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Potassium channels modulate the action but not the synthesis of hydrogen sulfide in rat corpus cavernosum

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ABSTRACT

Aim: Hydrogen sulfide (H_2S) is a newly-introduced gasotransmitter in penile tissues. However, its exact mechanism of action in mediating penile erection is not fully elucidated. The major aim of this study was to examine the role of different K^+ channels in mediating the responses to H_2S in the corpus cavernosum.

Main methods: Tension studies using isolated rat corpus cavernosum strips were conducted. Endogenous H_2S production was measured using polarographic technique. Results are expressed as mean ± SEM.

Key findings: L-Cysteine (10^{-2} M) stimulated rat corpus cavernosum to produce H_2S. Blockade of CSE by BCA (10^{-3} M) reduced the concentration of H_2S produced from rat corpus cavernosum significantly. Addition of TEA (10^{-2} M) or 4-AP (10^{-3} M) didn't have a significant effect on the concentration of H_2S produced. L-Cysteine (10^{-6}–10^{-2} M) elicited a concentration-dependent relaxation response which was significantly reduced by blockade of CSE using BCA (10^{-3} M). TEA (10^{-2} M), 4-AP (10^{-3} M) and TEA (10^{-4} M) attenuated L-cysteine-induced relaxation significantly. At 10^{-4} M, L-cysteine resulted in percentage relaxation of 1.55 ± 0.63, 10.94 ± 1.93 and 1.93 ± 0.80 in presence of TEA (10^{-2} M), 4-AP (10^{-3} M) and TEA (10^{-4} M) respectively compared to 23.78 ± 2.71 as control. Both glibenclamide (10^{-5} M) and $BaCl_2$ (3×10^{-5} M) failed to reduce these relaxations significantly.

Significance: H_2S-induced relaxation of rat corpus cavernosum may be mediated - at least in part - through BK_{ca} and K_V channels not by K_{ATP} and K_{ir} channels. It also seems that K^+-channels do not contribute to the synthesis of H_2S.

1. Introduction

Relaxation of the corpus cavernosum (CC) smooth muscle is an absolute prerequisite to penile erection which leads to decreased penile vascular resistance; allowing dilatation of the cavernosal arteries and increase in blood flow [1]. The innervation of the genitosexual tract consists of autonomic, somatic [2], and non-adrenergic non-cholinergic innervation such as vasoactive intestinal peptide, substance P, adenosine, ATP, gaseous neurotransmitters such as carbon monoxide, hydrogen sulfide (H_2S) and nitric oxide (NO) and other factors as dopamine and prostaglandins [3]. Nitric oxide is - by virtue of all previous reports - the main component that acts in the penis to mediate the erectile response through NO/cGMP signal transduction pathway [1,4].

Research in the last two decades has thrown H_2S into the spotlight and it is now widely referred to as the so-called third gasotransmitter after carbon monoxide and NO [5]. The first evidence, indicating H_2S as an endogenous mediator was published in 1996 by Abe and Kimura [6]. It is produced endogenously from L-cysteine in a reaction catalyzed by three different enzymes: Cystathionine γ-lyase (CSE), Cystathionine β-synthase (CBS) or 3-Mercaptopyruvate sulfurtransferase (3-MST) [7]. The main H_2S-producing enzyme in the central nervous system is CBS while in the vascular system, it is CSE [8]. Hydrogen sulfide is involved in vasoregulation, neuromodulation, reduction of oxidative stress, protection from myocardial ischemia-reperfusion injury, and anti-inflammation [9]. The first evidence showing the existence of the L-cysteine/H_2S system in penile tissue was published by Srilatha et al. [10]. Subsequently, the involvement of H_2S in facilitating erectile function has been reported [11]. In 2009, both CBS and CSE were reported present and active in human CC [12].

Four major types of potassium channels are expressed in smooth muscles: large conductance Ca^{2+}-activated (BK_{Ca}, IUPHAR: KCa1.1), adenosine triphosphate (ATP)-sensitive (K_{ATP}, IUPHAR: K_{IR}6.1 and 6.2), inwardly rectifying (K_{ir}, IUPHAR: K_{IR}1–7) and voltage-gated (K_V, IUPHAR: K_V1–12) [13]. Many reports indicate the presence of these channels in erectile tissues [14–16]. Phosphorylation of potassium channels leads to hyperpolarization and relaxation of CC, which makes

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them the end target for many effectors such as NO and ACh [2,17–19]. Moreover, many studies suggested a pre-junctional modulatory role of K_V channels in nitrergic neurotransmission [20–22].

Although potassium channels have been identified as downstream effectors of both ACh and NO in CC [23], the role of potassium channels in mediating relaxant responses induced by other transmitters, namely H_2S is not elucidated. Therefore, the aim of the current study is to investigate the contribution of each different type of potassium channels to the relaxations induced by H_2S in CC, as well as to examine the possible interactions between potassium channels and H_2S synthesis in CC.

2. Methods

This set of experiments has been conducted on male Wistar rats weighing from 300 to 350 g, all protocols adhere to international Animal Care and Use guidelines and approved by Faculty of Pharmacy - Alexandria University ACUC.

2.1. The isolated rat corpus cavernosum

The isolated rat corpus cavernosum was prepared according to the method described by Keegan et al. [24] and Paskaloglu et al. [25]. The penis isolated from male albino rats weighing 300–350 g was pinned out in a dish containing chilled Krebs solution aerated with 95% O_2 and 5% CO_2, the urethra and the dorsal vein were removed. Each CC was excised by separation from the thick medial septum and a longitudinal slit was made in each CC to aid drug penetration. Each CC yielded a single strip 2 × 2 × 15 mm. Strips of rat CC were tied at each end with cotton threads and mounted in a 10 ml organ chamber. Tissue baths containing Krebs solution were kept at 37 °C and constantly bubbled with 95% O_2 and 5% CO_2. The initial resting tension was set to 1 g. Changes in force were recorded by isometric force displacement transducer (Narco F-60, Houston, TX, USA) and physiographs (Linseis, Paris, France). Acquisition and processing of the data were performed with the IOX software (EMKA, Paris, France). Each strip was submaximally contracted with PE (10^{-5} M). After the PE contractile response has stabilized, relaxation responses to different treatments were recorded in a cumulative fashion. The relaxation responses were assessed as a percentage of the PE-induced contractile response.

2.2. Measurement of L-cysteine-induced hydrogen sulfide production by rat corpus cavernosum

Endogenous H_2S production was measured using a polarographic sensor (ISO-H_2S-2, WPI) together with an Apollo 1000 free radical analyser (WPI) according to the method described by Doeller et al. [26]. Acquisition and processing of the data were performed with LabScribe software (iWorx/CB Sciences, Inc.). The polarographic sensor uses a cylindrical platinum anode that is polished on the tip and electrically insulated along the side from a platinum cathode. The cathode consisted of a coiled platinum wire held in position with epoxy. The core was housed in a stainless-steel cylindrical sleeve that served as an electrical shield and to which an H_2S permeable membrane was fixed to cover the tip. The reservoir between the sleeve and the core was filled with an electrolyte consisting of $K_3[Fe(CN)_6]$. The electrolyte chemistry is initiated as H_2S diffuses from the sample solution through the membrane and dissociates to HS^- which in turn reduces ferricyanide to ferrocyanide that subsequently donates electrons to the anode creating a current proportional to sample sulfide concentration.

Rat CC were preincubated for 1 h at room temperature in RPMI medium. Specimens were subsequently placed in a 4 ml organ bath containing RPMI medium with the polarographic sensor for a 30 min equilibration period. After the equilibration period, L-cysteine (10^{-2} M) was added to the organ bath. The current corresponding to the amount of hydrogen sulfide produced from the CC was displayed as a peak. The concentration of hydrogen sulfide produced from each sample was calculated from the standard curve constructed daily using freshly prepared NaSH solution.

2.3. Statistical analysis

The results obtained are expressed as mean ± SEM. Throughout the manuscript, numbers between parentheses indicate number of animals. The student *t*-test was used for the analysis of paired/unpaired data whenever applies. The analysis of variance (ANOVA) followed by Bonferroni's post-test was used for multiple comparisons. The criterion for statistical significance was set at $p < 0.05$.

3. Results

3.1. L-Cysteine-induced hydrogen sulfide production and its modulation by some potassium channels blockers

Addition of L-cysteine (10^{-2} M) to the organ bath, stimulated rat corpus cavernosum to produce H_2S. Blockade of CSE by BCA (10^{-3} M) prior to addition of L-cysteine reduced the concentration of H_2S produced from rat corpus cavernosum significantly (Fig. 1).

On the other hand, addition of TEA (10^{-2} M) or 4-AP (10^{-3} M) to the organ bath prior to L-cysteine did not produce a significant effect on the concentration of H_2S produced from rat corpus cavernosum (Figs. 2 and 3).

3.2. Effect of L-cysteine-induced relaxation and its modulation by different potassium channels blockers

Addition of PE (10^{-5} M) to strips of rat CC produced contractile responses. L-Cysteine (10^{-6}–10^{-2} M) elicited a concentration dependent relaxation response of the PE precontracted rat CC. Blockade of CSE by BCA (10^{-3} M) significantly reduced L-cysteine-induced relaxations of rat corpus cavernosum at all concentrations (Fig. 4).

L-Cysteine (10^{-6} M) produced percentage relaxation that amounted to 5.13 ± 0.89. TEA (10^{-2}, 10^{-4} M), 4-AP (10^{-3} M) reduced the percentage relaxation induced by L-cysteine to 0.89 ± 0.41, 1.29 ± 0.66 and 1.75 ± 0.69 respectively. The inhibitory effect of TEA (10^{-2} M, non-selective) was significantly higher than that of 4-AP (10^{-3} M) when compared at 10^{-3} and 10^{-4} M L-cysteine. On the other hand, none of these blockers affected the relaxation induced by the highest concentration used of L-cysteine (10^{-2} M). Similarly, both

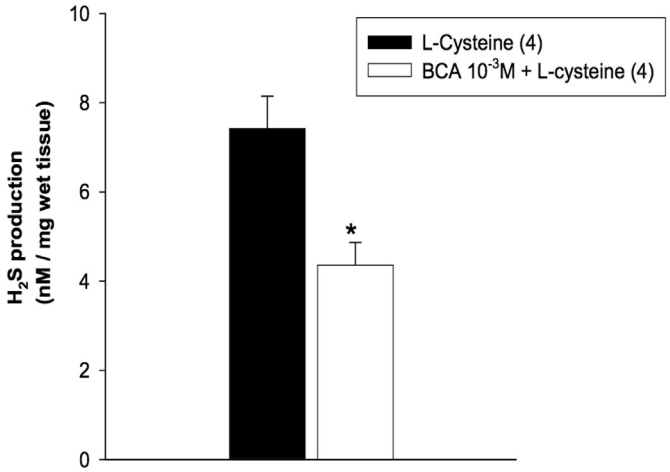

Fig. 1. Hydrogen sulfide production from rat corpus cavernosum induced by stimulation with L-cysteine (10^{-2} M) in absence and in presence of β-cyano-L-alanine (BCA, 10^{-3} M). Values are expressed as mean ± SEM. Values between parentheses indicate the number of observations. *Denotes significant difference compared to L-cysteine at the level $p < 0.05$.