ORIGINAL RESEARCH

Resveratrol Stimulates Hydrogen Sulfide (H_2S) Formation to Relax Murine Corpus Cavernosum

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ABSTRACT

Introduction. Resveratrol (RVT) found in red wine protects against erectile dysfunction and relaxes penile tissue (corpus cavernosum) via a nitric oxide (NO) independent pathway. However, the mechanism remains to be elucidated. Hydrogen sulfide (H_2S) is a potent vasodilator and neuromodulator generated in corpus cavernosum.
Aims. We investigated whether RVT caused the relaxation of mice corpus cavernosum (MCC) through H_2S.
Methods. H_2S formation is measured by methylene blue assay and vascular reactivity experiments have been performed by DMT strip myograph in CD1 MCC strips.
Main Outcome Measures. Endothelial NO synthase (eNOS) inhibitor Nω-Nitro-L-arginine (L-NNA, 0.1 mM) or H_2S inhibitor aminooxyacetic acid (AOAA, 2 mM) which inhibits both cystathionine-β-synthase (CBS) and cystathionine-gamma-lyase (CSE) enzyme or combination of AOAA with PAG (CSE inhibitor) has been used in the presence/absence of RVT (0.1 mM, 30 min) to elucidate the role of NO or H_2S pathways on the effects of RVT in MCC. Concentration-dependent relaxations to RVT, L-cysteine, sodium hydrogen sulfide (NaHS) and acetylcholine (ACh) were studied.
Results. Exposure of murine corpus cavernosum to RVT increased both basal and L-cysteine-stimulated H_2S formation. Both of these effects were reversed by AOAA but not by L-NNA. RVT caused concentration-dependent relaxation of MCC and that RVT-induced relaxation was significantly inhibited by AOAA or AOAA + PAG but not by L-NNA. L-cysteine caused concentration-dependent relaxations, which are inhibited by AOAA or AOAA + PAG significantly. Incubation of MCC with RVT significantly increased L-cysteine-induced relaxation, and this effect was inhibited by AOAA + PAG. However, RVT did not alter the effect of exogenous H_2S (NaHS) or ACh-induced relaxations.
Conclusions. These results demonstrate that RVT-induced relaxation is at least partly dependent on H_2S formation and acts independent of eNOS pathway. In phosphodiesterase 5 inhibitor (PDE-5i) nonresponder population, combination therapy with RVT may reverse erectile dysfunction via stimulating endogenous H_2S formation.
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Key Words. Corpus Cavernosum; Resveratrol; Phosphodiesterase 5 Inhibitor; Hydrogen Sulfide; Erectile Dysfunction

Introduction

Erectile dysfunction (ED) is an important indicator of poor health, which reduces quality of life. In United States alone, ED affects over 30 million men, and the prevalence of ED increases with age [1]. Normal penile erection is under the control of multiple factors and signaling pathways. Nitric oxide (NO) is known to regulate penile erection [2,3], and phosphodiesterase 5 inhibitors (PDE-5i) have significantly improved the quality of many men's sexual life with an efficacy about 70% [4], although it is significantly lower in difficult-to-treat subpopulations (e.g., diabetes mellitus, radical prostatectomy), and there are remaining over 30% of patients who are classified as PDE-5i nonresponders [5].

The "French Paradox" of reduced cardiovascular events despite a high cholesterol-containing diet in French population has been atrributed to the protective effect of resveratrol (RVT) in red wine [6]. RVT has phytotherapeutic potential with antioxidant, calorie-restricting, anti-aging, cardioprotective effects [7]. Recent studies showed RVT can induce vasorelaxant effect in penile tissue [8–10] and appears to have beneficial effects in ED induced by hypertension, hypercholesterolemia, and diabetes [8,11,12]. Although RVT has been shown to induce relaxation of mice corpus cavernosum (MCC) independent of the NO pathway [13], the exact mechanism remains unknown.

A vasodilator and neuromodulator H_2S is generated by the enzymes cystathionine-gamma-lyase (CSE), cystathionine-β-synthase (CBS), and 3-mercaptopyruvate sulphurtransferase (MPST) enzymes in corpus cavernosum [14–16]. We therefore investigated whether the vasodilator effect of RVT in MCC could be due to H_2S release.

Methods

Animals

The present study was approved by Institutional Review Board (7.201.2014.0002) as well as Animal Care and Use Committee of Ege University (February 26, 2014, number 2014-018), in agreement with the Institute of Laboratory Animal Research Guide for the Care and Use of Laboratory Animals. All experiments were conducted on 10–12 weeks of age male CD1 mice (n = 65) obtained from Breeding Center of Experimental Animals in Ege University (ARGEFAR). The animals were stunned by inhalation of CO_2, sacrificed by decapitation, and exsanguinated.

Drugs and Treatments

In some experiments, isolated MCC strips or homogenates were incubated with NOS inhibitor Nω-Nitro-L-arginine (L-NNA, 0.1 mM, 30 minutes) or CBS and CSE inhibitor (AOAA, 2 mM, 30 minutes) or combination of AOAA with CSE inhibitor (AOAA 2 mM + PAG 10 mM, 30 minutes) before vehicle or RVT exposure (0.1 mM, 30 minutes). RVTs were acquired from Santa Crus Biotechnology, Inc. (Santa Cruz, CA, USA). Unless otherwise stated, all other chemicals were obtained from Sigma.

Isolation of Penile Tissue and Treatments

The penis was excised at its base with removal of the glans penis and connective and adventitial tissues along the shaft. Two individual MCC strips ($1 \times 1 \times 10$ mm) from one animal were separated, mounted in 5-mL organ bath of strip myograph for isometric force recording (Danish Myograph Technology, Aarhus, Denmark) coupled to a PowerLab 8/SP data acquisition system (Chart 5.0 software; ADInstruments, Colorado Springs, CO, USA), and bathed in carboxygenated (95% O_2; 5% CO_2) modified Krebs–Ringer solution NaCl, 130 mM; $NaHCO_3$, 14.9 mM; dextrose, 5.5 mM; KCl, 4.7 mM; KH_2PO_4, 1.18 mM; $MgSO_47H_2O$, 1.17 mM; and $CaCl_22H_2O$, 1.6 mM at 37°C. Tissues were allowed to equilibrate for 90 min under a resting tension of 5 mN. Experiments were done in strips with endothelium as confirmed by relaxation more than 50% to ACh (1 μM) after contraction with phenylephrine (Phe, 10 μM). One concentration–response curve was obtained in each MCC. In the later series of experiments, relaxant responses to RVT (50–100–200–400 μM), ACh (10^{-9}–10^{-4} M), exogenous H_2S donor NaHS (10^{-6}–3.10^{-2} M), or endogenous H_2S donor L-cysteine (10^{-5}–3.10^{-5} M) were obtained in Phe-precontracted MCC.

Measurement of H_2S Level by Methylene Blue Assay

To assess the activity of CBS and CSE in MCC, H_2S determination was evaluated according to Stipanuk and Beck [17] in the presence/absence of AOAA. Since the MCC is small, at least two pair of MCC was combined, homogenized with lysis buffer containing potassium phosphate buffer (100 mM, pH 7.4), sodium orthovanadate (10 mM), and proteases inhibitors. Protein concentration was determined using the Bradford