Enhanced effects of sulfur dioxide on voltage-gated potassium channels in rat vascular smooth muscle cells

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ABSTRACT

Previously we observed vasodilator effects of gaseous sulfur dioxide on rat vascular smooth muscle cells. The main purpose is to further investigate enhanced effects of sulfur dioxide on voltage-gated potassium channel. The results showed that sulfur dioxide derivatives significantly increased the outward potassium currents ($I_{KV}$) with a certain degree of voltage-dependent. 10 μmol/L sulfur dioxide derivatives made current-voltage curve significantly shift up. And sulfur dioxide derivatives increased $I_{KV}$ in a dose-dependent manner. It increased $I_{KV}$ to some extent after applying 5 mmol/L 4-aminopyridine (4-AP). Tetraethylammonium chloride (TEA) could inhibit the increased amplitude $I_{KV}$. Moreover, 10 μmol/L sulfur dioxide derivatives could significantly shift $I_{KV}$ activation curve to the hyperpolarized direction, while did not affect the slope factor. In conclusion, sulfur dioxide derivatives increased outward $I_{KV}$ amplitude and activated potassium currents in advance. This might be one of the mechanisms about the depressing blood pressure effect of sulfur dioxide and derivatives. TEA and 4-AP can antagonize the augmentation of potassium currents in vascular smooth muscle cells induced by sulfur dioxide derivatives.

INTRODUCTION

Cardiovascular system is the most important part of human body. Meanwhile cardiovascular diseases have been the deadliest killer in developed countries, and the incidence of these diseases in China has also been increased. It has been demonstrated that sulfur dioxide ($SO_{2}$) lead to some diseases such as arrhythmia, is chemic heart disease etc.[1,2]. $SO_{2}$ derivatives spoil rat myocardial cell mainly by damaging the process of free radicals oxidative, especially the process of peroxo radical oxidative[3]. Studies also showed that $SO_{2}$ could lead to vasodilatation and the drop of blood pressure[4,5,6]. $SO_{2}$ derivatives activated the signal pathway of prostacyclin-cyclic adenosine monophosphate-protein kinase A (PGI$_2$-c-AMP-PKA), thus leading to vasodilatation[7,8,9]. Additionally, at physiological concentrations and low concentrations, the vasodilatation effect of $SO_{2}$ was related to potassium channels and large-conductance calcium (BK$_{Ca}$) activated potassium channels, which was endothelium-dependent. In contrast, at high concentrations, this effect of $SO_{2}$ was related to...
adenosine triphosphate (ATP) sensitive potassium channels (K\textsubscript{ATP}) and L-type calcium channels, which is endothelium-independent\cite{10}.

In the present study, it recorded the effects of low-concentration SO\textsubscript{2} on the voltage-dependent potassium currents in rat thoracic aortic vascular smooth muscle cells (VMSCs) using the whole-cell patch-clamp technique in order to probe into the possible mechanisms.

**EXPERIMENTAL SECTION**

**Cell preparation**

Male Wistar rats, 150-250g, clean grade, provided by the Animal Center of Shan Xi Institute of Traditional Chinese Medicine. After the Wistar rats were anesthetized with pentobarbital sodium\(\text{30 mg/kg, ip}\), we cut open the chest and abdomen of rats along the midline, cut off the diaphragm, and turned over the left lung lobe to expose the thoracic aorta in front of the spine. The aorta was carefully separated and put into a culture dish containing pre-cool physiological saline solution (PPS). The surrounding connective tissue and adventitia were removed and the thoracic aorta was quickly separated and cut along the longitudinal axis in an ice bath. Then, the aorta was cultivated in calcium free PSS at room temperature for 20 min. The process above was operated under mixed gas containing 95% O\textsubscript{2} and 5% CO\textsubscript{2}. The thoracic aorta was then cut into 1 mm\(\times\)1 mm blocks, put into calcium free PSS which contained Collagenase type IV 2 g/L, Papain 4 g/L, Bovine serum albumin (BSA) 2 g/L, Dithiothreitol (DTT) 1 mmol/L, and digested in 37\(^\circ\)C water bath for 30 min. The tissue blocks were gently washed with calcium free PSS three times to terminate the enzyme digestion. To get single VSMC, the digested remains were re-suspended with low calcium PSS and gently blew with polished wide mouth Pasteur pipette for 20 min. Cell physiological activity was confirmed by observing the contraction of VSMCs after adrenaline stimulation. The separated VSMCs were stored at 4\(^\circ\)C and prepared for patch clamp experiments within 6 h.

**Experiment requiring solution**

PSS was composed with (in mmol/L): NaCl 130, KCl 5, MgCl\textsubscript{2} 1.2, CaCl\textsubscript{2} 1.5, Hepes 10, glucose 10, and pH was adjusted to 7.4 with NaOH. Calcium free PSS: besides without CaCl\textsubscript{2}, the ingredients were as same as PSS. Low calcium PSS: besides with CaCl\textsubscript{2} 20 \(\mu\)mol/L, the ingredients were the same as PSS. Extracellular fluid: calcium free PSS. Pippette Solution was compounded with (in mmol/L): KCl 125, Na\textsubscript{2}ATP 5, Na\textsubscript{2}GTP 0.5, MgCl\textsubscript{2} 3, Hepes 10, EGTA 10, TEA 1, and pH was adjusted to 7.2 with KOH.

2.5 mmol/L NaHSO\textsubscript{3} and 7.5 mmol/L Na\textsubscript{2}SO\textsubscript{3} were mixed into a 10 mmol/L SO\textsubscript{2} derivatives mother liquor and the liquor was stored in a refrigerator (0\(^\circ\)C). Before the experiment, the mother solution was diluted into the required concentration. The solution was applied to the Y-tube by vacuum pump. The distance between Y tubes and cells was about 0.2 mm; drug application rate was about 2 mL/min. To keep the drug changing process within 200 ms, the distance between Y-tube mouth and cells was kept at about 2 mm, while the vacuum degree was generally at 0.5 atm. The diameter and inclination of Y-tube was 100-200 \(\mu\)m and 30\(^\circ\)-45\(^\circ\) respectively. All chemicals unless otherwise specified were purchased from Sigma.

**Whole-cell patch-clamp recording**

Voltage-clamp recordings were made in the whole-cell patch-clamp technique. The patch-clamp electrodes were pulled by a micropipette pullers (P97 type Shutter, USA) with a tip resistance of 5-8 M\(\Omega\). The seal resistance was >1 G\(\Omega\). Neurons with an inadequate seal were excluded from analysis. Currents were filtered at 2 kHz and stored in a computer using digidata 1200B interface and pCLAMP 10.0 software (Axon Instrument). Drug application was done using a “Y-tube method” and the corresponding signal was collected again in 1 min after giving drug.

**Statistical methods**

Data was analyzed using of pCLAMP CLAMPFIT procedures (Axon Instrument) and Origin8.0 Software (Microal software, USA). Results were presented as mean±S.D. and statistical comparisons were made using the paired Student’s t-test. The activation and inactivation curves of potassium current were fitted with the Boltzmann equation of the form: 

\[ I / I_{\text{max}} = [1+\exp (V_{1/2} - V_c)/k]^{-1}. \]

where \(I_{\text{max}}\) is the maximal conductance, \(V_{1/2}\), the voltage at which the current is half activated or in-
activated, and \( k \) is the slope factor describing the slope of the curves. Dose-effect curves were fitted by Logistic equation: 
\[
y = \frac{(A_1 - A_2)}{[1 + (x/x_0)^p]} + A_2, 
\]
where \( A_1 \) is the final value of \( SO_2 \) increase, \( A_2 \) is the initial value of \( SO_2 \) increase, \( x_0 \) is half effective concentration (EC\(_{50}\)), and \( p \) is the power.

**RESULTS AND DISCUSSION**

**Record of potassium current of VSMCs**

Potassium currents (\( I_{Kv} \)) were elicited by a 400 ms depolarizing pulses from -40 mV to +60 mV, with an increment of 10 mV at a holding potential of -70 mV. To minimize the activation of potassium channels by intracellular calcium, calcium-free electrode internal fluid and calcium-free PSS (contains 1 mmol/L TEA) perfusion were adopted and intracellular calcium was chelated with 10 mmol/L EGTA. In addition, the electrode internal fluid contains 5 mmol/L \( Na_2ATP \), which suppresses \( K_{ATP} \) current. In this condition, the recorded current was activated with a membrane potential above-40 mV, while the inactivation at 400 ms was not significant (Figure 1A). Adding 5 mmol/L 4-AP (main blocking agent of \( I_{Kv} \)) after the formation of whole-cell recording showed significantly inhibitory effect (Figure 1B) on the outward currents at +60 mV recording voltage, with a (63.4±4.7)% \((P<0.01, n=10)\) blockage of peak current, while the current mostly recovered after the blocking agent was eluted, which indicated that the recorded currents should be \( I_{Kv} \).

**The effect of \( SO_2 \) derivatives on potassium current of VSMCs**

First of all, the outward potassium current (\( I_{Kv} \)) under normal circumstances was recorded after the formation of the whole-cell state. Then, 1, 10, 100 \( \mu \)mol/L \( SO_2 \) derivatives were respectively added to the dishes and then the current above was recorded again after 1 min. The result showed that \( SO_2 \) derivatives increased \( I_{Kv} \) significantly (Figure 2B). Meanwhile, we recorded the \( I_{Kv} \) changes after adding 4-AP. When the whole-cell state was formed, significant reduction (Figure 2E) of current was observed after adding 5 mmol/L of 4-AP, while the current increased after applying 10 \( \mu \)mol/L \( SO_2 \) derivatives, which was shown in Figure 2F. After eluting with standard extracellular solution and adding 10 \( \mu \)mol/L \( SO_2 \) derivatives, the \( I_{Kv} \) was found to be significantly increased (Figure 2G) compared to the control group. The \( I_{Kv} \)-\( V \) curves of control group and 10 \( \mu \)mol/L \( SO_2 \) derivative group were fitted by current amplitudes of every pulse and their corresponding membrane potentials. 10 \( \mu \)mol/L \( SO_2 \) derivatives significantly shifted up the \( I-V \) curve (Figure 2C), which indicated the increment of \( I_{Kv} \), with certain voltage dependence. \( SO_2 \) derivatives increased \( I_{Kv} \), showing dose-
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**Figure 2**: The increscent effects of SO$_2$ derivatives on potassium current in VSMCs (A) Control of Test one; (B) 10 μmol/L SO$_2$ derivatives; (C) I-V graph; (D) Control of Test two; (E) 5 mmol/L 4-AP; (F) 5 mmol/L 4-AP and 10 μmol/L SO$_2$ derivatives; (G) After washout 5 mmol/L 4-AP and 10 μmol/L SO$_2$ derivatives and only add 10 μmol/L SO$_2$ derivatives.

dependent manner (Figure 3A). The dose-response curve in the Figure could be well fitted by Logistic equation, and the EC$_{50}$ for a 50% increase of $I_{Kv}$ was thereby calculated to be probably 41 μmol/L SO$_2$ derivatives.

To study the effect of SO$_2$ derivatives on activation of $I_{Kv}$, the cells were stimulated with the same voltage above and current activation curves were fitted by Boltzmann equation. The graph showed that after adding 10 μmol/L SO$_2$ derivatives, the $I_{Kv}$ activation curve significantly shift to the hyperpolarization direction, while the slope factor did not change (Figure 3B). As shown in Figure 3, before and after application of 10 μmol/L
SO_2_ derivatives, the activation curves were both S-shaped, and the V_{1/2} of both groups was (15.8±1.3) mV and (-4.8±1.0) mV (P<0.05, n=11), with k of (33.7±1.8) mV and (29.8±1.8) mV (P>0.05, n=11) respectively. It indicated that 10 μmol/L SO_2_ derivatives significantly moved the activation curve of I_{Kv} to the left, while did not change its slope.

The effect of TEA on the increase of I_{Kv} caused by SO_2_ derivatives:

TEA, a blocking agent of delayed rectifier potassium current channel, can inhibit the outward potassium current of VSMCs. When the cell was clamped at -70mV and stimulated as mentioned above, the outward potassium current increased significantly by adding 10 μmol/L SO_2_ derivatives, while this current was reduced from (2619.9±754) pA to (1865.62±609) pA (Figure 4) after adding 20 mmol/L TEA, with an inhibitory rate of (28.8±23.2)%. The significant difference indicated that TEA inhibited the increase of I_{Kv} caused by SO_2_ derivatives.

Angiostasis which is an important determinant for the peripheral resistance of circulatory system mainly comes from the contraction of VSMCs. Moreover, the ion channels play a central role in regulating the contraction activity of VSMCs[11]. The membrane potential is significantly affected by the ion channels on the membrane. Present studies have shown that VSMCs expressed one–two kinds of voltage-gated calcium channels and four kinds of potassium channels. These ion channels were involved in the regulation of angiostasis through regulating VSMCs membrane potential and intracellular calcium changes. The potassium channel’s opening and closing impose a significant effect on membrane potential and angiostasis. In details, the opening of potassium channel hyperpolarizes the cellular membrane while the closing of it depolarizes the membrane.

Voltage-gated calcium channel plays a central role...
in the regulation of angiotaxis[14]. The opening and closing of voltage-gated calcium channels is controlled by the membrane potential. Membrane hyperpolarization closes the channel and causes vasoconstriction, while membrane depolarization opens the channel and leads to vasoconstriction. Thus, potassium channel regulates angiotaxis through affecting the membrane potential and voltage-gated calcium channel activity. Potassium channels opening prompts the increment of membrane potential and the hyperpolarizes the membrane, and thus reduces the activity of vascular smooth muscle and vascular tension. Conversely, potassium channel closing increases angiotaxis. SO\textsubscript{2} derivatives increased the amplitude of voltage-dependent potassium current on VSMCs and brought forward the activation curve. SO\textsubscript{2} derivatives also pre-activated the potassium channel, increased the conductivity of potassium channel and hyperpolarized the cell, which inhibited cell excitability. It regulated the membrane excitability through affecting potassium channels and reduced the depolarization speed of membrane. As a depolarization damper, it inserted an intermittent time between the action potentials. Thus, the duration of “contraction-relaxation” action of vascular smooth muscle was prolonged and vascular smooth muscle tension was reduced, leading to depressing blood pressure, which might be one of the mechanisms of vasoconstriction caused by SO\textsubscript{2} and its derivatives. Our result was consistent with the facts that SO\textsubscript{2} derivatives could reduce blood pressure[4,6,10] which had been proved by many studies.

CONCLUSION

In conclusion, SO\textsubscript{2} derivatives affected the rat thoracic aortic VSMCs, increasing the amplitude of the outward potassium current and pre-activating the potassium current. Potassium channel opening hyperpolarizes the cell membrane and shuts down the voltage-gated calcium channel. Thus the calcium ion channel closing caused vasodilation and depressed blood pressure, which was one of the mechanisms of the antihypertensive effect of SO\textsubscript{2} and its derivatives.

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REFERENCES

pressure associates with the remodelling of inward rectifier K+ channels in mice mesenteric vascular smooth muscle cells. J. Physiol. 590(Pt 23), 6075-91 (2012).