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The vasodilator mechanisms of sodium metabisulfite on precontracted isolated aortic rings in rats: Signal transduction pathways and ion channels

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ABSTRACT

Sodium metabisulfite (SMB) is most commonly used as a food additives, however few study was performed on the vasodilator effect of SMB. In the present paper, the vasodilator effects of SMB and roles of Ca^{2+} and K^+ channels as well as the cGMP pathway on isolated rat aortic rings were studied. The results show that: (1) SMB could relax isolated aortic rings precontracted by norepinephrine in a concentration-dependent manner. The maximal endothelium-dependent vasorelaxation was approximately 20% whereas that not depending on the presence of the endothelium was more than 90%. (2) The vasorelaxant effects induced by 50 or 200 μM SMB were partially inhibited by iberiotoxin, NS-2028 or L-NNA. The vasorelaxation of 1000 μM SMB was partially inhibited by nifedipine or glibenclamide. The SMB induced vasorelaxation was partially inhibited by tetraethylammonium. These results led to the conclusions that the vasorelaxation of SMB at low concentrations (<400 μM) was endothelium-dependent and mediated by the cGMP pathway and BK_{Ca} channel, but at high concentrations (>500 μM) was endothelium-independent and mediated by K_{ATP} channel and L-type Ca^{2+} channel. The maximal allowable concentration from China and the acceptable daily intake level from WHO of SMB as a food additive should be revised.

1. Introduction

Food spoiling is caused by the action of microorganisms (such as bacteria and molds) and by chemical changes due to enzyme activities or autoxidation (Turkoglu, 2007). Sodium metabisulfite was also known as disodium sulfite, disodium salt, sodium pyrosulfite, sodium disulfite or sodium sulfite anhydrous. SMB inhibits proliferation of microorganisms, this character led to its wide use as a preservative in food products, such as biscuit, chocolate, jam, sausage, and salami, and in drugs such as parenteral amino acid solutions (Jamieson et al., 1985; Elmas et al., 2005). Some substances used as food additives are genotoxic in test systems (Hayashi et al., 1988). Several studies have been published demonstrating the genotoxic effect exerted by the majority of the chemicals (Luca et al., 1987; Rencuzogullari et al., 2001; Gomurgen, 2005).

SMB is a double-edged sword with antioxidant as well as prooxidant properties (Lavoie et al., 1994). Sun et al. (1995) have suggested that SMB can induce bronchoconstriction in asthmatic patients. Chromosome aberrations and sister chromatid exchanges have been reported following SMB exposure (Rencuzogullari et al., 2001). Potential genotoxic effects of SMB have been reported by Kayraldiz and Topaktas (2000). SMB may cause allergic reactions in those who are sensitive to sulfites, including respiratory reactions in asthmatics, anaphylaxis and other allergic reactions in sensitive individuals (Metcalfe et al., 2003). SMB also can significantly increase neuronal death at 10 and 100 μM concentrations (Dani et al., 2007).

Meng and Nie (2005a,b) found that SMB had neuronal toxicity by increasing the excitability of neurons and its mechanism might involve the oxidative damage on ion channels. SMB affected transient outward potassium current and delay rectifier potassium currents, and it would decrease the excitability of hippocampal neuron by increasing potassium currents. SMB might oxidize potassium channels, which relate to adjusting pain sensitivity in pain-sensing dorsal root ganglion neurons (Nie et al., 2009). SMB induced lipid peroxidation and apoptotic on gastric tissue in dose-dependent manner (Ercan et al., 2010).

In our previous report, we found that sulfur dioxide (SO_2) and SO_2 derivatives (mixture of sodium sulfite and sodium bisulfite, 3:1 M/M) could relax isolated rat aortic rings precontracted by NE in a concentration-dependent manner (Meng and Zhang, 2007; Zhang and Meng, 2009; Li and Meng, 2009). To date, the vasorelaxant effect of SMB has not been reported. Therefore, the purpose of the present study was to investigate the vasorelaxant effect of SMB on isolated rat aortic rings, and the signal transduction pathways as well as ion channels.

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2. Materials and methods

2.1. Chemicals and solution preparation

Acetylcholine (Ach), norepinephrine (NE), glibenclamide, 4-aminopyridine (4-AP), nifedipine, iberiotoxin, apamin, 4H-8-bromo-1,2,4-oxadiazolo(3,4-d)benz(b)(1,4)oxazin-1-one (NS-2028), tetraethylammonium (TEA), *N*-nitro-L-arginine (L-NNA), propranolol, indomethacin, staurosporine and SMB were purchased from Sigma (St. Louis, MO, USA). Krebs solution contains (mM) NaCl 120.6, KCl 5.9, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, $CaCl_2$ 2.5, and glucose 11.5.

SMB solution was freshly prepared before each experiment to achieve a solution at concentrations ranging from 0 to 100 mM.

2.2. Preparation of isolated rat thoracic aorta rings

Animal care and experimental protocols complied with the Animal Management Rule of the Ministry of Health, People's Republic of China (Documentation 55, 2001) and the Animal care Committee of Shanxi University.

Male Wistar rats weighing about 220–250 g were obtained from Hebei Medical University (Shijiazhuang, China). Rats were killed by anesthetic overdose (intraperitoneal injection of pentobarbital sodium). The thoracic aorta rings were prepared carefully so that the endothelial cells were not damaged. The thoracic aorta was removed immediately and dissected fat and connective tissues, then cut into rings about 3 mm long. The rings were placed in a bath of Krebs solution at pH 7.4, 37 °C under a resting optimal tension of 1.5 g; 95% O_2 and 5% CO_2 were bubbled through the solution. Tensions were recorded with a MedLab Biological Signal Collection System (Medease Science and Technology, Nanjing, China) during the experiment. The aortic rings in bath tubes were allowed to equilibrate for 1 h before experiment and the Krebs solution was changed every 15 min. The viability of the ring preparation was assessed by contracting vessels with 60 mM KCl before each experiment. The artery endothelium viability and integrity were checked by dilatory response of the ring to acetylcholine (10^{-6} M) as described by Furchgott and Zawadzki (1980) and Fiscus et al. (1991). All rings with endothelium tested had the dilatory response to acetylcholine.

2.3. Characterization of the SMB-induced vasorelaxation

To study the vasodilator effects of SMB, isolated rat thoracic aorta rings were precontracted by 10^{-6} M NE, when the vasoconstriction curves of rings reached the plateau phase of the maximum tension, SMB at 0–2000 μM were added, and the tensions were recorded. Percentage of dilation was calculated when the vasodilator curve reached the plateau phase of the minimum tension. The maximum constriction caused by 10^{-6} M NE was about 80% of the maximum constriction response induced by 10^{-4} M NE (data not shown). In this study, NE at 10^{-6} M was used, therewith vasodilator effect of SMB was expressed as a percentage of relaxation to maximum constriction induced by 10^{-6} M NE. Saline was used as a control group.

To investigate the role of endothelium in the relaxation response, endothelium was removed by gently scraping with a cotton ball. Verification of endothelium removal was confirmed by failure of the vessel to relax >20% in response to 10^{-6} M acetylcholine (Furchgott and Zawadzki, 1980; Fiscus et al., 1991).

2.4. Involvement of L-type Ca^{2+} channel in the vascular effects of SMB

To elucidate whether an L-type Ca^{2+} channel was the target of SMB vascular action, rings with intact endothelium and denuded endothelium were pre-incubated with nifedipine (1 μM), an L-type Ca^{2+} channel blocker, for 20 min prior to the application of 50, 200 or 1000 μM SMB (Shen et al., 2000; Zhang and Meng, 2009). In our pre-experiment, we found that 1 μM nifedipine incubating for about 20 min could completely abolish the $CaCl_2$ induced contraction, suggesting that nifedipine inhibited the L-type Ca^{2+} channels (data not shown).

2.5. Involvement of K^+ channels in the SMB-induced vasorelaxation

To determine the involvement of K^+ channels in the vasorelaxant effects of SMB, rings with intact endothelium and denuded endothelium were pre-incubated with TEA (10 mM), 4-AP (2.5 mM), iberiotoxin (100 nM), apamin (50 nM) and glibenclamide (10 μM) for 20 min prior to the application of 50, 200, or 1000 μM SMB, respectively (Leung et al., 2007; Zhao et al., 2001). TEA is known to block many different types of K^+ channels (Nelson and Quayle, 1995). 4-AP was a specific K_V channel inhibitor (Remillard and Leblanc, 1996). Iberiotoxin was a large-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) blockers (Satake et al., 1996). Apamin was a small-conductance K_{Ca} channel inhibitor (Blatz and Magelby, 1986). Glibenclamide was a selective ATP-sensitive K^+ channel blocker (Beech et al., 1993). Although these compounds are not entirely specific for these K^+ channels, at the concentrations and the time of incubation used in these studies, these blockers are relatively selective for the K_{Ca}, K_{ATP} and K_V channels, respectively, and are widely used in studies of vascular K^+ channel function (Nelson and Quayle, 1995; Zhao et al., 2001).

2.6. The signal transduction pathways of SMB-induced vasorelaxation

Further studies were carried out to identify the involvement of various signal transduction pathways in the vascular effect of SMB at low and high concentrations. The isolated rat thoracic aorta rings with intact endothelium and denuded endothelium were preincubated with 10^{-4} M L-NNA (NO synthase inhibitor) (Furfine et al., 1993), 10^{-5} M NS-2028 (an inhibitor of sGC) (Olesen et al., 1998), 10^{-5} M propranolol (an antagonist of β-noradrenoceptor) (Meng and Zhang, 2005), 10^{-5} M indomethacin (an inhibitor of cycloxygenase, one of PGI_2-synthetases) (Rodriguez-Martinez et al., 1998), 30 nM staurosporine (an inhibitor of PKC) (Hattori et al., 1995), and saline (control) for 20 min prior to the application of 50, 200 or 1000 μM SMB, respectively.

2.7. Statistical analysis

The concentration–response curves of SMB were built and fitted with a Hill equation, from which EC_{50} was calculated (Weiss, 1997). All values were expressed as mean ± standard deviation, and student's *t*-test for unpaired samples was used to compare the mean values between two groups. A level of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. The vasodilator effects of SMB

Fig. 1 shows that SMB caused relaxation of isolated rat aortic rings in a concentration-dependent manner for both endothelium-intact (EC_{50}, 829.66 ± 96.82 μM) and endothelium-denuded aortic rings (EC_{50}, 1087.26 ± 138.62 μM). These vasorelaxant effects of SMB at all concentrations tested were reversible to the levels as before the administration of SMB after SMB was washed out with Krebs solution. Fig. 1 also shows that SMB at low concentrations (<400 μM) caused relaxation of endothelium-intact aortic rings, but not for endothelium-denuded aortic rings. At high concentrations (>500 μM), SMB caused relaxation of both endothelium-intact and endothelium-denuded aortic rings.

3.2. Involvement of L-type Ca^{2+} channel in the vascular effects of SMB

Nifedipine alone significantly decreased the basal tension of the rat aortic tissue precontraction with 10^{-6} M NE by 34% (data not shown). From Fig. 2 we can see that the vasorelaxant effects of 1000 μM SMB on both endothelium-intact and endothelium-denuded rings were partially inhibited by nifedipine, an L-type Ca^{2+} channel blocker. But the vasorelaxant effect of 50 or 200 μM SMB on the endothelium-intact rings was not affected by nifedipine.

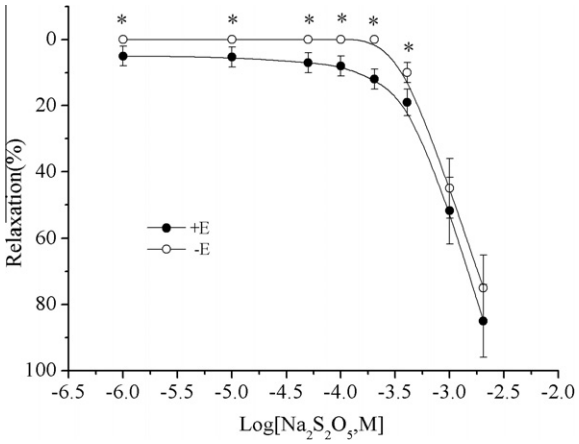

Fig. 1. Vasorelaxant effects of SMB on the endothelium-denuded or endothelium-intact rat aortic rings precontracted by 10^{-6} M NE. The relaxation effect was expressed as a percentage of decrement to the maximum tension caused by 10^{-6} M NE. $n = 6$ (six isolated aortic rings from six rats), compared with the SMB group of endothelium-intact rat aortic rings, $^*P < 0.05$.