Hydrogen Sulphide: A Novel Endogenous Gasotransmitter Facilitates Erectile Function

Balasubramanian Srilatha, MD, PhD,* P. Ganesan Adaikan, PhD, DSc,* Ling Li, BSc,† and Philip K. Moore, PhD†

*Department of Obstetrics & Gynaecology, National University Hospital, National University of Singapore, Singapore; †Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

DOI: 10.1111/j.1743-6109.2007.00561.x

ABSTRACT

Introduction. In a pilot study, we found that the novel gasotransmitter, hydrogen sulfide (H_2S), had a vasodilatory and proerectile action on the cavernosum. In the present work, we explored the ability of the cavernosum to synthesize H_2S and its mechanism on the cavernosal pathways.

Aim. To evaluate the physiopharmacological responses and mechanism in the erectile function of H_2S in rabbit cavernosum.

Methods. Rabbit corpus cavernosum (CC) smooth muscle tissue (N = 5) was homogenized and incubated with L-cysteine (10 mM) and pyridoxal 5′-phosphate (2 mM) to detect H_2S formation. In isometric tension studies on rabbits (N = 12), the effect of sodium hydrogen sulfide (NaHS; stable H_2S donor, 100 μM–3.2 mM) was evaluated on the relaxant and contractile pathways in the cavernous smooth muscle using standard pharmacological tools.

Main Outcome Measures. In vitro evidences for cavernosal H_2S formation and proerectile pharmacological effects.

Results. H_2S was readily synthesized in the rabbit CC (2.1 ± 0.4 nmol/mg protein). In organ bath studies, NaHS consistently relaxed the rabbit CC in a concentration-dependent manner. MDL 12,330A and 1-H-[1,2,4]-oxadiazolo-[4,3-a]-quinoxalin-1-one inhibited the NaHS relaxation by 22.5% and 14.7%, respectively. All three enzyme inhibitors (aminooxyacetic acid [AOAA], β-cyanoalanine [β-CA], and DL-propargylglycine [PAG] [1 mM]) markedly increased the noradrenergic contractile neurotransmission of CC strips to field stimulation with minimal reversal by cysteine (1 mM) indicating the possible inherent inhibition of the relaxant H_2S formation. AOAA, β-CA, or PAG had no significant effect on nitrergic relaxation of noradrenaline-precontracted CC strips.

Conclusion. These pioneering studies provide evidence for the endogenous formation of H_2S and its proerectile relaxant effect on the cavernosum, with the possibility of involvement of the cyclic adenosine monophosphate pathway. **Srilatha B, Adaikan PG, Li L, and Moore PK. Hydrogen sulphide: A novel endogenous gasotransmitter facilitates erectile function. J Sex Med 2007;4:1304–1311.**

Key Words. Hydrogen Sulphide; Erectile Dysfunction; Corpus Cavernosum; Neurotransmitter; Penile Physiopharmacology

Introduction

Erectile physiology is a complex interaction of number of neurologic, vascular, endocrine, and cellular inputs, and erectile dysfunction (ED), therefore, may arise from a variety of risk factors affecting any of these systems/pathways. Intact autonomic neurotransmitter function has been shown to be an essential prerequisite for the erectile process consisting of the adrenergic system (α-adrenoceptor activity) contributing to antierectile action and nitric oxide/cyclic guanosine monophosphate (NO/cGMP) pathway being acknowledged as a primary mediator in the generation of an erectile response [1]. In addition, the cyclic adenosine monophosphate (cAMP) can also contribute to pharmacological erection as exemplified by the effective intracavernosal (I/C) therapy of ED with prostaglandin E_1 [2]. While exploiting the NO/cGMP pathway with phosphodiesterase 5

inhibition has revolutionized the treatment of ED, nonresponsiveness to existing oral pharmacotherapy is still estimated to be around 30% [3].

Recently, H_2S has been established as a potential neurotransmitter in the central and in some peripheral systems [4], being formed endogenously from L-cysteine by the activity of two enzymes, viz. cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) [5]. While CBS is mainly active in the brain [6], smooth muscle and vascular tissues have shown both CBS and CSE activities [7] with a key role probably played by CSE [8]. Studies indicate that in brain homogenates, CBS activity increases to threefolds in the presence of Ca^{2+}/calmodulin [9], and the H_2S produced in response to neuronal excitation [6] activates adenylate cyclase or potassium adenosine triphosphate (K^+ATP) channels [4]. Alternatively, testosterone and S-adenosyl-L-methionine (a CBS activator) also regulate H_2S increase [10], although this effect cannot be subsequently proven [11]. In the periphery, H_2S acts as a smooth muscle relaxant in rabbit and guinea pig ileum and rat vas deferens [12]. Both exogenously administered and endogenous H_2S have short durations of action suggestive of rapid metabolism mediated by either chemical or enzymatic degradation (putative H_2S metabolizing enzymes include rhodanese and thiol S-methyltransferase) [13].

Experimental evidence points to possible crosstalk between the two gaseous neurotransmitters, NO and H_2S, in various physiological systems [14]. For instance, reduction in vascular H_2S generation was demonstrated in experimental hypertension induced by nitric oxide synthase (NOS) blockade [15], while the NO donor, sodium nitroprusside, enhanced H_2S formation by activating CBS in neuronal cell cultures [16]. Furthermore, NOS blockade could also interfere with antinociceptive [17] and cardioprotective effects [18] of H_2S. Overproduction of NO and H_2S may be implicated in hepatic cirrhosis, and the formation of a novel nitrosothiol for both NO and H_2S in liver homogenates has been demonstrated [19,20].

In a pilot study, we reported the proerectile effect of H_2S, which was characterized by significant increases in penile length, blood flow, and intracavernous pressure (ICP) in primates, while a CSE inhibitor, DL-propargylglycine (PAG), decreased cavernous nerve-mediated pressure in rats [21]. Although the fact remains that NO has a lead role in erectile pathophysiology, we believe that delineation of possible involvement of H_2S in the existent pathways (cGMP) or through an independent system in the corpus cavernosum (CC) in turn may identify a new pharmacological approach for ED.

Methods

Assay of Cavernous Tissue H_2S Synthesis

Penile erectile tissue was obtained from five New Zealand White (NZW) male rabbits (3- to 4-kg body weight) following euthanasia with 100 mg/kg intravenous pentobarbitone sodium (Abbott, Parramatta, Australia). Corpus cavernosal smooth muscle was carefully dissected out and snap frozen in liquid nitrogen. Cavernous tissue H_2S synthesizing activity was determined essentially as described elsewhere [22]. This is a standard assay that has been employed by research groups worldwide as a means to monitor H_2S synthesizing activity in cells and tissue homogenates. The assay utilizes optimized concentrations of substrate (cysteine) with cofactor (pyridoxal phosphate) at standardized time duration and temperature to ensure that total measurable H_2S is detected. Briefly, the tissue was thawed and homogenized (Ultra-Turrax (IKA Works, Staufen, Germany)) in 100-mM ice-cold potassium phosphate buffer (pH 7.4). Optimal (w/v) ratios of 3:10 were determined from preliminary experiments. The reaction mixture (total volume, 500 μL) contained L-cysteine (10 mM, 20 μL), pyridoxal 5′-phosphate (2 mM, 20 μL), saline (30 μL), and tissue homogenate (430 μL). The reaction was performed in tightly parafilmed Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and initiated by transferring the tubes from an ice bath to a water bath at 37°C. In some experiments, the enzymatic reaction was stopped immediately by the addition of trichloroacetic acid (10% [w/v], 250 μL) to denature protein prior to the addition of cysteine. After incubation for 45 min, zinc acetate (1% [w/v], 250 μL) was added to trap evolved H_2S followed by trichloroacetic acid. Subsequently, *N*,*N*-dimethyl-*p*-phenylenediamine sulfate (20 μM, 133 μL) in 7.2-M HCl and $FeCl_3$ (30 μM, 133 μL) in 1.2-M HCl were added, and the absorbance of the resulting solution (670 nm) measured for 10 min thereafter using a 96-well microplate reader (Tecan Systems Inc., San Jose, CA, USA). Basal concentration of H_2S was determined in incubates in which trichloroacetic acid was added at zero time prior to incubation with L-cysteine (37°C, 45 minutes). At the end of this period, zinc acetate was added and the H_2S generation measured spectrophotometrically. All samples were