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Sulfur dioxide derivatives increase a hyperpolarization-activated inward current in dorsal root ganglion neurons

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Abstract

The effect of derivatives of sulfur dioxide (SO_2), a common air pollutant, which exists in vivo at equilibrium between bisulfate and sulfite, was studied on hyperpolarization-activated cation current (I_h) in cultured post-natal dorsal root ganglion (DRG) neurons using the whole cell configuration of patch-clamp technique. SO_2 derivatives increased I_h current in a dose and voltage-dependent manner. The EC_{50} value was 25 μM and the Hill coefficient was 1.44. 50 μM SO_2 derivatives significantly shifted the activation curve of I_h in the hyperpolarizing direction by 5.5 mV. The reversal potential of I_h was shifted to 5.2 mV in positive direction by 10 μM SO_2 derivatives. According to the functional role of I_h, the increase of I_h should result in an enhanced neuronal excitability, which was possibly the basis for neuropathic pain.

Keywords: Sulfur dioxide derivatives; Hyperpolarization-activated cation current(I_h); DRG; Rat

SO_2 is one of the most common air pollution, resulting into the atmosphere from the combustion of fossil fuel. Previous studies had demonstrated that SO_2 is a systemic toxic including neurotoxic material (Meng, 2003; Shapiro, 1977). For example, SO_2 inhalation may cause changes of oxidative stress and antioxidation status in various organs of mice, damage the brain of rats and guinea pigs, induce apoptosis of mouse spleen lymphocytes, and bring about lipid peroxidation, which is believed to be involved in several disease states, such as diabetes and neurodegenerative diseases as well as the aging process. In addition, SO_2 derivatives can alter cell's excitability by increasing the frequency firing of potassium channels in hippocampal neurons and ventricular myocytes (Du and Meng, 2004a,b; Nie and Meng, 2005a,b). SO_2 derivatives can enhance somatic nociceptive signals through the TTX-resistant sodium channels and mediate nociceptive information triggered by high-threshold afferents (Du and Meng, 2004a,b,2006).

The hyperpolarization-activated cation current (I_h) consists of a slow, non-inactivating inward current carried by K^+ and Na^+ which is modulated by internal cAMP as well as by external K^+ (Banks et al., 1993; Surges et al., 2002). I_h is an important contributor to the rhythmic (or pacemakers) activity in neurons and heart cells. The presence of I_h has been described in different neurons (Gabriel et al., 1998; Wang et al., 1997). It was reported that I_h plays an important role in the control of electrical activity in dorsal root ganglion (DRG)

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neurons during hyperpolarization (Wang et al., 1997). I_h was inhibited by Cs^+ and ZD7288 (Harris and Constanti, 1995; Satoh and Yamada, 2000) and 1 mM Ba^{2+} could block the anomalous rectifier K^+ current which express in I_h. However, little is known about the effects of sulfur dioxide derivatives on I_h. Therefore, the principal purpose of the present study was to determine the effects of acute application of sulfur dioxide derivatives on I_h and discuss possible mechanisms underlying the SO_2 derivatives action.

1. Experimental procedures

Dorsal root ganglion neurons were prepared from 10 to 16 days Old Wistar rats of either sex. Animals were killed according to institutional and national guidelines. All experiments conformed to local and international guidelines on ethical use of animals and all efforts were made to minimize the number of animal used and their sufferings. The thoracic and lumbar segments of the vertebrate column were removed and cut longitudinally. The dorsal root ganglia were immediately incubated in the fresh Dulbecco's modified Eagle's medium (DMEM Gibco) solution, which was added with 1 mg/ml type/collagenase (Sigma) for 50 min at 32 °C and then in a solution of 0.5 mg/ml type XI trypsin (Sigma) for 30 min. After enzyme treatment, ganglia were rinsed three times with DMEM solution, and the ganglia were transferred to standard external solution. Single cells were mechanically dissociated by triturating with a series of fire-polished Pasteur pipettes. DRG neurons were placed in dishes. Fifty minutes later, when the neurons were attached to the bottom, they were used for recordings at room temperature (20–25 °C).

External solution contained (in mM): 140 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2{\cdot}6H_2O$, 10 HEPES, 10 D-glucose and 0.5 μM tetrodotoxin, pH 7.4. Electrodes were filled with (in mM): 140 KCl, 4 NaCl, 2 Na_2-ATP, 1 $MgCl_2{\cdot}6H_2O$, 10 EGTA and HEPES, pH 7.2. We dissolved 2.5 mM $NaHSO_3$ and 7.5 mM Na_2SO_3 in distilled water at a concentration of 10mMderivatives (pH 7.4) for only a stock solution and added the diluted solution to the external solution just before each experiment. All chemicals unless otherwise specified were purchased from Sigma. All experiments were carried out at room temperature (20–25 °C). Drug application was done by using a "Y-tube method" and the outlet tip of Y-tube (0.1 mm diameter) was set about 0.2 mm away from the neuron under recording. The solution was applied to the Y-tube by vacuum pump.

Patch electrodes were made by a PP-830 micropipette puller (Narishige, Japan) with resistance of 3–6 MΩ. Membrane currents were digitized at 0.5 ms/sample, filtered at 2 kHZ during voltage clamp experiments. (Digidata 1200, Axon Instruments). When whole-cell configuration was established, the capacitance and series resistance were compensated. Leakage was subtracted on line. Neurons with an inadequate seal were excluded from data analysis. Data analysis was performed by using Origin 6.0 (Microcal software, Inc. USA).

To examine dose dependence of the SO_2 derivatives-increase currents, experimental data were fitted with the logistic equation: $I = (I_1 - I_0)/[1 + (X/X_0)^p] + I_0$, where I_0 is initial value of sulfur dioxide (SO_2) derivatives increase, I_1 the final value of sulfur dioxide (SO_2) derivatives increase, X_0 corresponds to the EC_{50} value and p is the Hill coefficient.

Activation curves were plotted as a function of the membrane potential and fitted with the following Boltzmann equation: $I/I_{max} = \{1 + \exp[(V - V_{1/2})/k]\}^{-1}$, where I_{max} is fitted maximal current amplitude. $V_{1/2}$ and k represent the voltage at which the current is half activated and slope factor of the I–V curve around the point $V_{1/2}$, respectively.

Results were presented as means ± S.D. and statistical comparisons conducted by using the paired Student's t-test, and probabilities less than 0.05 were considered significant.

2. Results

The effect of SO_2 derivatives on the membrane currents was examined in cultured 30–40 μm-diameter DRG neurons. The hyperpolarization-activated cation current (I_h) was evoked by 800 ms voltage step to −150 mV in 15 mV increments from a holding potential of −60 mV. We recorded I_h in 84.3% of medium-size DRG neurons and it was comprised of an initial instantaneous inward current (I_{ins}) and a subsequent slow activating inward current (I_{ss}). The amplitude of I_h was determined by subtracting the instantaneous current amplitude at the beginning of the voltage step from the steady state current at the end of the step. I_h displayed reproducible time and voltage-dependent activation patterns similar to those described by Mayer and Westbrook (1983). The amplitude of I_h was significantly increased after application of 10 μM SO_2 derivatives for 5 min at least. Augmentation of SO_2 derivatives led to a substantial and voltage-dependent increase in I_h (Fig. 1.), which was (89 ± 6)% reversible after washout.

Local application of SO_2 derivatives rapidly increased the H current and the increase was dose-dependent, as shown in Fig. 2. EC_{50} on I_h was approximately 25 μM, and the Hill coefficient was 1.44.

We analyzed the current–voltage relationship between I_{ins} and I_{ss} corresponding to membrane potential in control and in the presence of 50 μM SO_2 derivatives. Sulfur dioxide derivatives increased I_{ss} significantly, but had little effect on I_{ins} (Fig. 3A). The current–voltage relationship showed that the amplitude of I_h increased with the increasing amplitude of the command potential, especially from −75 to −150 mV (Fig. 3B).

The time course of activation was best fitted with the sum of two exponential terms. The short time constant τ_{short} as well as the long time constant τ_{long} were voltage dependent. In the absence of SO_2 derivatives, the τ_{short}

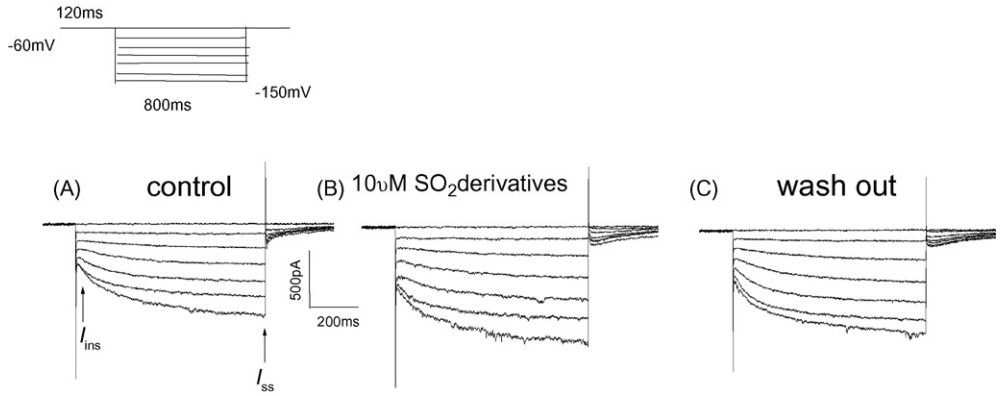

Fig. 1. SO_2 derivatives increases the amplitude of I_h. (A) In voltage-clamp mode, whole-cell currents of a medium-sized (32 μM diameter) DRG neuron were recorded in response to 800 ms voltage steps from a holding potential of −60 mV in 15 mV increments (left). Arrows indicate the positions where I_{ins} and I_{ss} were measured. (B) After application of 10 μM SO_2 derivatives, the amplitude of I_h was enhanced.

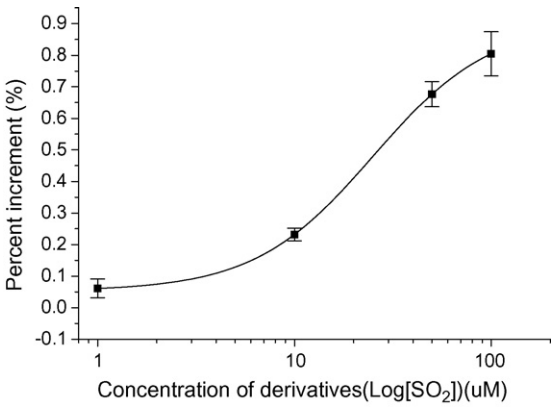

Fig. 2. Dose-response curves for the effects of SO_2 derivatives on I_h. Percent increment values were plotted against the SO_2 derivatives dose, fitted with the Hill function. Each point represents mean ± S.D. ($n = 23$). The EC_{50} value was 25 μM and the Hill coefficient was 1.44.

value was 191 ± 17 ms and 41 ± 6 ms ($n = 10$) and τ_{long} value was 600 ± 37 ms and 181 ± 13 ms at −75 mV and −150 mV, respectively. In comparison, in the presence of 50 μM SO_2 derivatives, the τ_{short} value was 206 ± 26 ms and 37 ± 7 ms ($n = 10$) and τ_{long} value was 630 ± 44 ms and 167 ± 16 ms at the same final voltages. Thus, 50 μM SO_2 derivatives did not significantly alter the I_h activation process at hyperpolarizing voltage ranging from −75 to −150 mV ($n = 10$; $P > 0.05$).

To show the voltage dependency of I_h activation, the current amplitudes were normalized to the maximal amplitude and the resulting data points were fitted with Boltzmann equation (Fig. 4). Under control conditions $V_{1/2}$ and k were −97.7 ± 1.1 mV and 15.1 ± 1.2, respectively ($n = 13$). After application 50 μM SO_2 derivatives $V_{1/2}$ and k were −103.2 ± 1.7 mV ($P < 0.05$) and 14.9 ± 1.9 ($P > 0.05$), respectively. Thus, SO_2 derivatives produced a 5.5 mV hyperpolarizing shift in the

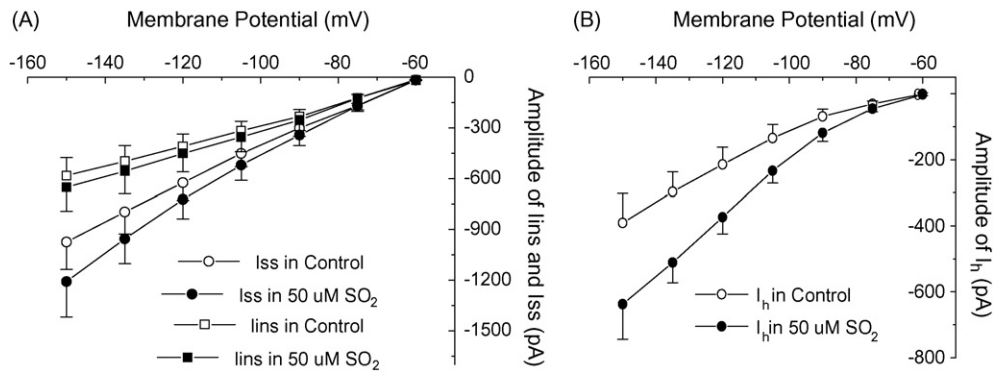

Fig. 3. Voltage-dependent effects of the SO_2 derivatives on I_h. (A) Plots of I_{ins} and I_{ss} against the membrane potential ($n = 12$). Note a lot of change has seen in the I_{ins} and I_{ss} before and after application of 50 μM SO_2 derivatives. (B) Plots of the difference between I_{ins} and I_{ss} in the control and application of 50 μM SO_2 derivatives against the membrane potential. Note the difference between I_{ins} and I_{ss} corresponds to I_h.

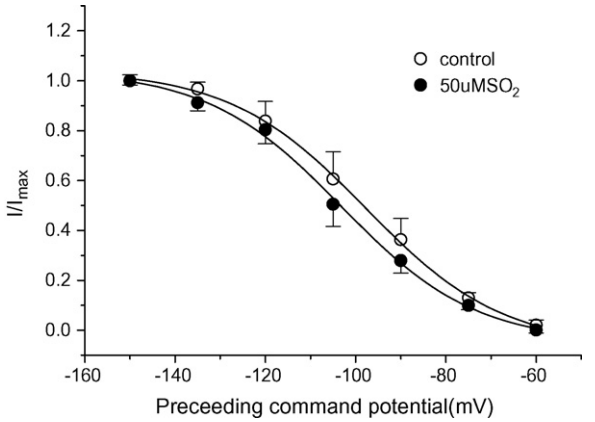

Fig. 4. Effects of 50 μM SO_2 derivatives on the activation of I_h. The activation curve was shifted in the negative direction after application of SO_2 derivatives (shift of V_{50} induced by 50 μM SO_2 derivatives from about −97.7 to −103.2 mV; paired *t*-test; $P < 0.05$; $n = 11$).

activation curve of I_h without a significant change in the slope factor.

To estimate the effects of SO_2 derivatives on the reversal potential of I_h in DRG neurons, a hyperpolarizing voltage pulse to −150 mV from a holding potential of −60 mV was applied for 800 ms and from this lever depolarizing voltage pulses from −80 to −20 mV in 10 mV steps were given for 200 ms (Fig. 5A). In order to avoid the effect of other voltage-dependent currents on the record of I_h, we added 200 μM Cd^+, 1 μM TEA and 3 mM 4-AP to external solution and tried to block the Ca^{2+} and K^+ ionic currents. The instantaneous current amplitudes during the steps from −80 mV to the more depolarized levels were plotted against the corresponding command potentials after leakage subtraction and the obtained data points were submitted to linear regression analysis. The intercept of the resulting curve with the *X*-axis and the slope represented Erev and conductance, respectively (Fig. 5B). Under control conditions Erev and the slope conductance was 37.7 ± 4.3 mV and 14.8 ± 0.3 nS ($n = 6$), indicating that I_h is a slow, non-inactivating inward current carried by K^+ and Na^+, which is similar to those described by the previous reports (Meng, 2003; Du and Meng, 2006; Banks et al., 1993). Application 10 μM SO_2 derivatives the Erev was shifted in positive direction by 5.2 mV ($P < 0.05$; $n = 6$) and the slope conductance was increased by 23% ($P < 0.05$).

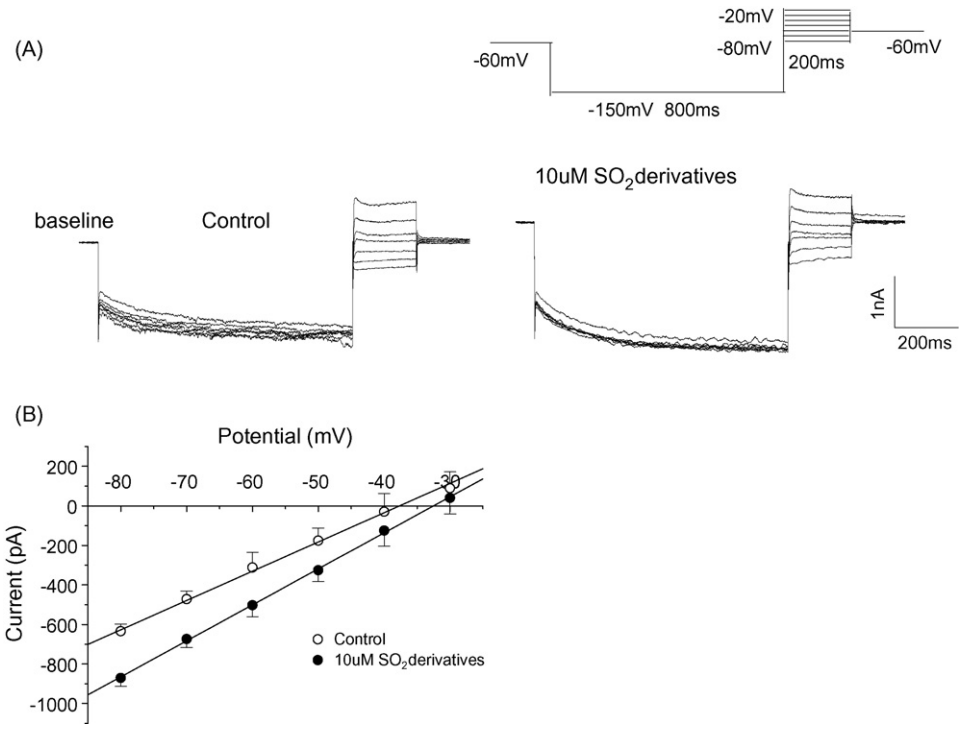

Fig. 5. Effects of SO_2 derivatives on the reversal potential of I_h in DRG neurons. (A) To determine Erev, after I_h reached its maximal value, the membrane potential was stepped to depolarizing voltages (*V*) from −80 to −20 mV in increments of 10 mV. The left traces represent currents under control conditions, the right traces at 10 μM SO_2 derivatives, respectively. (B) The intercept of resulting curve with the *X*-axis and the slopes represented Erev and slope conductance, respectively. Erev was shifted by ∼5.2 mV in the positive direction in the presence of 10 μM SO_2 derivatives.

3. Discussion

We report in the present study that a hyperpolarizing-activated current can be observed in rat DRG neurons. The main characteristics such as the voltage dependence, reversal potential, activation kinetics and non-inactivation of the current described here are consistent with the previous observations (Ingram and Wartenson, 1994; Liu et al., 2000).

In this present study, we have shown that SO_2 derivatives reversibly increased the amplitude of I_h current in rat dorsal root ganglion neurons, and the increase was dose-dependent with an EC_{50} value of 25 μM. We have also shown that the SO_2 derivatives increase was voltage-dependent, with greater increase at hyperpolarization. SO_2 derivatives also affected gating kinetics of I_h current and made activation course significantly shifting to a hyperpolarizing direction. SO_2 derivatives increased I_{ss} significantly, but had little effect on $I_{ins.}$ These implied that SO_2 derivatives altered the Na^+ and K^+ ionic selectivity of I_h channel in hyperpolarizing potential. In addition, TTX in external solution did not block TTX-resistant sodium action potentials in DRG neurons and SO_2 derivatives increased the reversal potential of TTX-resistant sodium currents (Du and Meng, 2004a,b). These might be the reasons for SO_2 derivatives shifted the hyperpolarizing current's reversal potential to a positive direction.

It was reported that I_h plays an important role in the control of electrical activity in DRG neurons during hyperpolarization (Surges et al., 2002; Wang et al., 1997). The major functional roles of I_h are: contribution to the resting membrane potential; control of the rhythmic-oscillatory activity; maintenance of the membrane potential toward depolarization; contribution to the production of after hyperpolarization (McCormick and Pape, 1990; Pape, 1996; Scroggs et al., 1994).

SO_2 derivatives increasing the amplitude of I_h current might be due to: an increase in slope conductance (I_h); positive shift of the reversal potential which increase the driving force; increasing the activating course by shifting the activating curve to a hyperpolarization direction (Surges et al., 2002). It is possible that the increase of I_h by SO_2 derivatives lead to an elevation of the depolarizing influence after the initial phase of after hyperpolarization. The enhancement of I_h by SO_2 derivatives may increase the discharge frequency of active DRG neurons, and advance initiation of the next action potential, resulting in the enhancement of the membrane conductance and frequency of the repetitive action potential evoked by a depolarizing current (McCormick and Pape, 1990; Pape, 1996). According to the functional role of I_h, the increase of I_h should result in an elevation of neuronal activity. The effects of SO_2 derivatives on DRG neurons were similar to the elevation of external K^+ on CA1 pyramidal cells (Surges et al., 2002). Since CA1 pyramidal cells are subjected to sustained elevations of external K^+ in epileptic tissue, our data might indicate SO_2 derivatives enhanced I_h perhaps involved in neuropathic pain (Surges et al., 2002; Deng et al., 2002).

In summary, our data provided the first quantitative analysis of the effect of SO_2 derivatives on I_h properties in DRG neurons. We demonstrated that SO_2 derivatives modulate not only the conductance of I_h, but also its gating properties. SO_2 derivatives acted through different mechanisms to alter cell excitability, which include increasing the frequency firing of potassium channels in neurons and cardiac myocytes (Du and Meng, 2004a,b; Nie and Meng, 2005a,b), modulation of sodium and calcium currents in the rat dorsal roof ganglion neurons (Du and Meng, 2004a,b,2006), and increasing a hyperpolarizing-activated cation current. Hence, our study provides a reasonable explanation for the neural toxicity of SO_2 derivatives.

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