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Modulation of L-type calcium current in rat cardiac myocytes by sulfur dioxide derivatives

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Abstract

The effects of sulfur dioxide (SO_2) derivatives (bisulfite and sulfite, 1:3 M/M) on voltage-dependent L-type calcium current ($I_{Ca,L}$) in isolated rat ventricular myocytes were studied using the whole cell patch-clamp technique. SO_2 derivatives increased $I_{Ca,L}$ in a concentration-dependent manner. SO_2 derivatives shifted both the steady-state activation and the inactivation curves of $I_{Ca,L}$ to more positive potentials, the effect on the latter being more pronounced. SO_2 derivatives markedly accelerated the recovery of $I_{Ca,L}$ from inactivation. SO_2 derivatives also significantly shortened the fast and slow time constants of inactivation. These results suggested that SO_2 inhalation might cause cardiac myocyte injury through increasing intracellular calcium via voltage-gated calcium channels.

Keywords: Cardiomyocyte; L-type calcium current; Patch-clamp technique; Sulfur dioxide

1. Introduction

Sulfur dioxide (SO_2) is a common air pollutant released into the atmosphere from the combustion of fossil fuel. Inhaled SO_2 can easily be hydrated to produce in the respiratory tract sulfurous acid, which subsequently dissociates to form its derivatives, bisulfite and sulfite (1:3 M/M, in neutral fluid) (Shapiro, 1977). The derivatives can be absorbed into blood or other body fluid. In addition, bisulfite/sulfite enters the body via foods, beverages and drugs because sulfiting agents (sulfur dioxide, metabisulfite, bisulfite and sulfite) are widely used as preservatives (Gould and Russell, 1991). Endogenous bisulfite/sulfite is generated during the normal processing of sulfur-containing amino acids (Ubuka et al., 1990) and can be formed by the metabolism of sulfur-containing drugs, including *N*-acetylcysteine (Cotgreave et al., 1987). The natural SO_2 concentration is 0.04–0.45 mg/m^3 in atmosphere. Epidemiological studies have linked SO_2 exposure with many respiratory diseases such as lung cancer (Atkinson et al., 1993) when SO_2 concentration exceeds 0.6 mg/m^3. SO_2 inhalation may induce chromosomal aberrations (CA), sister chromatid exchanges (SCE), and micronuclei (MN) in human peripheral blood lymphocytes. (Rencüzoğullari et al., 2001; Meng and Zhang, 1990, 1992). SO_2 inhalation may cause changes of oxidative stress and

Abbreviations: TEA-Cl, tetraethylammonium chloride; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; TTX, tetrodotoxin; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; CA, chromosomal aberrations; SCE, sister chromatid exchanges; KB, Krafteburhe; $I_{Ca,L}$, L-type calcium current; L-Glu, L-glutamic acid; Tau, Taurine; τ_f, fast time constant; τ_s, slow time constant; PKA, protein kinase A; PKI, protein kinase inhibitor; VDCC, voltage-dependent calcium channel; cAMP, cyclic adenosine monophosphate; AC, adenylate cyclase; VSMC, vascular smooth muscle cells.

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antioxidation status in various organs of mice, especially in brain (Meng, 2003). SO_2 inhalation can damage the brain cell of rat (Yargicoğlu et al., 1999). The studies in our laboratory have indicated that SO_2 is a systemic oxidative damage agent and toxic to all organs tested in the mouse body, not only to the respiratory system (Meng, 2003).

Biological membranes are essential in maintaining cell integrity and function. Ion channels in cell membrane are targets for many toxins and drugs. Three prominent voltage-gated ionic currents are expressed in cardiac ventricular muscle, the tetrodotoxin (TTX)-resistant sodium current (I_{Na}), the L-type calcium current ($I_{Ca,L}$) and the transient outward potassium current (I_{to}). These currents contribute in a precisely timed and regulated manner to the development, maintenance and termination of the action potential (Walsh and Parks, 2002). Voltage-gated L-type calcium channel is extremely important for cardiac contraction, because this kind of channel is the major pathway for calcium entry during excitation. The L-type calcium current ($I_{Ca,L}$) in heart cells triggers intracellular calcium release, which initiates and regulates the force of the muscular contraction, i.e., excitation–contraction coupling (Xiao et al., 2000). Calcium influx plays important physiological roles, including mediation of cell contraction, secretion, protein phosphorylation and gene transcription. During sarcolemmal membrane depolarization, the L-type calcium channel will open to permit calcium influx and trigger calcium-induced calcium release, leading to cell contraction (Fan et al., 2000).

Recently, we have found that the SO_2 derivatives can cause changes of sodium and potassium currents in the hippocampal CA1 neurons and dorsal root ganglion neurons from rats (Meng and Sang, 2002; Du and Meng, 2004a,b). However, little is known about the effects of SO_2 on L-type calcium currents of mammalian cells. In the present study, we examined the effects of SO_2 derivatives on L-type calcium currents in rat ventricular cardiac myocytes by means of whole cell patch-clamp technique in order to probe into the possible mechanisms of SO_2 on cardiac myocyte. Our results indicated that SO_2 derivatives increased $I_{Ca,L}$ in a concentration-dependent manner, shifted the steady-state activation curve and inactivation curve of $I_{Ca,L}$ to more positive potentials, with main effect on the inactivation kinetics. The recovery from inactivation and time course of inactivation were both accelerated by SO_2 derivatives.

2. Materials and methods

2.1. Isolation of single ventricular myocytes

Single ventricular myocytes were isolated from the heart of adult rats (200–300 g body weight, Wistar) by a modified enzymatic dissociation technique (Isenberg and Klöckner, 1982). Rats were purchased from Experimental Animal Center of Shanxi Medical University (Grade II, Certificate No. 070101). Briefly, The rats were stunned by heavy blow on the head. The heart was rapidly removed and placed in oxygenated ice-cold Ca^{2+}-free Tyrode's solution, and then the excised heart was mounted on a modified Langendorff apparatus for perfusion of the coronary arteries. Blood was removed by a 4-min period of perfusion with oxygenated 37 °C Tyrode's solution, which was followed by 5 min of perfusion with a nominally Ca^{2+}-free Tyrode's solution. Enzymatic digestion was initiated by 25 min of perfusion with 50 ml Ca^{2+}-free Tyrode's solution containing 15 mg collagenase (Type P, Boehringer Mannheim, Roche). At the end of enzyme perfusion, the heart was sequentially washed with 50 ml 0.2 mM Ca^{2+} Tyrode's solution plus 1 mg/ml bovine serum albumin. The ventricles were then cut off, chopped into small chunks and stirred in a small vessel containing 'Krafteburhe' (KB) solution until elongated, striated myocytes dissociated from the tissue pieces. Myocytes were harvested after filtering the cell-containing suspension through a nylon mesh (200 μm). They were washed three times in storage solution and then maintained at room temperature in KB solution for at least 1 h before the electrophysiological experiment. The concentration of Ca^{2+} in Tyrode's solution was gradually increased to 1.8 mmol/l. All experiments were performed within 12 h after isolation.

2.2. Electrophysiological measurements

Isolated ventricular myocytes were placed in the experimental chamber mounted on the stage of an inverted microscope (Olympus IX50, Japan). After setting to the bottom of chamber, the cells were superfused with external solution for 10 min at a rate of 2–3 ml/min at 25 °C. Transmembrane currents were recorded with Axopatch 200B patch clamp amplifier (Axon Instruments, CA, USA). Glass microelectrodes were made using a micropipette puller (PP 830, Narishige, Japan) and had a resistance of 2–5 MΩ, when filled with electrode internal solution. Only the rod shaped cells with visible striations were used for experiments. Liquid junction potential between the pipette solution and external solution was corrected after the pipette tipped into the external solution. After forming a conventional ''giga-seal'', the membrane was ruptured with a gentle suction to obtain the whole cell voltage-clamp configuration. To minimize the duration of capacitive current, membrane capacitance and series resistance were compensated after membrane rupture. The external solution was changed to Na^+-free solution in which Na^+ was replaced by equimolar tetraethylammonium chloride (TEA-Cl). Sodium current was also inactivated at the holding potential of −40 mV and blocked by tetrodotoxin (TTX,