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Enhancing effects of diethyldithiocarbamate on increase of sodium channel by sulfur dioxide derivatives in ventricular myocytes

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Abstract

The enhancing effects of diethyldithiocarbamate (DDC) on increase of sodium channel by sulfur dioxide derivatives in ventricular myocytes were studied using the whole cell patch-clamp technique to probe the mechanism of SO_2 on the cardiovascular system in this study. Firstly, the effects of DDC and/or sulfur dioxide (SO_2) derivatives on the activities of superoxide dismutase (SOD) were studied. The results showed that DDC decreased SOD activities significantly and SO_2 derivatives had no significant effect on SOD activities; however, DDC and SO_2 derivatives combined led to a significant decrease of SOD activities. In the electrophysiological test, DDC (1−100 mM) increased sodium current (I_{Na}) in a concentration-dependent manner and the concentration for half-maximum increase (EC_{50}) was 20 mM. Addition of 20 mM DDC to the SO_2 derivatives-containing medium significantly shifted the voltage-dependent activation curve of I_{Na} toward the hyperpolarizing direction (V_h are −51 mV, −53 mV and −54 mV, respectively) and shifted the steady-state inactivation curve to more positive potentials (V_h are −74 mV, −71 mV and −65 mV, respectively) compared with the control and 10 μM SO_2 derivatives exposure. These results indicated that DDC could enhance the increasing effects on Na^+ channels induced by SO_2 derivatives, and suggested that the toxicity of SO_2 on ventricular myocytes of rats was realized by free radical, especially O_2^-.

Keywords: Ventricular myocytes; Patch-clamp technique; Sodium current; Diethyldithiocarbamate; Sulfur dioxide

1. Introduction

Sulfur dioxide (SO_2) is a systemic oxidative damage agent, which may cause oxidation damage and DNA damage in various organs of mice and rats, especially in lung, brain and heart (Meng, 2003; Meng and Zhang, 2003). 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 the natural fluid) (Shapiro, 1977). The derivatives can be absorbed into blood or other body fluid and reach other tissue and organs. Over the past decade, many studies suggested that SO_2 inhalation might have relation with cardiovascular diseases. Epidemiological studies in Asian cities (Hong Kong, Beijing, Shenyang, Taipei, Seoul, etc.) demonstrated that SO_2 increased the risk of cardiovascular disease and mortality due to cardiovascular disease (Xu et al., 2000; Wong et al., 2002; Chang et al., 2003; Changa et al., 2005). Some studies also suggested that inhalation of SO_2 or intake of food containing sulfite (bisulfite, metabisulfite, etc.) might increase cardiac contractility or cause several kinds of cardiomyopathies (Xu et al., 1994; Vedal et al., 2004). Moreover, the decrease of blood pressure in rats induced by SO_2 has been reported (Meng et al., 2003).

Voltage-gated sodium channel (VGSC) is a prominent voltage-gated ion channel in cardiac ventricular muscle, which plays a crucial role in regulating the electrical excitability of animal cells, being primarily responsible for the depolarization phase of the action potential (Goldin, 2003). Recently, Nie found that SO_2 derivatives significantly enhanced VGSC in a concentration-dependent manner in isolated adult rat ventricular myocytes, and may lead to a series of injury to cardiac

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muscle (Nie and Meng, 2005). However, study of rat hippocampal neurons indicated that three kinds of antioxidase, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxide (Gpx), could partly inhibit the enhancement effect of SO_2 and sulfite on the sodium channel (Meng et al., 2005). The toxicity of SO_2 has been attributed to its oxidative damage. Many studies of our laboratory suggested that the toxicity of SO_2 or sulfite/bisulfite is due to the production of free radical, which may cause enhancement in lipid peroxidation (LPO) and lead to disorder of ion channels in ventricular myocytes of rats, but the detailed mechanism is not clear as yet.

SOD is a kind of antioxidant enzyme existing widely in the living body, which can clear away excessive superoxide anion ($O_2^{-\cdot}$). Diethyldithiocarbamate (DDC) is a well-known inhibitor of Cu,Zn-SOD, a thiol-containing molecule and a potent metal ion-chelating agent (Iqbal and Whitney, 1991; Arnelle et al., 1997), which has been described both to increase superoxide concentration and to inhibit the detoxification of reactive oxygen species (ROS) (Siwik et al., 1999; Didion et al., 2001). Therefore, we examined the effects of SO_2 derivatives on the sodium channel from the point of view of free radical oxidative damage using the whole cell patch-clamp technique to investigate the mechanism of oxidative stress induced by SO_2 derivatives in the cardiovascular system, and provide more academic evidence for the prevention and cure of cardiovascular diseases.

2. Materials and methods

2.1. Cell isolation

Single ventricular myocytes were obtained from the hearts of adult rats (250–300 g) by a modified enzymatic dissociation technique (Isenberg and Klöckher, 1982). Rats were purchased from the Experimental Animal Center of Shanxi Medical University (Grade II, Certificate No. 070101). The animals were stunned by a heavy blow on the head, their hearts quickly excised and mounted on a Langendorff perfusion apparatus for perfusion through the aorta. Hearts were perfused for 5 min at 37 °C with a nominally Ca^{2+}-free Tyrode's solution containing (mM): NaCl 137, KCl 5, $MgCl_2$ 1, NaH_2PO_4 0.33, $CaCl_2$ 1.8, HEPES 10, glucose 10, pH 7.4. The perfusate was then replaced with enzymatic digestion, being 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, then the heart was removed from the cannula, the atria were discarded and the ventricles were chopped and stirred in a small vessel containing 'Krafteburhe' (KB) solution containing (mM): L-Glu 50, KCl 30, Tau 20, KH_2PO_4 30, $MgCl_2$ 1, HEPES 10, glucose 10, EGTA 0.5, pH 7.4, 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 to1.8 mmol l^{-1}. All experiments were performed within 12 h after isolation.

All procedures met the local and international guidelines on ethical use of animals and all efforts were made to minimize the number and suffering of animals used.

2.2. Assay of SOD

When myocytes were dissociated from the tissue pieces, a 1 ml aliquot of ventricular myocytes suspension was vortexed strongly to break the membranes, and the mixture was centrifuged at 4000 rpm at 4 °C for 15 min; the supernatant obtained was used for spectrophotometric assays. Total SOD activity was determined using modification of a previously described method. Briefly, the reaction mixture contained 20 μM cytochrome *c* and 50 μM xanthine in 50 mM phosphate buffer (pH 7.8) and the reaction was initiated with xanthine oxidase. SOD activity was determined by adding lysate containing 40 μg of protein to the reaction mixture. In order to standardize SOD activity, a curve was generated by plotting known concentrations of bovine SOD; the results of this enzymatic assay were shown in units of SOD (U ml^{-1}) where one unit of SOD is defined as the amount able to inhibit 50% cytochrome *c* reduction at 25 °C. Absorbance measurements were made at 550 nm with a Hitachi model U-3010 dual beam spectrophotometer (Hitachi Instruments, San Jose, CA, USA).

An experimental regime of DDC and SO_2 derivatives was established (Table 1) in order to test the activities of SOD and the sodium current. DDC concentrations (0, 10, 20, 50, 100 mM) were set out and the concentration of SO_2 derivatives was 10 μM, which was the concentration for a half-maximum increase (EC_{50}) of sodium current (I_{Na}) (Nie and Meng, 2005).

2.3. Electrophysiological techniques and data analysis

Isolated ventricular myocytes were continuously superfused with appropriate solutions in a recording chamber mounted on the stage of an inverted microscope (Olympus, Japan) at room temperature (22–24 °C). Membrane potential and whole-cell currents were recorded using the whole-cell configuration of the patch-clamp technique (Belles et al., 1987), using an Axopatch 200B patch clamp amplifier (Axon Instruments, Foster City, CA, USA). Recording pipettes, made from borosilicate

Table 1
Experimental regime of DDC and SO_2 derivatives

SO_2 derivatives (μM)	DDC (mM)				
	0	10	20	50	100
0	0 + 0	10 + 0	20 + 0	50 + 0	100 + 0
10	0 + 10	10 + 10	20 + 10	50 + 10	100 + 10