CYSTATHIONINE GAMMA–LYASE AS A REGULATOR OF RESISTANCE ARTERY CONTRACTION UNDER NORMAL AND HYPERGLYCEMIC CONDITIONS

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Abstract


The aim of our study is to clarify the contribution of cystathionine gamma–lyase (CSE) that produces H2S in smooth muscle and perivascular adipose tissues in the regulation of resistance size rat artery contraction. In this research the isometric contraction of de-endothelised rat gracilis artery preparations was measured with Small Vessel Myograph (DMT 410M, Denmark). Half of the isolated rings were incubated in 20 mmol/l D-glucose-containing bath and the others in normal glucose solution (5.5 mmol/l). Increasing concentrations of serotonin (5-hydroxytryptamine) from 10^-10 to 10^-5 mmol/l were applied to induce gradual constriction of circular artery segments. The absence of endothelium was confirmed by the lack of the relaxation to acetylcholine. The extra cellular glucose concentration of 20 mmol/l did not influence the contractile effect of serotonin. The inhibition of CSE by DL-propargyl glycine significantly enhanced the maximal force of serotonin-induced contraction under normal and hyperglycemic conditions. It is concluded that CSE is an important regulator of endothelium denuded rat resistance arteries. It is suggested that CSE in smooth muscle cells of artery wall released mediator that antagonized the constricting action of serotonin. This regulation is insensitive to extra cellular glucose concentration.

Key words: artery, contraction, cystathionine gamma–lyase, hyperglycemia


Introduction

Hydrogen sulfide (H2S) has recently been identified as a new gasotransmitter alongside nitric oxide (NO) and (CO) in the mammals, particularly in the central nervous system and the circulatory system (Zhao et al., 2001, Li et al., 2009). There are three enzymes that synthesize hydrogen sulfide (H2S) from cysteine – cystationine-beta-synthase, cystationine-gamma lyase and 3-mercaptosulfur-transferase. In the cardiovascular system, H2S is generated mainly by cystathionine gamma-lyase (CSE) (Kimura, 2011). Hypertension is observed in CSE knockout mice, confirming that H2S is a smooth muscle relaxant and suggesting that it may regulate blood pressure (Yang et al., 2008). H2S exerts anti-hypertensive effects by vasorelaxation by activation of voltage-sensitive potassium channels (KCNQ type, also know as Kv7) (Schleifenbaum et al., 2010; Kohn et al., 2012) and KATP channels (Zhao et al., 2001). KCNQ channels regulate excitability of neuronal, sensory and muscular cells (Soldovieri et al., 2011). Abnormal metabolism and functions of the CSE/
H₂S pathway have been linked to various cardiovascular diseases including atherosclerosis and hypertension (Yang et al., 2010). The inability of the vascular endothelial cells to produce vasorelaxant mediators plays a key role in the pathogenesis of various diabetic complications. It is an early indicator of the development of the micro- and macroangiopathy associated with diabetes (Triggle and Ding, 2010). Substitution of H₂S protects against the development of endothelial dysfunction (Szabo, 2007; Wagner, 2009; Asdaq and Inamdar, 2010). The paracrine (vasocrine) H₂S signaling into the artery wall may represent a potential therapeutic target for obesity- and diabetes-associated vascular dysfunction. The aim of our study is to clarify the importance of H₂S producer CSE of skeletal artery smooth muscle cells as an antagonist of serotonin-induced contraction.

Materials and Methods

Male rats (200–300 g) were killed under ether. The gracilis arteries (a. gracilis) were removed, quickly transferred to cold (4°C) physiological salt solution (PSS). The isometric contractions of de-endothelised rat a. gracilis were measured with Small Vessel Myograph (DMT 410M, Denmark). Isometric wire myography is an in vitro technique that examines the functional responses and vascular reactivity of isolated vessel rings with small diameters. In our study rat a. gracilis were dissected of approximately 4 mm ring preparations in chilled PSS. Perivascular adipose tissue, connective tissue and endothelial layer were removed. The organ bath was filled with PSS containing (in mmol/l): 119 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 25 NaHCO₃, 1.2 MgSO₄, 5.5 or 20 glucose, 1.6 CaCl₂. The bath solution was continuously oxygenated with a gas mixture of 95%O₂ and 5%CO₂, and kept at 37°C (pH = 7.4). Half of the isolated rings were incubated in PSS with 20 mmol/l D-glucose and the others – in 5.5 mmol/l. After 1h of equilibration the contractile force was measured under isometric conditions. The arterial contraction is expressed as a percentage of the steady-state tension (100%) obtained with isotonic PSS containing 60 mmol/l KCl (i.e. 55 mmol/l NaCl has been replaced by 55 mmol/l KCl). Increasing concentrations of serotonin (5-hydroxytryptamine) from 10⁻¹⁰ to 10⁻⁵ mol/l were applied to induce gradual constriction of circular artery segments. The absence of endothelium was confirmed by the lack of relaxation to acetylcholine of 60 mmol/l KCl-contracted arteries. The relaxing effect of CSE was inhibited by 1 micromol/l DL-propargyl glycine (PGG). All drugs were added into the bath solution (PSS). Statistical analyses were performed by SPSS. All results are given as means ± S.E.M of six separate experiments. Statistical significance was determined using Student t-test.

Results and Discussion

Increasing concentrations of serotonin from 10⁻¹⁰ to 10⁻⁵ mol/l induced dose-dependently increase of force of contraction of a. gracilis preparations. Under hyperglycemic conditions when extra cellular glucose concentration was enhanced to 20 mmol/l the contractile effect of serotonin remained statistically unchanged (Figure 1). The inhibition of CSE by 10⁻⁶ mol/l PGG significantly enhanced the maximal force of serotonin-induced contraction under normal and hyperglycemic conditions (Figures 2 and 3). PGG is a selective inhibitor of CSE (Yang et al., 2008) that is routinely used as a pharmacological tool for CSE participation in physiologi-
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Cal regulations. We applied low concentration of this substance to minimize its influence on other target molecules. Nevertheless, the use of PPG reveals a powerful increase of force of skeletal muscle artery contractions induced by serotonin in a wide range of its concentrations (from 10 nmol/l to 10 micromol/l). The increase of extracellular glucose from 5 to 20 mmol/l leaves the endothelium-denuded rat resistance artery preparations as sensitive to PGG as under normal glucose concentration (Figure 4). Therefore, neither hyperglycemia nor the enhanced osmotic pressure by 15 mOsm changes the significant activity of CSE \textit{a. gracilis} preparations. Thus, CSE is an important regulator of their serotonin-induced contractions in this tissue. It is concluded that CSE of smooth muscle cells of skeletal muscle artery wall releases mediator, most probably \(H_2S\) that antagonizes the constricting action of serotonin. This regulation remains unchanged in normal and hyperglycemic conditions.

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\textbf{References}


\begin{figure}
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\includegraphics[width=\textwidth]{fig3.png}
\caption{Maximal force of contraction in 20 mM glucose vs. 20mM glucose with PGG (* p < 0.05)}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{Maximal force of contraction in 5.5 mM glucose with PGG vs. 20mM glucose with PGG (n/s)}
\end{figure}