Effects of sulfur dioxide and its derivatives on the functions of rat hearts and their mechanisms

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Abstract

The present study was designed to investigate the mechanisms underlying the effects of SO\textsubscript{2} on functions of the isolated perfused hearts in rats. The results suggest that both SO\textsubscript{2} and SO\textsubscript{2} derivatives (sulfite: bisulfite, 3:1, M/M) elicited a negative inotropic effect. At high concentrations, the effects of SO\textsubscript{2} or its derivatives on heart functions might be related to the increasing of reactive oxygen species (ROS) contents and decreasing of ATPase activities as well as the potentially damaging effects on the hearts; while at low concentrations, SO\textsubscript{2} or its derivatives might modulate heart functions mainly through the NO signal transduction pathway.

Keywords: sulfur dioxide; SO\textsubscript{2} derivatives; heart function; inotropic effect

1. Introduction

The epidemiological studies have revealed that SO\textsubscript{2} exposure is linked to cardiovascular diseases \cite{1, 2}. In recent years, our studies indicated that both SO\textsubscript{2} and SO\textsubscript{2} derivatives (sulfite: bisulfite, 3:1, M/M) could lower rat blood pressure and promote a concentration-dependent vasodilatation in the isolated rat aorta \cite{3-5}. SO\textsubscript{2} showed a higher vasorelaxant effect than SO\textsubscript{2} derivatives. In our previous studies, the EC\textsubscript{50} values of vasorelaxation effects induced by SO\textsubscript{2} derivatives and SO\textsubscript{2} were 7.28 ± 0.12 mM and 1.25 ± 0.10 mM, respectively \cite{5-8}. In addition, by using the whole cell patch-clamp technique, we investigated the effects of SO\textsubscript{2} derivatives on the voltage-dependent calcium current and potassium current in the isolated adult rat ventricular myocyte. Results showed that SO\textsubscript{2} derivatives might cause cardiac myocyte injury through...
the increase of intracellular calcium by modulating voltage-gated calcium channels and increase of extracellular potassium by modulating voltage-gated potassium channels [9, 10]. SO₂ also could cause DNA damage in heart cells [11].

Moreover, we carried out another study to identify the roles of Ca²⁺ and K⁺ channels in the negative inotropic effects of SO₂ on isolated hearts in rats. The results demonstrated that the negative inotropic effect induced by SO₂ might be related to the ATP-sensitive K⁺ (K<sub>ATP</sub>) channel and L-type Ca²⁺ channel [12]. However, the roles of the NO signal transduction pathway, reactive oxygen species (ROS) levels and ATPase activities in the effects of SO₂ on rat hearts remains unknown. Therefore, we did this study to investigate the roles of the NO signal transduction pathway, ROS contents and ATPase activities in the effects of SO₂ or SO₂ derivatives on hearts functions of the isolated rat hearts.

2. Material and methods

2.1. Chemicals and solution preparation

Sodium bisulfite, sodium sulfite were purchased from Sigma (St. Louis, MO, USA). Assay kits for NO, NO synthase (NOS), Adenosinetriphosphatase (ATPase), reduced glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), anti-superoxide anion radical (O₂⁻), hydroxyl radical (OH), and hydrogen peroxide (H₂O₂) were from Nan Jing Jian Cheng Company of Biological Technology (Nanjing, China). Other chemical reagents were of analytical grade.

To prepare the SO₂ solution, pure SO₂ gas (purity: 99.99%) obtained from the Beijing He-Pu-Bei-Fen Gas Company, Ltd. (Beijing, China) was used. SO₂ solution was freshly prepared before each experiment by bubbling saline with the pure SO₂ gas to achieve a stock solution containing SO₂ at concentrations ranging from 6.0 to 91 mM. Previous experiments indicated that the concentration of SO₂ in the stock solution and the Krebs-Henseleit (K-H) buffer was relatively stable. SO₂ derivatives were sodium sulfite and sodium bisulfite neutral solution (about 3:1, M/M) [13].

2.2. Preparation of isolated rat hearts

The isolated rat hearts were prepared as described previously [12]. Male Wistar rats, weighing about 220–250 g, were used. The hearts were quickly excised and perfused on a Langendorff apparatus with a modified K-H buffer under a 100cm H₂O pressure at 37°C, gassed with 95% O₂–5% CO₂. Heart rate, coronary flow, left ventricular developed pressure (LVDP=left ventricular systolic pressure-left ventricular diastolic pressure) and maximal of left ventricular pressure development (±LVdp/dt<sub>max</sub>) were measured by a MedLab Biological Signal Collection System (Medease Science and Technology, Nanjing, China) with the left ventricular diastolic pressure pro-stabilized at 10 mmHg.

2.3. Inotropic effect of SO₂ or SO₂ derivatives in the rat hearts

To study the negative inotropic effects of SO₂ or SO₂ derivatives in the isolated perfused rat hearts, SO₂ or SO₂ derivatives (10, 300 and 1000 μM) were added into the perfused fluid. The concentration of SO₂ in the K-H buffer in the Langendorff apparatus was measured using pararosaniline hydrochloride spectrophotometry [14]. The parameters of cardiac function in the various groups were measured.

2.4. Preparation of myocardial tissue and measurement of biochemical indexes

Rat hearts were allowed to stabilize for 20-30 min, and then SO₂ or SO₂ derivatives (10, 300 and 1000
µM) were added into the perfused fluid for 10 min. The hearts were quickly frozen in liquid nitrogen. Then the hearts were used for measuring the levels of GSH, MDA, O₂⁻, OH, H₂O₂, NO, protein and activities of SOD, ATPase, NOS.

2.5. Statistical analysis

All values were expressed as mean ± standard deviation. Student’s t-test for unpaired samples was used to compare the mean values between the control and tested groups. Multiple comparisons were made with one-way ANOVA followed by a post hoc analysis (Tukey’s test). Statistical significance was set at P < 0.05.

3. Results

3.1. The negative inotropic effects of SO₂ or SO₂ derivatives

Table 1 presents the changes in heart rate, coronary flow, LVDP and ±LVdp/dt max of the isolated hearts exposed to increasing concentrations of SO₂ or its derivatives. Both SO₂ and SO₂ derivatives elicited a negative inotropic effect in a concentration-dependent manner with the maximum response shown at the highest concentration tested (1000 µM). SO₂ caused a higher negative effect than the SO₂ derivatives. SO₂ decreased the LVDP by around 50%, while the SO₂ derivatives decreased the LVDP by around 17% at 1000 µM. Heart rate was significantly decreased by SO₂ at 1000 µM, but SO₂ derivatives had no significant effect on heart rate of the treated hearts over the concentration range applied. Coronary flow did not change in response to SO₂ at 10 µM, but significantly increased when the high concentrations (300 and 1000 µM) of SO₂ were infused. The similar results were seen when the hearts were treated with SO₂ derivatives, and the coronary flow significantly increased when the high concentration (1000 µM) of SO₂ derivatives were infused (Table 1). The perfusion with SO₂ or SO₂ derivatives to the isolated rat hearts obviously inhibited the ±LVdp/dt max in a concentration-dependent manner. Cardiac function recovered rapidly after the perfusion of SO₂ or SO₂ derivatives stopped (>90% of original values, the data were not shown).

Table 1. Alterations of functional parameters before and after different concentrations SO₂ or SO₂ derivatives perfusion in vitro.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>10 µM</th>
<th>300 µM</th>
<th>1000 µM</th>
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</thead>
<tbody>
<tr>
<td><strong>SO₂</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>248.3±12.36</td>
<td>244.29±14.29</td>
<td>237.15±10.28</td>
<td>226.82±19.25*</td>
</tr>
<tr>
<td>CF</td>
<td>12.28±0.79</td>
<td>12.14±1.52</td>
<td>12.89±0.82*</td>
<td>14.77±1.18*</td>
</tr>
<tr>
<td>LVDP</td>
<td>98.89±5.23</td>
<td>90.78±3.21**</td>
<td>79.63±2.34***</td>
<td>49.07±4.29***</td>
</tr>
<tr>
<td>+dP/dt max</td>
<td>3429.2±136.4</td>
<td>3165.3±116.3**</td>
<td>2836.6±145.8***</td>
<td>1926.9±102.7***</td>
</tr>
<tr>
<td>-dP/dt max</td>
<td>-2012.3±113.8</td>
<td>-1816.4±102.6***</td>
<td>-1649.4±122.7***</td>
<td>-1069.3±113.9***</td>
</tr>
<tr>
<td><strong>SO₂ Derivatives</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>246.73±16.73</td>
<td>249.35±15.27</td>
<td>239.89±11.87</td>
<td>243.72±13.88</td>
</tr>
<tr>
<td>CF</td>
<td>11.97±1.43</td>
<td>12.21±1.37</td>
<td>12.21±0.97</td>
<td>13.57±1.57*</td>
</tr>
<tr>
<td>LVDP</td>
<td>101.03±4.97</td>
<td>95.39±2.97**</td>
<td>89.21±2.99**</td>
<td>83.24±3.77**</td>
</tr>
<tr>
<td>+dP/dt max</td>
<td>3367.7±129.3</td>
<td>3257.9±105.4''</td>
<td>3086.8±114.4''</td>
<td>2643.8±105.1***</td>
</tr>
<tr>
<td>-dP/dt max</td>
<td>-1977.6±104.1</td>
<td>-1875.6±115.7''</td>
<td>-1724.3±109.6''</td>
<td>-1479.7±96.2''***</td>
</tr>
</tbody>
</table>

Note: Values were means ± SD (n=6), *P < 0.05, **P < 0.01, ***P < 0.001, compared with the control. HR, heart rate; CF, coronary flow; LVDP, left ventricular developed pressure.
3.2. Effects of SO\textsubscript{2} or SO\textsubscript{2} derivatives on the GSH, MDA contents and SOD activities in perfused hearts

The GSH levels, MDA contents and SOD activities in the rat hearts perfused by SO\textsubscript{2} and SO\textsubscript{2} derivatives were assessed (Fig. 1). The GSH levels and SOD activities in the hearts were significantly decreased by SO\textsubscript{2} (10, 300 and 1000 µM) or its derivatives (1000 µM). At the concentrations of 300 and 1000 µM, SO\textsubscript{2} caused a larger decrease of the GSH content than SO\textsubscript{2} derivatives. However, the MDA contents in the hearts were significantly increased by SO\textsubscript{2} (10, 300 and 1000 µM) or its derivatives (1000 µM). At the concentrations of 300 and 1000 µM, MDA contents were significantly higher in the SO\textsubscript{2} group than the SO\textsubscript{2} derivatives group.

3.3. Effects of SO\textsubscript{2} or SO\textsubscript{2} derivatives on the \textit{O}_2\textsuperscript{•−}, \textit{OH}, \textit{H}_2\textit{O}_2 formation in perfused hearts

SO\textsubscript{2} at the concentrations of 300 and 1000 µM significantly increased the \textit{OH} and \textit{H}_2\textit{O}_2 levels and decreased \textit{O}_2\textsuperscript{•−} levels in the isolated perfused hearts. At the concentrations of 300 and 1000 µM, SO\textsubscript{2} derivatives significantly increased the \textit{H}_2\textit{O}_2 levels in the isolated perfused hearts. In addition, SO\textsubscript{2} derivatives at 1000 µM significantly increased the \textit{OH} levels and decreased the \textit{O}_2\textsuperscript{•−} levels (data not shown).

3.4. Effects of SO\textsubscript{2} or SO\textsubscript{2} Derivatives on the Activities of NOS, ATPase and the Levels of NO in Perfused Hearts

The total NOS (tNOS) and inducible NOS (iNOS) activities in the hearts were significantly increased by increasing concentrations of SO\textsubscript{2} or its derivatives. At the concentrations of 10 µM, SO\textsubscript{2} caused a larger increase of the tNOS and iNOS activities than SO\textsubscript{2} derivatives (Fig. 2).
Fig. 3 depicts the Na⁺K⁺-ATPase and Ca²⁺Mg²⁺-ATPase activities in the rat hearts perfused by SO₂ or SO₂ derivatives. The Na⁺K⁺-ATPase activities in the hearts were significantly decreased by SO₂ at 300 and 1000 µM, whereas the Na⁺K⁺-ATPase activities were significantly decreased by SO₂ derivatives at only 1000 µM. At the concentration of 1000 µM, both SO₂ and SO₂ derivatives significantly decreased the Ca²⁺Mg²⁺-ATPase activities in the hearts.

![Fig. 3. Effects of SO₂ or SO₂ derivatives on the activities of Na⁺K⁺-ATPase (A) and Ca²⁺Mg²⁺-ATPase (B) in myocardium of the isolated hearts. Compared with control group, *P < 0.05.](image)

The NO levels were assessed in the rat hearts perfused by SO₂ or SO₂ derivatives (Fig. 4). The NO levels in the hearts were significantly increased by increasing concentrations of SO₂ or its derivatives. In addition, SO₂ at 10, 300 and 1000 µM caused a larger increase of the NO levels than SO₂ derivatives.

![Fig. 4. Effects of SO₂ or SO₂ derivatives on the levels of NO in perfused rat hearts. Compared with control group, *P < 0.05; compared with SO₂ derivatives group at the same concentration, # P < 0.05.](image)

4. Discussion

In this study, we utilized the isolated perfused heart model to elucidate the effects of SO₂ or SO₂ derivatives on the heart functions. The results showed that both SO₂ and SO₂ derivatives elicited a negative inotropic effect in a concentration-dependent manner. SO₂ induced a higher negative effect than the SO₂ derivatives. For example, SO₂ decreased the LVDP by around 50%, while SO₂ derivatives decreased the LVDP by around 17% at 1000 µM. In addition, heart rate was significantly decreased by SO₂ at high concentration (1000 µM), but SO₂ derivatives had no significant effect on heart rate of the treated hearts over the concentration range applied (Table 1). The different effects of SO₂ and SO₂ derivatives on the perfused rat hearts might be resulted from the different chemical property of them. In
SO$_2$ solution, SO$_2$ exists in water mostly in a state of SO$_2$-nH$_2$O, and only a little HSO$_3^-$ is found [15]. Therefore, the negative effect caused by exposure to SO$_2$ solution was actually the effect of SO$_2$ molecules on the perfused hearts. In contrast, SO$_2$ derivatives are the mixture of sulfate and bisulfite (in neutral solution 3:1, M/M), which are the product of SO$_2$ metabolism in the body. SO$_2$ and SO$_2$ derivatives at high concentrations could significantly increase coronary flow, which might be due to the vasorelaxant effects of SO$_2$ and SO$_2$ derivatives on the coronary artery [5-8]. Our results are consistent with the previous report. For example, Zhang et al. reported that SO$_2$ derivatives had a negative inotropic effect on the isolated perfused rat hearts and suggested that the mechanism of SO$_2$ derivatives-induced negative inotropic effects might be related to the voltage-gated calcium channel [16].

In normal conditions, cells cope with ROS using such enzymatic and nonenzymatic defenses as SOD, GSH, and so on. The decreases of SOD activities might predispose the various organs to increase ROS damage, because SOD can catalyze decomposition of superoxide radicals to produce H$_2$O$_2$. SOD is considered as the first line of defense against oxygen toxicity [17]. GSH is a key component of the defense cascade against injury caused by ROS, and the tissue concentration of GSH serves as an indicator of oxidative stress. The level of MDA has been shown to be a good indicator of endogenous lipid peroxidation [18]. Lipid peroxidation is known to have deleterious effects on structure and functions of cell membrane. Recent studies have found that although ROS are involved in pathology, they are also integral to modulating functional responses. ROS are now being recognized as important regulators of cell function by altering the redox state of proteins. They are believed to interact with cell signalling pathways by way of modification of key thiol groups on proteins that possess regulatory functions. These proteins may be second messengers such as serine/threonine, tyrosine and MAP kinases, growth factors and transcription factors such as NF-kB [19, 20]. In addition, it is now recognized that cardiac ion channel function is regulated by ROS [21, 22]. In the present study, SO$_2$ or its derivatives at high concentrations could significantly decrease the GSH, O$_2^.$ contents, SOD activities and increase the MDA, OH, H$_2$O$_2$ contents in the isolated perfused hearts. Similarly, some previous studies also demonstrated that one-electron oxidation of bisulfite produced the sulfur trioxide radical anion, which reacted rapidly with molecular oxygen in cells to form a highly biologically reactive peroxyl radical [23, 24]. All these results indicate that the negative inotropic effects induced by SO$_2$ or its derivatives at high concentrations are probably related to the increasing of ROS levels in isolated hearts. And the increasing of ROS levels further regulated the cardiac ion channel functions and heart functions. Of course, SO$_2$ or its derivatives might have an oxidative damage effect on the isolated hearts at high concentrations.

The NO levels and NOS activities in the hearts were significantly increased by different concentrations of SO$_2$ or its derivatives. These results strongly suggested that the NO signal transduction pathway played a significant role in the effects that SO$_2$ and SO$_2$ derivatives-mediated. A possible mechanism underlying the effects was that NO production exerted a negative inotropic effect which was mediated by the further production of cGMP. These results are consistent with our previous report in which the cGMP levels in the hearts were significantly increased by different concentrations of SO$_2$ or SO$_2$ derivatives [12]. Similarly, some studies have shown that a large increase in cGMP level elicits a depression of contractility, which is mediated by activation of cGMP-dependent protein kinase, in isolated rat ventricular myocytes [25, 26].

Ca$^{2+}$Mg$^{2+}$-ATPase and Na$^+$K$^+$-ATPase on plasma membrane play important roles in maintaining the normal transport state of positive ions such as Na$^+$, K$^+$ and Ca$^{2+}$. Inhibition of them may lead to the elevation of plasma membrane permeability for positive ions, possibly resulting in the increase of extracellular K$^+$ concentration and abnormal change of cytosolic Ca$^{2+}$ concentration [27]. In the present study, SO$_2$ or its derivatives at the concentration of 1000 $\mu$M significantly decreased the Na$^+$K$^+$-ATPase and Ca$^{2+}$Mg$^{2+}$-ATPase activities in the hearts. In a previous study, we also found that the negative inotropic effects of SO$_2$ and SO$_2$ derivatives at high concentrations could be partially inhibited by a K$\text{ATP}$
channel inhibitor [12]. All these results suggested that the negative inotropic effects of SO\textsubscript{2} or its derivatives at high concentrations were partially achieved by increasing the extracellular K\textsuperscript{+} concentration. The opening of the K\textsubscript{ATP} channel is associated with the potassium efflux, polarization of cell membrane and shortening of action potential duration [28]. These effects reduce Ca\textsuperscript{2+} influx via L-type Ca\textsuperscript{2+} channels and decrease the time that the Na\textsuperscript{+}-Ca\textsuperscript{2+} exchange operating in the reverse mode. The resultant decrease in Ca\textsuperscript{2+} influx would be expected to lead to a reduction of the cardiomyocyte contraction, inducing the negative inotropic effect of SO\textsubscript{2} or its derivatives.

5. Conclusion

We have shown that both SO\textsubscript{2} and its derivatives elicited a negative inotropic effect and SO\textsubscript{2} produced a higher negative inotropic effect than SO\textsubscript{2} derivatives on isolated perfused rat hearts. The mechanisms of the effects of SO\textsubscript{2} and its derivatives on heart function at high concentrations were different from those at low concentrations. At high concentrations, the effects of SO\textsubscript{2} or its derivatives on heart function might be related to the increasing of ROS levels and decreasing of ATPase activities as well as the damage effect on the hearts; while at low concentrations, SO\textsubscript{2} or its derivatives might modulate heart functions mainly through the NO signal transduction pathway.

Acknowledgements

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