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Nucleoside monophosphorothioates as the new hydrogen sulfide precursors with unique properties

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ABSTRACT

Hydrogen sulfide (H_2S) is the gasotransmitter enzymatically synthesized in mammalian tissues from L-cysteine. H_2S donors are considered as the potential drugs for the treatment of cardiovascular, neurological and inflammatory diseases. Recently, it has been demonstrated that synthetic nucleotide analogs, adenosine- and guanosine 5′-monophosphorothioates (AMPS and GMPS) can be converted to H_2S and AMP or GMP, respectively, by purified histidine triad nucleotide-binding (Hint) proteins. We examined if AMPS and GMPS can be used as the H_2S donors in intact biological systems. H_2S production by isolated rat kidney glomeruli was measured by the specific polarographic sensor. H_2S production was detected when glomeruli were incubated with AMPS or GMPS and ionotropic purinergic $P2X_7$ receptor/channel agonist, BzATP. More H_2S was generated from GMPS than from equimolar amount of AMPS. Nucleoside phosphorothioates together with BzATP relaxed angiotensin II-preconstricted glomeruli. In addition, infusion of AMPS or GMPS together with BzATP into the renal artery increased filtration fraction and glomerular filtration rate but had no effect on renal vascular resistance or renal blood flow. AMPS but not GMPS was converted to adenosine by isolated glomeruli, however, adenosine was not involved in AMPS-induced H_2S synthesis because neither adenosine nor specific adenosine receptor agonists had any effect on H_2S production. AMPS, but not GMPS, increased phosphorylation level of AMP-stimulated protein kinase (AMPK), but AMPK inhibitor, compound C, had no effect on AMPS-induced H_2S production. In conclusion, nucleoside phosphorothioates are converted to H_2S which relaxes isolated kidney glomeruli in vitro and increases glomerular filtration rate in vivo. AMPS and GMPS can be used as the H_2S donors in experimental studies and possibly also as the H_2S-releasing drugs.

1. Introduction

Hydrogen sulfide (H_2S) is produced endogenously from L-cysteine in most, if not all, mammalian tissues by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) or cysteine aminotransferase and 3-mercaptopyruvate sulfurtransferase (3-MST) [1]. Endogenous H_2S regulates multiple physiological processes such as neurotransmission, cell proliferation and apoptosis, inflammatory and immune response, vascular tone, gastrointestinal function and glucose metabolism [2]. One of the proposed signaling mechanisms of H_2S is sulfhydration of protein cysteine residues from thiol (–SH) to persulfide (–SSH) form, which is often associated with the activation of target proteins [3,4]. Alterations of H_2S level were observed in many diseases and H_2S and its donors are either protective or detrimental in experimental models of these disorders [5,6]. The unique feature of H_2S among other gasotransmitters, nitric oxide and carbon monoxide, is that it is enzymatically metabolized in mitochondria in oxygen concentration-dependent manner [7]. Whereas high H_2S concentrations are highly toxic by inhibiting cytochrome *c* oxidase, physiological amounts of H_2S are rapidly oxidized by sequential action of sulfide:quinone oxidoreductase (SQR), sulfur dioxygenase (protein deficient in ethylmalonic encephalopathy, ETHE1) and sulfite oxidase, and is the only inorganic

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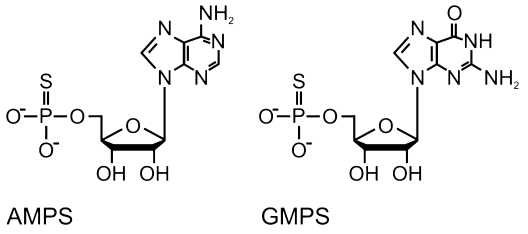

Fig. 1. Structure of adenosine (left) and guanosine (right) monophosphorothioates.

substrate which can provide electrons for mitochondrial respiratory chain [7].

H_2S research is still in its early phase and any further progress in this field is highly dependent on the availability of specific H_2S donors as well as inhibitors of H_2S-synthesizing enzymes. Currently available H_2S donors have significant disadvantages. Inorganic sulfide salts such as NaHS and Na_2S provide high, supraphysiological amounts of H_2S for a short time, and may exert toxic rather than physiological effects even at moderate concentrations [5,6]. Some organic slowly-releasing H_2S donors such as (*p*-methoxyphenyl)morpholino-phosphinodithioic acid (GYY4137) have been synthesized which in aqueous solutions spontaneously decompose to H_2S. However, the compound which originates after H_2S release may potentially have additional H_2S-independent effects in biological systems. Several H_2S-releasing derivatives of non-steroidal anti-inflammatory and other drugs have been obtained and may be potentially useful clinically but they clearly evoke H_2S-independent effects of their parent moieties [5,6].

Adenosine- and guanosine monophosphorothioates (AMPS and GMPS) are synthetic nucleotide analogs with one oxygen atom of the phosphate group replaced by sulfur (Fig. 1). Previously, it has been demonstrated that AMPS and GMPS may be decomposed to H_2S and AMP and GMP, respectively, by two histidine triad nucleotide-binding proteins; cytoplasmic Hint1 [8,9] and mitochondrial Hint2 [10]. These proteins are the evolutionary conserved homodimers that bind and hydrolyze such uncommon nucleotides as natural adenosine 5′-monophosphoramidate and some other nucleotidyl derivatives. Although these analogs are in general membrane-impermeable, they can enter the cells through the opened purinergic ionotropic receptor/channel $P2X_7$ [11]. However, it was unclear if nucleoside monophosphorothioates can be converted to H_2S in intact cells.

Herein, we examined if H_2S can be produced from AMPS and GMPS by isolated kidney glomeruli and if these nucleotide monophosphorothioates (NMPS) can affect renal function in vivo. We have chosen this experimental model for several reasons. First, endogenous H_2S is produced in the kidney by abundantly expressed CBS, CSE and 3-MST [12,13]. Second, H_2S regulates renal vascular tone and tubular transport [14,15]. Third, H_2S is protective in various kidney pathologies including ischemia-reperfusion injury, diabetic nephropathy, kidney fibrosis induced by ureteral obstruction, and chronic kidney disease [16–19]. In addition, $P2X_7$ receptor is expressed in glomerular mesangial cells [20]. In these in vitro and in vivo studies we provide the first evidence that AMPS and GMPS can be converted to H_2S in intact biological systems.

2. Materials and methods

2.1. Animals

Study was performed in adult male Wistar rats weighing 234 ± 6 g before the experiment. Animals were fed standard rat chow (Agropol, Motycz, Poland) ad libitum and had free access to tap water. They were kept at a temperature of 20 ± 2 °C and 12-hour light/dark cycle (lights on at 7.00 AM). The study was approved by the Bioethical Committee of the Lublin Medical University.

2.2. Isolation of glomeruli from the kidney

Rats were anesthetized with sodium pentobarbital (50 mg/kg ip.). The abdominal cavity was opened and a thin polyethylene catheter was inserted to the abdominal aorta proximally to the renal arteries. Then 10 ml of phosphate-buffered saline (PBS, pH 7.4) was infused slowly to perfuse the kidneys and remove blood from renal vasculature. Kidneys were removed and decapsulated, renal cortex was dissected and sliced with a razor blade to small pieces 1–2 mm in size. Tissue slices were pressed with a spatula through a stainless steel sieve (pore size: 250 μm) and rinsed with PBS through successive two sieves (pore sizes 150 and 74 μm, respectively). Glomeruli were collected on the top of 74 μm sieve, suspended in 3 ml PBS and centrifuged at 200 × *g* for 5 min. The pellet was re-suspended in PBS and this washing/centrifugation procedure was repeated three times until the supernatant became clear. The final preparation contained decapsulated glomeruli without afferent or efferent arterioles and tubular contamination was below 5% as assessed by light microscopy.

2.3. Measurement of H_2S production

Generation of H_2S by isolated glomeruli was measured by H_2S-selective sensor (ISO-H2S-2; World Precision Instruments, Sarasota, FL, USA) connected to Four-Channel Free Radical Analyzer (TBR-4100) and Lab-Trax-4/24T data acquisition system (World Precision Instruments) according to the manufacturer's instruction. Each sensor was first calibrated by putting in the incubation solution without glomeruli and recording the current after adding increasing concentrations of Na_2S. The calibration curve was constructed to calculate the sensor's sensitivity (increase in current per unit of sulfide concentration).

After the final wash, glomeruli were suspended to 2 mg protein/ml in the buffered Krebs-Ringer solution (114 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.0 mM $CaCl_2$, 17 mM $NaHCO_3$, 5.6 mM glucose and 16 mM Tris, pH 7.4) and placed in 2-ml vials closed with tight caps. H_2S sensor and a thin needle (for application of tested substances) were mounted through the caps such that their ends remained 5 mm above the vial bottom. Before suspending glomeruli, the incubation solution was pre-bubbled with 80% N_2/20% O_2, 95% N_2/5% O_2, or 99% N_2/1% O_2 gas mixtures to measure H_2S production at different O_2 concentrations. Glomeruli were allowed to equilibrate for 5 min and then tested substances were added to the vial at appropriate concentrations (see Section 3). Increase in current over time between 1 and 5 min after addition of H_2S donor was monitored and the regression line (current = $a \times$ time + b) was fitted to the data by the least-square method. H_2S production was calculated from the slope ("a") according to the sensitivity of the respective sensor.

2.4. Contractility of isolated glomeruli

Glomeruli were transferred to coverslips in 35-mm Petri dishes, incubated at 37 °C for 30 min, and visualized under an inverted microscope (Nikon, TE-2000S, Nikon, Melville, NY, USA). Images were captured before, 5 min after, and 10 min after addition of 1 μM angiotensin II. In separate experiments, H_2S donors were applied 5 min after addition of angiotensin II. Surface area of glomeruli was calculated using the ImageJ software (National Institutes of Health, USA). Glomerular volume (V) was calculated from the formula: $V = (4A/3)/(A/\pi)^{1/2}$ where A is glomerular spherical surface area.