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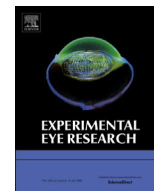

Inhibitory action of novel hydrogen sulfide donors on bovine isolated posterior ciliary arteries

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ABSTRACT

In the present study, we investigate the inhibitory effect of novel H_2S donors, AP67 and AP72 on isolated bovine posterior ciliary arteries (PCAs) under conditions of tone induced by an adrenoceptor agonist. Furthermore, we examined the possible mechanisms underlying the AP67- and AP72-induced relaxations. Isolated bovine PCA were set up for measurement of isometric tension in organ baths containing oxygenated Krebs solution. The relaxant action of H_2S donors was studied on phenylephrine-induced tone in the absence or presence of enzyme inhibitors for the following pathways: cyclooxygenase (COX); H_2S; nitric oxide and the ATP-sensitive K^+ (K_{ATP}) channel. The H_2S donors, NaSH (1 nM – 10 μM), AP67 (1 nM – 10 μM) and AP72 (10 nM – 1 μM) elicited a concentration-dependent relaxation of phenylephrine-induced tone in isolated bovine PCA. While the COX inhibitor, flurbiprofen (3 μM) blocked significantly ($p < 0.05$) the inhibitory response elicited by AP67, it had no effect on relaxations induced by NaSH and AP72. Both aminooxyacetic acid (30 μM) and propargylglycine (1 mM), enzyme inhibitors of H_2S biosynthesis caused significant ($p < 0.05$) rightward shifts in the concentration–response curve to AP67 and AP72. Furthermore, the K_{ATP} channel antagonist, glibenclamide (300 μM) and the NO synthase inhibitor, L-NAME (100 μM) significantly attenuated ($p < 0.05$) the relaxation effect induced by AP67 and AP72 on PCA. We conclude that H_2S donors can relax pre-contracted isolated bovine PCA, an effect dependent on endogenous production of H_2S. The inhibitory action of only AP67 on pre-contracted PCA may involve the production of inhibitory endogenous prostanoids. Furthermore, the observed inhibitory action of H_2S donors on PCA may depend on the endogenous biosynthesis of NO and by an action of K_{ATP} channels.

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1. Introduction

For decades, hydrogen sulfide (H_2S) was best known as toxic environmental pollutant until recent studies provided evidence that this gas can serve as a key regulator of several physiologic and pathophysiological processes in animals. H_2S is endogenously produced in mammalian tissues from amino acids, L-cysteine and homocysteine in the presence of enzymes of the sulfur metabolic network i.e. cystathionine β-synthase (CBS), cystathionine λ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST) along with cysteine aminotransferase (CAT). The biological roles of H_2S as a gaseous transmitter are multiple and rapidly expanding in most organ systems including the central and peripheral nervous systems, and the cardiovascular, gastrointestinal and respiratory systems. H_2S was recently described as a vasodilator and an endothelium-hyperpolarizing factor (EDHF) candidate produced in the vascular tissues, mesenteric arteries, hepatic arteries and aorta of various species like rat, mouse and rabbits (Kimura, 2010). This vasodilatory response can be due to a variety of mechanisms like activation of phospholipase A_2, inhibition of phosphodiesterase, secretion of adipocyte-derived relaxing factor (that opens voltage-dependent Kþ (Kv) channels), activation of large-conductance

Abbreviations: H_2S, Hydrogen sulfide; CBS, Cystathionine β-synthase; CSE, Cystathionine γ -lyase; PAG, Propargylglycine; AOAA, Aminooxyacetic acid; SAM, S-adenosyl-L-methionine; L-NAME, L-nitroarginine methyl ester.

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Ca^{2+}-activated K^+ (BK_{Ca}) channels and activation of ATP-sensitive K^+ (K_{ATP}) channels (Mustafa et al., 2009a, 2009b).

In the eye, studies from our laboratory showed that H_2S can be endogenously produced in ocular tissues using H_2S-releasing compounds and that this gas can induce relaxation of pre-contracted porcine irides, alter sympathetic and excitatory amino acid transmission and regulate signal transduction processes in mammalian retina (Chitnis et al., 2013; Kulkarni et al., 2009, 2011; Monjok et al., 2008; Njie-Mbye et al., 2010, 2012; Ohia et al., 2010; Opere et al., 2009). In a recent study, we found that both slow generating- (GYY4137) and fast-generating (NaSH) H_2S donors can relax the precontracted isolated long posterior ciliary artery (PCA) (Chitnis et al., 2013). Furthermore, we showed that the GYY4137-mediated relaxation of pre-contracted PCA was dependent upon the production of excitatory endogenous prostanoids and that this response was due, at least in part, on the endogenous biosynthesis of H_2S by CBS and CSE and by an action of the release gas on K_{ATP} channels (Chitnis et al., 2013). There is evidence that H_2S can interact with other gaseous molecules such as nitric oxide (NO) and carbon monoxide (CO) in exerting physiological and pharmacological actions in ocular tissues (Bucolo and Drago, 2011; Pong and Eldred, 2009; Salomone et al., 2014). In the rabbit ophthalmic artery, Salomone et al. (2014) reported that CO can synergize with NO to induce relaxations whereas H_2S counteracted the actions of endogenous NO. Taken together, it appears that the pharmacological actions induced by H_2S donors in the ocular vasculature may involve a cross talk between H_2S, NO and CO.

The overall objective of the present study was to investigate the pharmacological actions of other slow releasing H_2S donors, AP67 {(4-methoxyphenyl)pyrrolidin-1-ylphosphinodithioc acid} and AP72 {(4-methoxyphenyl)piperidin-1-ylphosphinodithioc acid)} on isolated bovine long posterior ciliary arteries (PCAs). We also compared the pharmacological actions of AP67 and AP72 with that of the fast releasing H_2S donor, NaSH. Furthermore, we studied the mechanism of action of AP67 and AP72 in eliciting relaxations of isolated pre-contracted bovine PCAs. The chemical structures of AP67 and AP72 are illustrated in Fig. 1. Parts of the work presented in this paper has been communicated in an abstract form (Ohia et al., 2014).

2. Methods

2.1. Chemicals

Propargylglycine (PAG), aminooxyacetic acid (AOAA), NaSH, S-adenosyl-L-methionine (SAM), glibenclamide and L-nitroarginine methyl ester (L-NAME) were all purchased from Sigma Aldrich, St. Louis, MO. Flurbiprofen was procured from Cayman Chemicals, Ann Arbor, MI. All test agents were freshly prepared immediately before use on the day of the study. Stock solution of NaSH, AP67 and AP72 were prepared in distilled water whilst all other compounds were prepared in 70% ethanol. Vehicle controls were prepared using 0.1% saline.

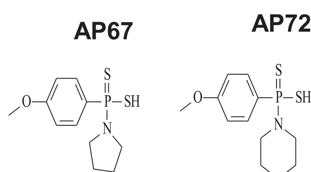

Fig. 1. Chemical Structure of AP67 {(4-methoxyphenyl)pyrrolidin-1-ylphosphinodithioc acid} and AP72 {(4-methoxyphenyl)piperidin-1-ylphosphinodithioc acid)}.

2.2. Tissue preparation

Bovine eyes were obtained from Vision Tech, Dallas, TX within 24 h following enucleation and transported to the laboratory (shipped on ice). The medial or lateral long posterior ciliary arteries were dissected free from the optic nerve at a distance of 2 cm from the posterior aspect of the globe of each eye. The fatty and connective tissues were removed and the vessel with attached endothelium was cut into strips of 2 mm length and placed in oxygenated Krebs solution maintained at 37 °C as described by Dalske (1974).

2.3. Measurement of isometric tension

Posterior ciliary artery rings (2 mm long) were prepared for muscle contraction/relaxation studies using a 25 ml organ bath containing oxygenated Krebs solution (pH 7.4) at 37 °C. Two vessel rings were prepared from each eyeball. In brief, vascular rings were mounted on two tungsten wires (with one wire immobilized and the other connected to a Grass FT03 transducer) for measurement of isometric tension using a Grass Polyview Software. The Krebs solution had the following composition (mM): potassium chloride, 4.8; sodium chloride, 118; calcium chloride, 2.5; potassium dihydrogen phosphate, 1.2; sodium bicarbonate, 25; magnesium sulfate, 2.0; and dextrose, 10. Each ciliary artery ring preparation was mechanically stretched to achieve a basal tension of 5.0 g and then allowed to equilibrate for 45 min before the start of the experiment. Ciliary artery rings were pre-contracted with sub-maximal concentrations of the adrenoceptor receptor agonist, phenylephrine to induce tone, and then exposed to different concentration of H_2S-donors for 2–5 min or until the development of a stable tone. When used, enzyme inhibitors, activator or ion channel blockers were incubated with the vascular segments for 30 min before exposure to H_2S-donors.

The relaxant actions of NaSH, AP67 and AP72 on phenylephrine-induced tone were studied in the absence or presence of an inhibitor of cyclooxygenase (flurbiprofen (3 μM). Furthermore, effects of AP67 and AP72 were studied in the absence or presence of inhibitors and activators of enzymes for the biosynthetic pathways of H_2S, and nitric oxide production and ion channels.: inhibitors of cystathionine γ-lyase, PAG (1 mM) and cystathionine β-synthase, AOAA (30 μM); an activator of cystathionine β-synthase, SAM (100 μM); an inhibitor of nitric oxide synthase, L-NAME (100 μM) and the inhibitor of K_{ATP} channel, glibenclamide (300 μM). The concentrations of PAG, AOAA, SAM, L-NAME and glibenclamide employed in these assays are based on published reports in literature (Chitnis et al., 2013; Dawe et al., 2008; Ghasemi et al., 2012; Monjok et al., 2008; Teague et al., 2002).

2.4. Data analysis

The response of each tissue is expressed as percentage of relaxation of the phenylephrine-induced tone. Cumulative concentration–response curves were constructed, analyzed and IC_{50} values (i.e. agonist concentrations that produced 50% inhibition of the observed maximum response) were determined using Graph Pad Prism 5.0 software (San Diego, CA). 0% relaxation depicts conditions whereby agonists failed to cause relaxation of the phenylephrine-induced tone. Comparison of controls and treatment groups (with inhibitor/activator agents) were performed on the same sets of tissues from different eyeballs. Values given are arithmetic means ± S.E.M. Significance of differences between control and test values were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's test (Graph Pad Prism software San Diego, CA). P values <0.05 were accepted as

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