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Hydrogen sulphide inhibits carbachol-induced contractile responses in β-escin permeabilized guinea-pig taenia caecum

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ABSTRACT

Hydrogen sulphide (H_2S) is an endogenous mediator producing a potent relaxation response in vascular and non-vascular smooth muscles. While ATP-sensitive potassium channels are mainly involved in this relaxant effect in vascular smooth muscle, the mechanism in other smooth muscles has not been revealed yet. In the present study, we investigated how H_2S relaxes non-vascular smooth muscle by using intact and β-escin permeabilized guinea-pig taenia caecum. In intact tissues, concentration-dependent relaxation response to H_2S donor NaHS in carbachol-precontracted preparations did not change in the presence of a K_{ATP} channel blocker glibenclamide, adenylate cyclase inhibitor SQ-22536, guanylate cyclase inhibitor ODQ, protein kinase A inhibitor KT-5720, protein kinase C inhibitor H-7, tetrodotoxin, apamin/charybdotoxin, NOS inhibitor L-NAME and cyclooxygenase inhibitor indomethacin. We then studied how H_2S affected carbachol- or Ca^{2+}-induced contractions in permeabilized tissues. When Ca^{2+} was clamped to a constant value (*pCa6*), a further contraction could be elicited by carbachol that was decreased by NaHS. This decrease in contraction was reversed by catalase but not by superoxide dismutase or N-acetyl cysteine. The sarcoplasmic reticulum Ca^{2+}-ATPase pump inhibitor, cyclopiazonic acid, also decreased the carbachol-induced contraction that was further inhibited by NaHS. Mitochondrial proton pump inhibitor carbonyl cyanide p-trifluromethoxyphenylhydrazone also decreased the carbachol-induced contraction but this was not additionally changed by NaHS. The carbachol-induced Ca^{2+} sensitization, calcium concentration–response curves, IP_3- and caffeine-induced contractions were not affected by NaHS. In conclusion, we propose that hydrogen peroxide and mitochondria may have a role in H_2S-induced relaxation response in taenia caecum.

1. Introduction

Hydrogen sulphide (H_2S), similar to nitric oxide and carbon monoxide, is an endogenous gaseous mediator playing important roles in various physiological and pathophysiological events (Hosoki et al., 1997; Wang, 2002; Chen et al., 2007). In mammalian tissues, H_2S is mainly produced from L-cysteine by two pyridoxal-5-phosphate-dependent enzymes, cystathionine-ß-synthetase (CBS) and cystathionine-γ-lyase (CSE) (Wang, 2002). Recently, a third enzyme, 3-mercaptopyruvate sulfurtransferase, that can produce H_2S has been reported and there is also a metabolic pathway in which homocysteine can be converted to L-cysteine to form H_2S (Shibuya et al., 2009a,b; Carson and Konje, 2010). CBS is highly expressed in the central nervous system, liver and kidney whereas CSE is expressed mostly in the vascular tissues, liver, kidney, small intestine and pancreas (Abe and Kimura, 1996; Hosoki et al., 1997; Bao et al., 1998; Zhao et al., 2001; Kimura, 2002). Both CBS and CSE are also expressed in reproductive tissues (Patel et al., 2009). The third enzyme 3-mercaptopyruvate sulfurtransferase has been identified in neurons and vascular endothelium (Shibuya et al., 2009a,b). In terms of the gaseous product itself, H_2S has been detected in rat, human and bovine brain tissues (50–160 μM) and also in rat and human blood (10–100 μM) (Warenycia et al., 1989; Abe and Kimura, 1996; Richardson et al., 2000; Zhao et al., 2001). On the other hand, Olson (2009) has questioned the existence and the concentrations of H_2S in circulation (Olson, 2009).

H_2S produces a potent relaxant effect in vascular and non-vascular smooth muscles (Warenycia et al., 1989; Zhao et al., 2001; Wang, 2003). In the cardiovascular system, it relaxes isolated arteries and veins and lowers blood pressure by opening ATP-sensitive potassium channels and consequent membrane hyperpolarization (Hosoki et al., 1997; Zhao et al., 2001; Cheng et al., 2004). Furthermore, others have suggested that H_2S leads to vasorelaxation via metabolic inhibition and a fall in adenosine triphosphate levels (Kiss et al., 2008) or a decrease in the intracellular pH (Lee et al., 2007). However, the mechanism of H_2S-induced relaxation in non-vascular smooth muscle has not been identified.

H_2S-induced toxicity has been proposed to be related to mitochondrial inhibition. It has been suggested that cytochrome oxidase, which is the terminal enzyme in mammalian mitochondrial oxidative phosphorylation, is potently inhibited by H_2S (Smith et al.,

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1977; Holland and Kozlowski, 1986; Reiffenstein et al., 1992; Dorman et al., 2002). It is therefore interesting that inhibition of mitochondria affects the contractility of smooth muscle. For instance, in rats with mitochondria having impaired oxidative phosphorylation, both receptor and depolarization-induced contractions have been shown to decrease in gastric fundus and in mesenteric arteries (Jawien et al., 2008). Moreover, it has also been shown that reactive oxygen species play a role in H_2S-induced cytotoxicity in rat hepatocytes (Eghbal et al., 2004; Truong et al., 2006). Reactive oxygen species may change muscle contraction by interacting with excitation–contraction coupling. It has been known that in aorta, myocardium and bladder detrusor different types of reactive oxygen species decreased receptor- or depolarization-induced contractions (Wolin and Belloni, 1985; Duranteau et al., 1998; Aikawa et al., 2003; Durlu-Kandilci and Sahin-Erdemli, 2008).

In the present study, we investigated the mechanism of the smooth muscle relaxant effect of H_2S in guinea-pig taenia caecum by using intact and β-escin permeabilized tissues. When the smooth muscle cell membrane is permeabilized by using a chemical agent, the intracellular ionic composition can be modified by changing the bath medium since both the extracellular and the intracellular mediums contain the same ions (Nasu, 1989). Therefore, permeabilization of this tissue allowed us to inquire the role of sarcoplasmic reticulum Ca^{2+}-ATPase pump, IP_3 receptors, mitochondria and contractile proteins in changes induced by H_2S in Ca^{2+}- and carbachol-mediated contractions.

2. Material and methods

The study protocol was approved by the Hacettepe University Animal Ethics Committee (2008/72-3). Male albino guinea-pigs (350–400 g) were killed by dislocation of the neck and the taenia caecum were isolated.

2.1. Organ bath experiments

2.1.1. Tissue preparation

Taenia caecum strips of approximately 7–8 mm were prepared. Subsequently, strips were mounted under a resting tension of 1 g in 5-ml glass organ baths filled with Krebs–Henseleit solution (see below) gassed with 95% O_2–5% CO_2 at 37 °C and pH 7.4. Tissues were equilibrated for 1 h and washed by Krebs–Henseleit solution every 15 min before each experimental procedure. Isometric changes in the tension were recorded with an isometric force transducer (MAY95-transducer data acquisition system).

2.1.2. Experimental protocol

Sodium hydrogen sulphide (NaHS) was used as an H_2S donor and its aqueous solution was introduced directly into the organ bath by an automated pipette. NaHS dissociates to Na^+ and HS^- in aqueous solution, then HS^- associates with H^+ to form H_2S (Hosoki et al., 1997).

Taenia caecum strips were precontracted by 1 μM carbachol (60–80% of the maximum) and then the relaxation response to NaHS (0.1–3 mM) was evaluated by cumulative addition to the organ bath. In some experiments, NaHS (0.1–1 mM) response was elicited in the presence of ATP-sensitive potassium channel blocker glibenclamide (10 μM), adenylate cyclase inhibitor SQ-22536 (10 μM), guanylate cyclase inhibitor ODQ (100 μM), protein kinase A inhibitor KT-5720 (1 μM), protein kinase C inhibitor H-7 (30 μM), small conductance/intermediate and large conductance Ca^{2+} dependent K^+ channel blockers apamin (500 nM)/charybdotoxin (100 nM), Na^+ channel blocker tetrodotoxin (1 μM), nitric oxide synthase inhibitor L-NAME (100 μM) and cyclooxygenase inhibitor indomethacin (30 μM). At the end of each experiment papaverine (100 μM) is added to the organ bath to test the maximum relaxation of the tissue. Each concentration–response curve to NaHS was obtained in individual preparations.

2.1.3. Solutions used in organ bath experiments

The composition of the Krebs–Henseleit solution (in mM) was NaCl, 113; KCl, 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.6.

Stock solutions of all drugs used in organ bath experiments were made in distilled water except for glibenclamide, ODQ and KT-5720 which were dissolved in dimethyl sulfoxide (DMSO). The dilutions of the stock solutions are made with distilled water. DMSO at concentrations that match its dilutions did not alter the control responses.

2.2. Permeabilization experiments

A different organ bath set up was used for the permeabilization experiments.

2.2.1. Tissue preparation

Taenia caecum tissues were placed in Hepes-buffered modified Krebs' solution (see below). Small strips (150–250 μm in diameter, 3–4 mm in length) of smooth muscle were dissected from taenia caecum. A small hook was tied to one end of a strip to attach it to the transducer. A snare of 5/0 surgical silk captured the other end and was used to mount the strip in a fixed position in a 1000 μl chamber in one of a series of small chambers in a Perspex block. The chamber was filled with Hepes-buffered modified Krebs' solution at room temperature and the tissues were equilibrated for 30 min under a resting tension of 100 mg. Solution changes were made by moving the Perspex block. After stable responses had been achieved to 80 mM K^+ (see below) and 50 μM carbachol in intact tissues, they were moved into relaxing solutions (see below) and incubated for a few minutes. Then, the tissues were permeabilized with 80 μM β-escin in relaxing solution for 30 min at pH 6.8. This was followed by a 4 min wash in relaxing solution before beginning an experiment. Muscle fibers were accepted as permeabilized if the maximum tension obtainable by 100 μM Ca^{2+} after permeabilization was found to be greater than the tension produced by 80 mM K^+ applied in the same strip before permeabilization (Endo et al., 1977). In the experiments with H_2S donor NaHS, the strips were incubated with this substance for 3 min before the contractile responses were elicited. Whereas, with ROS scavengers or other inhibitors, the tissues were incubated with the substance for 15 min in relaxing solution and then the contraction was obtained. The contractile force was measured by a sensitive force transducer (Swema, Stockholm, Sweden) connected to a computer using Biopac Student Lab Pro 3.7 (Commat LTD, Turkey) software.

2.2.2. Experimental procedures

2.2.2.1. Carbachol-induced contractions. After permeabilization, the preparations were relaxed by exposure to the relaxing solution for 4 min. Intracellular Ca^{2+} stores were loaded by activating solution at pCa 6 for 10 min. Then, a contraction response was elicited by carbachol (50 μM) in the presence of GTP (100 μM) which was repeated in the presence of H_2S donor NaHS (300 μM) and with the ROS scavengers catalase (1000 U/ml), N-acetyl-L-cysteine (NAC; 100 μM) and superoxide dismutase (SOD; 150 U/ml). The same protocol was repeated in the presence of SR Ca^{2+}-ATPase pump inhibitor cyclopiazonic acid (CPA;1 μM) and mitochondrial proton pump inhibitor carbonyl cyanide p-trifluromethoxyphenylhydrazone (FCCP; 1 μM) and then both of them at the same time to induce Ca^{2+} sensitization (Durlu-Kandilci and Brading, 2006).

2.2.2.2. pCa tension curves. After permeabilization, the strips were relaxed by exposure to the relaxing solution for 4 min. Ca^{2+} response curves were elicited by Ca^{2+} applied cumulatively (pCa 7-3) in activating solution. The same protocol was repeated in the presence of H_2S donor NaHS (300 μM) in another strip, since after permeabilization the second response curve decreased compared to the first one.