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The molecular mechanisms of sodium metabisulfite on the expression of K_{ATP} and L-Ca^{2+} channels in rat hearts

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ABSTRACT

Sodium metabisulfite (SMB) is used as an antioxidant and antimicrobial agent in a variety of drugs and foods. However, there are few reported studies about its side effects. This study is to investigate the SMB effects on the expression of ATP-sensitive K^+ (K_{ATP}) and L-type calcium (L-Ca^{2+}) channels in rat hearts. The results show that the mRNA and protein levels of the K_{ATP} channel subunits Kir6.2 and SUR2A were increased by SMB; on the contrary, SMB at 520 mg/kg significantly decreased the expression of the L-Ca^{2+} channel subunits $Ca_v1.2$ and $Ca_v1.3$. This suggests that SMB can activate the expression of K_{ATP} channel by increasing the mRNA and protein levels of Kir6.2 and SUR2A, while it inhibits the expression of L-Ca^{2+} channels by decreasing the mRNA and protein levels of $Ca_v1.2$ and $Ca_v1.3$ in rat hearts. Therefore, the molecular mechanism of the SMB effect on rat hearts might be related to the increased expression of K_{ATP} channels and the decreased expression of L-Ca^{2+} channels.

1. Introduction

Sodium metabisulfite (SMB) as a preservative is commonly used in food, beverages, and drugs due to its ability to inhibit proliferation of microorganisms and its antioxidant properties (Rencüzoğullari et al., 2001). When ingested, SMB reacts with acids and water, releasing toxic sulfur dioxide (SO_2). However, several idiosyncratic reactions (e.g., bronchospasm, oculonasal symptoms, and urticaria/angioedema) have been associated with SMB as a food preservative in sulfite-sensitive individuals, and as an under-recognized hazard, the pathogenic mechanism of these reactions has not yet understood. In particular, there are several amino acid preparations utilized in total parenteral nutrition solutions that contain large amounts of sulfites (Lakamp and Dobesh, 2000). Five sulfite salts including SMB ($Na_2S_2O_5$), sodium sulfite (Na_2SO_3), sodium bisulfite ($NaHSO_3$), potassium sulfite (K_2SO_3), and potassium metabisulfite ($K_2S_2O_5$) as antimicrobial agents and antioxidants are commonly applied in food preparations (Gunnison and Jacobsen, 1987). Sun et al. (1995) have suggested that SMB can induce bronchoconstriction in asthmatic patients. Recent studies have reported that SMB can induce chromosomal aberrations (CA) and sister chromatid exchanges (SCE) in human lymphocytes (Rencüzoğullari et al., 2001), which is similar to the effect of SO_2 and its derivatives (sulfite and bisulfite, 3:1 M/M) (Meng and Zhang, 1990).

Our recent studies show that the negative inotropic effects of SO_2 and its derivatives in isolated perfused rat hearts might be related to ATP-sensitive K^+ (K_{ATP}) channels and L-type calcium (L-Ca^{2+}) channels (Zhang and Meng, 2012). However, the molecular mechanism involved in SMB-affected K^+ and Ca^{2+} channels is unknown. K_{ATP} channels are widely distributed in various tissues and may be associated with different cellular functions. In the heart, K_{ATP} channels appear to be activated during ischemic or hypoxic conditions, and responsible for the increase of K^+ efflux and shortening of the action potential duration (Fujita and Kurachi, 2000). Under the conditions of metabolic surplus, the cardiac K_{ATP} channels respond by closure while metabolic challenge provokes channel opening with the consequent K^+ efflux, action potential shortening, and limiting potential damage of intracellular Ca^{2+} loading (Terzic et al., 1995; Zingman et al., 2002). K_{ATP}

Abbreviations: CA, chromosomal aberrations; Ct, threshold cycle; EC, excitation–contraction; FASEB, Federation of American Societies for Experimental Biology; HE, hematoxylin–eosin; K_{ATP}, ATP-sensitive K^+; Kir6.x, inward-rectifying K^+ channel family; L-Ca^{2+}, L-type calcium; LD_{50}, median lethal dose; MAC, maximum allowable concentration; qPCR, quantitative polymerase chain reaction; SCE, sister chromatid exchanges; SMB, sodium metabisulfite; SO_2, sulfur dioxide; SUR, sulfonylurea receptor.

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channels can increase myocardial oxygen supply and decrease oxygen consumption, thus improving cardiac function and myocardial energy metabolism, preserving the normal structure of cells, regulating vascular tension, and changing ion distributions between both sides of cardiac myocytes (Ljubkovic et al., 2006).

L-Ca^{2+} channels in cardiomyocytes provide the main influx pathway for Ca^{2+}, an essential, versatile and universal intracellular messenger accompanying cardiomyocytes throughout their entire lifespan. The pore-forming protein is activated upon membrane depolarization, and it also triggers many processes including electrogenicity, contraction, biochemical and gene regulation (Ringer, 1883). L-Ca^{2+} channels are located in sarcolemma, including the T-tubules facing the sarcoplasmic reticulum junction, and are activated by membrane depolarization, but intracellular Ca^{2+}-dependent inactivation limits Ca^{2+} influx during action potential. The influx of Ca^{2+} through L-Ca^{2+} channels is important for heart function because it triggers excitation–contraction (EC) coupling, modulates action potential shape and results in cardiac arrhythmia (Jean-Pierre et al., 2010). In cardiac myocytes, L-Ca^{2+} channels are localized in multiple distinct subcellular compartments that impact their function and regulation. The significance of dyadic L-Ca^{2+} channels in EC coupling has long been recognized. Many cardiac diseases involve changes in subcellular architecture and organization, and then altere subcellular localization of L-Ca^{2+} channels together with associated changes in channel functions, which can produce aberrant electrophysiology resulting in arrhythmias and dysregulation of various Ca^{2+}-dependent cellular processes (Best and Kamp, 2012).

In our previous report, we investigated the effects of SO_2 derivatives on the K^+ and Ca^{2+} currents in the isolated adult rat ventricular myocytes with the whole cell patch-clamp technique and found that SO_2 derivatives might cause the cardiac myocytes injury by changing extracellular potassium via voltage-gated K^+ channels and intracellular calcium via voltage-gated Ca^{2+} channels (Nie and Meng, 2005a; Nie and Meng, 2006). In addition, we found that SMB might lead to the decrease of the excitability and even death of hippocampal neurons and adjust the pain sensitivity of dorsal root ganglion neuron through modulating K^+ and Ca^{2+} currents (Nie and Meng, 2005b; Nie et al., 2009; Wei and Meng, 2010). However, it is still unknown for the molecular mechanism by which SMB produces these effects through K^+ and Ca^{2+} channels. The purpose of this study is to investigate the effects of SMB on the gene and protein expression of K_{ATP} and L-Ca^{2+} channels in rat hearts.

2. Materials and methods

2.1. Chemicals

SMB was purchased from Sigma (St. Louis, MO, USA). Maxiama SYBR Green qPCR Master Mix kit, PrimeScript RT reagent kit, and TRIzol reagent were purchased from TaKaRa (TaKaRa, Dalian, China). Rabbit polyclonal $Ca_v1.2$, $Ca_v1.3$, β-actin and goat polyclonal Kir6.1, Kir6.2, SUR2A, SUR2B antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Preparation and treatment protocols of rat hearts

Healthy adult and clean grade male Wistar rats, weighing 200–250 g, were purchased from Animal Center of Hebei Medical University (Shijiazhuang, China). The rats were used complied with the guidelines approved by the Institutional Animal Care and Use Committee of Shanxi University and were housed in metallic cages under standard conditions (24 ± 2 °C and 50 ± 5% humidity) with a 12 h light–dark cycle. Rats were divided randomly into four groups of six for each. SMB (0, 130, 260, 520 mg/kg body weight) were respectively given to rats by intragastric administration for 1 week (Elmas et al., 2005; Hui et al., 1989; Nair and Elmore, 2003). After 24 h of the last treatment, rats were killed by anesthetic overdose (intraperitoneal injection of pentobarbital sodium 90 mg/kg). The hearts were removed immediately and then heart tissues from each group were fixed in 10% formaldehyde in PBS for the HE (hematoxylin and eosin) staining analysis. The remaining heart tissues were frozen in liquid nitrogen for mRNA and protein assay.

2.3. Histopathological observation

The above heart tissues in 10% formaldehyde in PBS were paraffin-embedded for HE staining. After routine processing, the paraffin sections were cut into 5 μm thickness, stained with HE, and observed under light microscopy (Olympus, Japan) with ×400 magnification (Li et al., 2010).

2.4. RNA isolation and real time PCR

Total RNA was isolated from heart tissues using TRIzol Reagent according to the manufacturer's instructions. RNA quality was assured by 1% gel electrophoresis (28S/18S RNA). The total RNA concentration was determined by spectrophotometric analysis at 260 nm. The OD_{260}/OD_{280} ratio was in the range of 1.9–2.1. The first-strand cDNA was synthesized using the First Strand cDNA Synthesis kit. The cDNA product was stored at −80 °C before use.

All PCR primers were designed by Primer designer software (Table 1). qRT-PCR was done by using an iCycler thermal cycler with an iQ5 qRT-PCR detection system (Bio-Rad, Hercules, CA, USA) and Maxima SYBR Green qPCR Master Mix kit (Takara, Dalian, China). The melting curve was established for each sample under the cycling conditions as following: 3 min at 95 °C, 40 cycles of 20 s at 94 °C, 20 s at 55–60 °C, and 20 s at 72 °C. Fluorescence data were acquired at 72 °C step. The threshold cycle (Ct) value for each dilution was then plotted against the logarithm of concentration, and Ct values for the experimental samples were plotted

Table 1
Primers sequences used in real-time RT-PCR.

Gene	Accession No.	Sequences
β-actin	NM_031144	
	Forward primer	5′-CCT CTA TGC CAA CAC AGT GCT GTC T-3′
	Reverse primer	5′-GCT CAG GAG GAG CAA TGA TCT TGA-3′
SUR2A	D83598	
	Forward primer	5′-GGA GTG CGA TAC TGG TCC AAA CCT-3′
	Reverse primer	5′-CCC GAT GCA GAG AAC GAG ACA CT-3′
SUR2B	AF087838	
	Forward primer	5′-CAT AGC TCA TCG GGT TCA CAC CAT T-3′
	Reverse primer	5′-GCA TCG AGA CAC AGG TGC TGT TGT-3′
Kir6.1	NM_017099	
	Forward primer	5′-ACG ACG CCA GAA GGA GAG-3′
	Reverse primer	5′-GCC ACT AGG AAG ATG TTA TTG C-3′
Kir6.2	NM_031358	
	Forward primer	5′-CAA CGT CGC CCA CAA GAA CAT C-3′
	Reverse primer	5′-CCA GCT GCA CAG GAA GGA CAT G-3′
$Ca_v1.1$	NM_053873	
	Forward primer	5′-ACA TCG CCC TGC TCG TCC TCT-3′
	Reverse primer	5′-CAC TCG CTG CCG TTG ATG G-3′
$Ca_v1.2$	NM_012517	
	Forward primer	5′-CAT CAT CAT CAT TGC CTT CTT C-3′
	Reverse primer	5′-ACT GGT GCT GGT TCT TGG-3′
$Ca_v1.3$	NM_017298	
	Forward primer	5′-CTT CCT CTT CAT CAT CAT CTT C-3′
	Reverse primer	5′-TCA TAC ATC ACC GCA TTC C-3′
$Ca_v1.4$	NM_053701	
	Forward primer	5′-AAC TGG GCC TAG TGA TGA TGA TGG-3′
	Reverse primer	5′-AGG GAC TTC CTG TTC CTC ATT CTG-3′