developed pressure; NA, noradrenaline; NO, nitric oxide; PhNCS, phenylisothiocyanate; PhNCS-CF_3, 2-trifluorometyl phenylisothiocyanate; PhNCS-CH_3, metyl-phenylisothiocyanate; PhNCS-COOH, 4-carboxyphenylisothiocyanate; PhNCS-iPr, 2-isopropyl phenylisothiocyanate; SBP, systolic blood pressure; SMGS, smooth muscle growth supplement; VSM, vascular smooth muscle.

* Corresponding author. Tel.: +39 0 50 2219589; fax: +39 0 50 2219609.
E-mail addresses: alma.martelli@farm.unipi.it (A. Martelli), lara.testai@farm.unipi.it (L. Testai), valentina.citi@libero.it (V. Citi), alice.marino@for.unipi.it (A. Marino), breschi@farm.unipi.it (M.C. Breschi), calderone@farm.unipi.it (V. Calderone).

1537-1891/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.vph.2013.11.003

viewed as useful tools for basic studies and promising drugs for cardiovascular diseases. Of course, the administration of gaseous H_2S is greatly limited for the risk of poor posologic control and overdose; the use of other appropriate chemicals, behaving as H_2S-releasing agents, is largely preferred. Sodium hydrogen sulfide (NaHS) is the prototypical example of H_2S-generating agent. It is a rapid H_2S-donor and the most widely used H_2S-donor for experimental purposes. However, this salt is not appropriate for clinical uses, since the quick release of H_2S may cause adverse effects, such as rapid and excessive lowering of blood pressure. Calcium sulfide (CaS) has been proposed as a possible alternative [9], but it should be noted that the rate and mechanism of H_2S release from these two inorganic salts are almost equivalent.

Ideal H_2S-donors should generate H_2S with slower releasing rates [10,11]. This pharmacological feature is exhibited by some natural derivatives, typically present in several plants belonging to the botanical family of Alliaceae. Recent and convincing evidences show that some organic polysulfides of garlic, such as diallyl disulfide (DADS; Fig. 1), act as H_2S-releasing compounds with relatively slow mechanism, requiring the presence of reduced glutathione [12].

In addition to these natural organic polysulfides, early examples of original synthetic H_2S-releasing agents have been described. Among them, the phosphinodithioate derivative GYY4137 (morpholin-4-ium-4-methoxyphenyl-morpholino-phosphinodithioate, Fig. 1) is currently used for pharmacological studies, since it ensures a sustained release of H_2S over a prolonged period [13]. Interesting H_2S-releasing feature of aminothiol [14] and aryl thioamide [15] derivatives has been also reported. As well, the H_2S-donor properties of dithiolethiones and thioamides have been widely used for the synthesis of multitarget drugs [16–18].

In contrast, although an isothiocyanate derivative (4-hydroxyphenyl isothiocyanate) has been reported as an example of potential H_2S-releasing side-chain of multitarget anti-inflammatory agents in a recent patent [19], its real H_2S-releasing properties have not been characterized. To date, no specific investigation has been focused on the isothiocyanate functional group as a potential source of H_2S, and its properties of H_2S-donor and H_2S-dependent effects on the vascular smooth muscle are unexplored.

Thus, this paper aimed to the evaluation and exploitation of the isothiocyanate moiety, as a suitable H_2S-donor moiety, by evaluating the H_2S-releasing properties of some aryl isothiocyanate derivatives (PhNCS, PhNCS–COOH, PhNCS–CH_3, PhNCS–CF_3, PhNCS–iPr; Fig. 1)

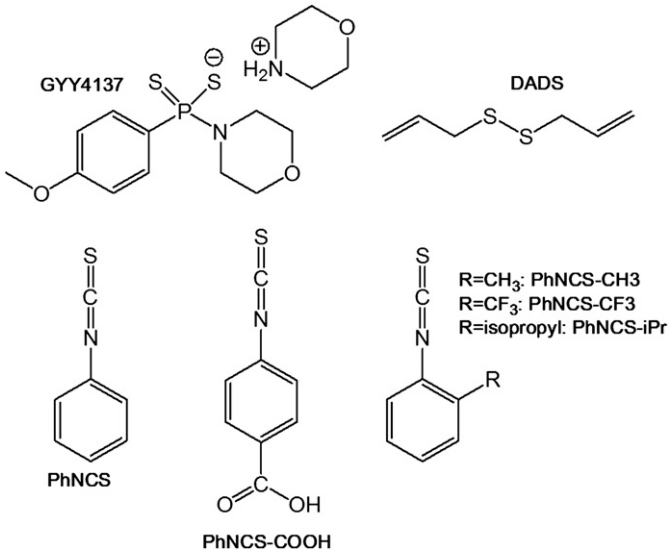

Fig. 1. Chemical structures. Chemical structures of DADS, GYY4137 and the tested isothiocyanates.

and the comparison with the properties exhibited by reference molecules already reported in literature. Moreover, selected isothiocyanate derivatives were submitted to further experimental protocols, aimed to evaluate the vasorelaxing effects on rat's aorta and coronary arteries and the membrane hyperpolarizing activity on human vascular smooth muscle cells.

2. Methods

2.1. Determination of H_2S

2.1.1. Amperometry

The characterization of the potential H_2S-generating properties of the tested compounds has been carried out by amperometric approaches, through an Apollo-4000 Free Radical Analyzer (WPI) detector and H_2S-selective minielectrodes. The experiments were carried out at room temperature. Following the manufacturer's instructions, a "PBS buffer 10×" was prepared ($NaH_2PO_4 \cdot H_2O$ 1.28 g, $Na_2HPO_4 \cdot 12H_2O$ 5.97 g, NaCl 43.88 g in 500 mL H_2O) and stocked at 4 °C. Immediately before the experiments, the "PBS buffer 10×" was diluted in distilled water (1:10), to obtain the assay buffer (AB); pH was adjusted to 7.4. The H_2S-selective minielectrode was equilibrated in 10 ml of the AB, until the recovery of a stable baseline. Then, 100 μl of a dimethyl sulfoxide (DMSO) solution of all the tested H_2S-releasing compounds was added (final concentration of the tested H_2S-donors 1 mM; final concentration of DMSO in the AB 1%). The eventual generation of H_2S was observed for 15 min. When required by the experimental protocol, L-Cysteine (0.33, 1, 2 or 4 mM) was added, before the H_2S-donors. The correct relationship between the amperometric currents (recorded in pA) and the corresponding concentrations of H_2S was determined by opportune calibration curves, which were previously obtained by the use of increasing concentrations of NaHS (1 μM, 3 μM, 5 μM, 10 μM) at pH 4.0.

The curves relative to the progressive increase of H_2S vs time, following the incubation of the tested compounds, were analysed by the equation

$$C_t = C_{max} - \left(C_{max} \cdot e^{-k \cdot t}\right)$$

where C_t is the instant concentration at time t, and C_{max} is the highest concentration achieved in the recording time. The constant k is $0.693/t_{hc}$, where t_{hc} (time for half concentration) is the time required to reach a concentration = ½ C_{max}. The values of C_{max} and t_{hc} were calculated by a computer fitting procedure (software: GraphPad Prism 4.0) and expressed as mean ± standard error; at least 5 different curves were performed for each compound. ANOVA and Student *t* test were selected as statistical analysis, $P < 0.05$ was considered representative of significant statistical differences.

2.1.2. Spectrophotometry

At the end of the above amperometric approach, an usual spectrophotometric method has been also used, in order to have further indication about the H_2S-releasing properties of PhNCS. Briefly, at the end of the amperometric recording, 800 μl of the AB containing PhNCS (in the absence or in the presence of L-Cysteine) were mixed with 50 μl of N,N-dimethyl-phenylen-diamine (Sigma-Aldrich) (20 mM in hydrochloric acid solution 7.2 M) and 50 μl of $FeCl_3$ (Sigma-Aldrich) (30 mM in hydrochloric acid solution 1.2 M). After 20 min, required for the methylene blue formation, the absorbance at 670 nm has been read through a spectrophotometer Novaspec Plus (Amersham Biosciences). The spectrophotometric measurements were converted to the corresponding concentrations of H_2S, by opportune calibration curves previously obtained by the use of increasing concentrations of NaHS (1 μM, 3 μM, 5 μM, 10 μM). The values of C_{max} were expressed as mean ± standard error, from at least 5 different measurements.