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Effects of AP39, a novel triphenylphosphonium derivatised anethole dithiolethione hydrogen sulfide donor, on rat haemodynamic parameters and chloride and calcium Ca_v3 and RyR2 channels

Lenka Tomasova [a,b], Michaela Pavlovicova [a], Lubica Malekova [a], Anton Misak [a], Frantisek Kristek [c], Marian Grman [a,d], Sona Cacanyiova [c], Milan Tomasek [e], Zuzana Tomaskova [a], Alexis Perry [f], Mark E. Wood [f], Lubica Lacinova [a], Karol Ondrias [a,1], Matthew Whiteman [g,*]

[a] Institute of Molecular Physiology and Genetics, SAS, Bratislava, Slovakia
[b] Faculty of Pharmacy, Comenius University, Bratislava, Slovakia
[c] Institute of Normal and Pathological Physiology, SAS, Bratislava, Slovakia
[d] Center for Molecular Medicine, SAS, Bratislava, Slovakia
[e] LOX Technologies, Bratislava, Slovakia
[f] Department of Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK
[g] University of Exeter Medical School, Exeter, UK

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ABSTRACT

H_2S donor molecules have the potential to be viable therapeutic agents. The aim of this current study was (i) to investigate the effects of a novel triphenylphosphonium derivatised dithiolethione (AP39), in the presence and absence of reduced nitric oxide bioavailability and (ii) to determine the effects of AP39 on myocardial membrane channels; Ca_V3, RyR2 and Cl^-. Normotensive, L-NAME- or phenylephrine-treated rats were administered Na_2S, AP39 or control compounds (AP219 and ADT-OH) (0.25–1 μmol kg^{-1} *i.v.*) and haemodynamic parameters measured. The involvement of membrane channels T-type Ca^{2+} channels $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ as well as Ca^{2+} ryanodine (RyR2) and Cl^- single channels derived from rat heart sarcoplasmic reticulum were also investigated. In anaesthetised Wistar rats, AP39 (0.25–1 μmol kg^{-1} i.v) transiently decreased blood pressure, heart rate and pulse wave velocity, whereas AP219 and ADT-OH and Na_2S had no significant effect. In L-NAME treated rats, AP39 significantly lowered systolic blood pressure for a prolonged period, decreased heart rate and arterial stiffness. In electrophysiological studies, AP39 significantly inhibited Ca^{2+} current through all three Ca_V3 channels. AP39 decreased RyR2 channels activity and increased conductance and mean open time of Cl^- channels. This study suggests that AP39 may offer a novel therapeutic opportunity in conditions whereby $^{\bullet}NO$ and H_2S bioavailability are deficient such as hypertension, and that Ca_V3, RyR2 and Cl^- cardiac membrane channels might be involved in its biological actions.

Abbreviations: ADT-OH, anethole dithiolethione, 5-[p-methoxyphenyl]3H-1,2-dithiole-3-thione; AP219, 9-(Carboxynonyl)triphenylphosphonium bromide; AP39, (10-oxo-10-(4-(3-thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)decyl)triphenylphosphonium bromide; ApoE, apolioprotein E; ARRIVE, Animal Research: Reporting In Vivo Experiments; ATP, [(2"R",3"S",4"R",5"R")-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methyl(hydroxyphosphonooxyphosphoryl)hydrogen phosphate, adenosine trishosphate; BP, blood pressure; BLM, bilayer lipid membrane; Ca^{2+}, calcium cation; $CaCl_2$, calcium chloride; $Ca(OH)_2$, calcium hydroxide; Cav3.1, low voltage activated calcium channel 3.1; Cav3.2, low voltage activated calcium channel 3.2; Cav3.3, low voltage activated calcium channel 3.3; CsCl, cesium chloride; CsOH, cesium hydroxide; Cl^-, chloride; CBS, cystathionine-β-synthase; CSE, cystathionine-γ-lyase; DMSO, dimethyl sulfoxide; EDHF, endothelium-derived hyperpolarising factor; eNOS, nitric oxide synthase 3; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, ethylene glycol tetraacetic acid; G 418, *O*-2-Amino-2,7-didesoxy-D-glycero-α-D-gluco-heptopyranosyl-(1→4)-*O*-(3-desoxy-4-*C*-methyl-3-(methylamino)-β-L-arabinopyranosyl- (1→6))-D-streptamin; GYY4137, *P*-(4-Methoxyphenyl)-*P*-4-morpholinylphosphinodithioic acid morpholine salt; H_2O, oxidane, water; H_2S, hydrogen sulfide; HEK, human embryonic kidney; HEPES, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid; I/R, ischaemia-reperfusion; logP, octanol:water partition coefficient; K^+, potassium cation; KCl, potassium chloride; L-NAME, (2S)-2-Amino-5-[[amino(nitramido)methylidene]amino] pentanoic acid, Nω-nitro-L-arginine; $MgCl_2$, magnesium chloride; min, minute; 3-MST, 3-mercaptopyruvate sulfurtransferase; NaCl, sodium chloride; NADPH, 2'-O-phosphonoadenosine 5'-{3-[1-(3-carbamoylpyridinio)-1,4-anhydro-D-ribitol-5-yl] dihydrogen diphosphate, Nicotinamide adenine dinucleotide phosphate; NaSH, sodium hydrosulfide; Na_2S, sodium sulfide; $^{\bullet}NO$, nitrogen monoxide; PDE5, phosphodiesterase 5; Phen, (*R*)-3-[-1-hydroxy-2-(methylamino)ethyl]phenol, phenylephirine; PW, pulse wave; RyR2, ryanodine receptor 2; SR, sarcoplasmic reticulum; TEA-Cl, *N*,*N*,*N*,*N*-Tetraethylammonium chloride, tetraethylammonium chloride; Tris, 2-Amino-2-hydroxymethyl-propane-1,3-diol, tris(hydroxymethyl)amioethane.

* Corresponding author. University of Exeter Medical School, St. Luke's Campus, Magdalen Road, Exeter EX1 2LU, England. Fax: +44 1392 722926.
E-mail address: m.whiteman@exeter.ac.uk (M. Whiteman).

[1] This author equally contributed to the work.

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1. Introduction

In humans and mammals, hydrogen sulfide (H_2S) has recently been identified as an endogenous mediator of blood pressure where it is synthesised from L-cysteine, homocysteine and cystathionine by the enzymes cystathionine-β-synthase (CBS) and cytathionine-γ-lyase (CSE) and from 3-mercaptopyruvate by 3-mercaptopyruvate sulfurtransfearase (3-MST) [1,2]. Perturbed synthesis and/or bioavailability of H_2S has been found in animal models with various pathological conditions such as myocardial ischaemia, spontaneous hypertension, obesity, and hypoxic pulmonary hypertension [1]. $CSE^{-/-}$ mice have been shown to be markedly hypertensive relative to wild type animals [3] and are predisposed to vascular remodelling and early development of atherosclerosis [4]. In humans, lower blood (serum or plasma) levels of H_2S have been reported in patients with obesity and type II diabetes [5,6], hypertension, coronary heart disease, cataractogenesis, atherosclerosis, choleresis and cirrhosis [7–10]. These studies suggest that vascular H_2S may be an endogenous regulator of vascular tone and strategies which increase H_2S bioavailability may represent a novel approach to the treatment of vascular disease. In keeping with this notion, animals treated with high concentrations of sodium hydrosulfide (NaSH) and sodium sulfide (Na_2S), as crude sources of H_2S, have been shown to protect heart tissue shown in various cardiovascular models [11]; e.g. against the damage caused by coronary artery ligation or injection with homocysteine or isoproterenol [12–14], myocardial ischaemia and ischaemia/reperfusion (I/R) injury [15–17] as well as contributes to the cardioprotective effects of preconditioning and post-conditioning [18,19]. Recently H_2S donor molecules such as GYY4137 have been developed and shown to dilate isolated blood vessels and reduce blood pressure in spontaneously hypertensive and nitric oxide deficient (e.g. L-NAME treated) rats [20]. More recently, in atherosclerosis prone apoE ($^{-/-}$) mice, GYY4137 restored aortic endothelium-dependent relaxation and decreased atherosclerotic plaque formation [21], suggesting H_2S donors may be therapeutically useful in the treatment of vascular disease.

Low-voltage activated T-type Ca^{2+} channels encompass three members $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$. Ca_V3 channels are characterised by relatively low voltage threshold for current activation, which may contribute to basal $(Ca^{2+})_i$ concentration in smooth muscle cells [22]. Ca_V3 channels have been identified in a wide variety of tissues including the heart in the sinoatrial nodal and conduction cells, brain, skeletal muscle, testis and spermatids, indicating multiple functions of these channels such as cardiac rhythm generation, neuronal excitability, hormone secretion, neurotransmitter release, vascular tone regulation, muscle contraction, gene expression, cell metabolism, differentiation, and proliferation [23–27]. Therefore, abnormal expression and function of Cav3 are associated with many diseases including cardiac hypertrophy and arrhythmia, hypertension, epilepsy, autism, and cancer [24,27,28]. High concentrations of H_2S (≥100 μM), albeit in the form of sulfide salts such as NaSH, have been shown to modulate T-type Ca^{2+} channel activity [29] suggesting H_2S may act as an endogenous regulator and pharmacological target of calcium signalling in excitable tissue.

The excitation-contraction coupling in the heart is regulated by the release of Ca^{2+} from the sarcoplasmic reticulum *via* the ryanodine receptor (RyR2) [30]. The amplitude of the Ca^{2+} transient generated by Ca^{2+} release *via* RyR2 determines contractile force in cardiomyocytes, controls cardiac muscle contraction and relaxation, and an impaired Ca^{2+} release causes heart dysfunction [30–33]. Mice with genetically reduced RyR2 exhibit a lower basal heart rate, decline in cardiac output and fatal arrhythmias [34]. Ryanodine receptors have also been suggested to be involved in the vascular effects of H_2S [35].

Accumulating evidence strongly supports an important role for Cl^- channels in vascular remodelling and stroke [36]. For example, Cl^- channels play a role in modulation of endothelium-derived hyperpolarising factor (EDHF)-mediated relaxation in rat mesenteric arteries [37], delayed relaxation of skeletal muscle [38], cardiovascular disease, hypertension, arrhythmogenesis, myocardial hypertrophy, heart failure and cardioprotection against ischaemia reperfusion [39–41]. Sulfide (as NaSH) has been found to inhibit intracellular Cl^- channels, derived mostly from lysosomal membranes, implying that Cl^- channels may play a role in the biological effects of H_2S [42] although it is not known whether H_2S influences Cl^- channel activity in cardiac tissue.

Given that H_2S is an important in the homeostasis of the cardiovascular system and the pathogenesis of cardiovascular disease [43], H_2S donors have a potential to be developed into an effective H_2S-based therapeutic agents [44–46]. With this in mind, we have developed a novel highly lipophilic triphenylphosphonium derivatised anethole dithiolethione H_2S donor, AP39 [47,48] (Fig. 1) and for the first time evaluated its effects *in vivo* on rat haemodynamics. Since H_2S from sulfide salts (albeit from supraphysiological concentrations NaSH or Na_2S; ≥ 100 μmol L^{-1}) are also reported to inhibit ion channels which regulate vascular haemodynamics e.g. cardiac T-type Ca^{2+} channels, RyR2 and intracellular Cl^- channels [29], we have also investigated the effect of AP39 on these channels *in vitro* to identify the possible mechanistic actions of AP39 *in vivo*.

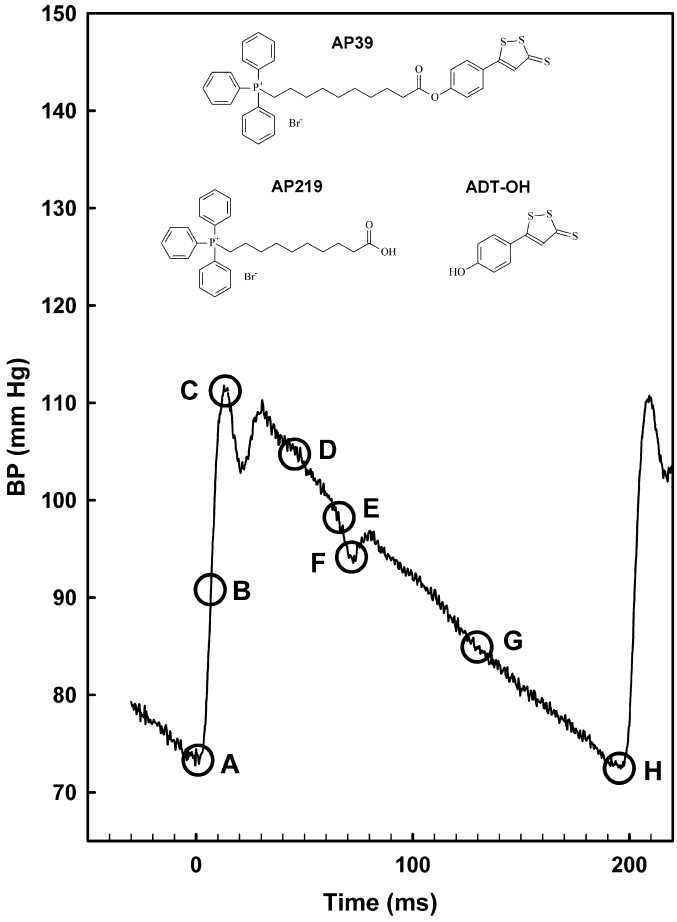

Fig. 1. Chemical structure of AP39, AP219 and ADT-OH and pulse carotid artery waveform (PW) in the anesthetised rat. PW Diastolic BP (A); dP/dt_{max} (B); systolic BP (C); BP (D) at (F-A)/2; dP/dt_{min} (E); Dicrotic notch (F); (G) at (H-F)/2.

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