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Biphasic effects of H_2O_2 on BK_{Ca} channels

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Abstract

The inhibitory or activating effect of H_2O_2 on large conductance calcium and voltage-dependent potassium (BK_{Ca}) channels has been reported. However, the mechanism by which this occurs is unclear. In this paper, BK_{Ca} channels encoded by mouse *Slo* were expressed in HEK 293 cells and BK_{Ca} channel activity was measured by electrophysiology. The results showed that H_2O_2 inhibited BK_{Ca} channel activity in inside-out patches but enhanced BK_{Ca} channel activity in cell-attached patches. The inhibition by H_2O_2 in inside-out patches may be due to oxidative modification of cysteine residues in BK_{Ca} channels or other membrane proteins that regulate BK_{Ca} channel function. PI3K/AKT signaling modulates the H_2O_2-induced BK_{Ca} channel activation in cell-attached patches. BK_{Ca} channels and PI3K signaling pathway were involved in H_2O_2-induced vasodilation and H_2O_2-induced vasodilation by PI3K pathway was mainly due to modulation of BK_{Ca} channel activity.

Keywords: *BK_{Ca} channels, H_2O_2, cysteine, PI3K, vasodilation*

Introduction

Large conductance calcium and voltage-dependent potassium (BK_{Ca}) channels are ubiquitously distributed and play a pivotal physiological role in vascular tone [1,2]. It provides a negative feedback mechanism that links to cellular membrane hyperpolarization. The BK_{Ca} channel function is generally regulated by oxidative stress, intracellular calcium concentration and others [3–5]. Hydrogen peroxide (H_2O_2) is an important product of oxidative metabolism. Although H_2O_2 has been depicted as damaging to cells, it also acts as a signaling molecule in physiological and pathological processes. Recent studies proposed two different roles of H_2O_2 on the mediation of BK_{Ca} channels in different cells. The BK_{Ca} channels function as an effector molecule to mediate the H_2O_2-induced vasodilation in coronary artery smooth muscles [6] and porcine coronary arteries [2]. However, H_2O_2 induced a decrease in both the number of active channels and the open probability of a multichannel patch (NP_O) in human BK_{Ca} channel α sub-unit [7]. It is not known whether the inhibitory and activating effects of H_2O_2 on BK_{Ca} channels appeared in the same cell line and in different patch configurations.

The mechanisms on the inhibitory or activating effects of H_2O_2 on BK_{Ca} channels have been detected in different cells and conditions. Wang et al. [8] indicated that redox modulation of cysteine thiol groups altered BK_{Ca} channel gating. Furthermore, researchers lately demonstrated that H_2O_2 inhibited BK_{Ca} channel current in inside-out patches by decreasing the Ca^{2+} sensitivity of channels [9,10] and the function was mediated by oxidative modification of cysteine residue 911 near the 'calcium bowl' in the pore-forming α sub-unit [10,11]. However, the mechanism of H_2O_2-induced BK_{Ca} channel activation is not fully understood. Some studies only showed that H_2O_2 opened BK_{Ca} channel currents via phospholipase A_2 (PLA_2)-arachidonic acid (AA) signaling pathway [6] in cell-attached patches and

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increased BK_{Ca} channel activity through cGMP pathway in whole-cell patches [12].

Phosphoinositide 3-kinase (PI3K) is a specific enzyme with a lipid kinase activity which phosphorylates phosphoinositides at the 3-hydroxyl [13]. Some studies showed that PI3K was an important modulator of vasodilation [14] and regulated the activities of some channels [15]. The extracellular H_2O_2 can significantly increase PI3K activity [16]. However, it is not known whether the PI3K signaling pathway modulates BK_{Ca} channel activation by H_2O_2. We have previously found that lipid phosphatase activity of PTEN (PI3K related protein) was involved in BK_{Ca} channel activation induced by H_2O_2 [17].

In this study, we detected two different effects of H_2O_2 on mouse *Slo* (m*Slo*) channel activity in HEK 293 cells using different patch configurations. The results showed that the distinct effects of H_2O_2 were related to the modes of action. Intracellular H_2O_2 inhibits BK_{Ca} channels, maybe by oxidative modification of cysteine residues in BK_{Ca} channels or other membrane proteins that regulate BK_{Ca} channel function. The PI3K/AKT signaling pathway may be involved in BK_{Ca} channel activation by extracellular H_2O_2. The PI3K signaling pathway or BK_{Ca} channels plays a role in H_2O_2-induced vasodilation and H_2O_2-induced vasodilation by the PI3K signaling pathway was mainly due to modulation of BK_{Ca} channel activity.

Materials and methods

Cell culture

Human embryonic kidney 293 (HEK 293) cells were cultured in Dulbecco's-modified-eagle's-medium (DMEM, Invitrogen, Carlsbad, CA, USA) containing 10% foetal bovine serum (FBS, Gibico BRL, Gland Island, NY, USA), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Measurement of intracellular ROS

Intracellular ROS were measured by fluorescent spectrophotometry (Hitachi F4500) using a specific fluorescence-labelled dye, 2,7-dichlorofluorescein diacetate (DCFH-DA, Molecular Probes, Eugene, OR). HEK 293 cells were washed with phosphate-buffered saline (PBS) and were pre-cultured with 10 μM DCFH-DA for 30 min at 37°C. The cells were washed three times with PBS and then treated with H_2O_2 for 30 min. The cells were washed again and homogenized in 200 μl 0.1% Triton X-100 by sonication on ice for 8 s. The lysates were centrifuged and the supernatants were used for measurement with excitation wavelength at 488 nm and emission wavelength at 510 nm.

Transfection

HEK 293 cells were cultured and then transfected with pcDNA3.1-EGFP-mSlo (mSlo) plasmid (provided by professor J. P. Ding, Huazhong University of Science and Technology, Wuhan, China) using Lipofectamine 2000 (Invitrogen). All experimental processes were done according to the manufacturer's instruction. Cells were used for electrophysiological experiments in 24–72 h after transfection.

Electrophysiology

Pipettes were pulled from borosilicate glass capillaries with a resistance between 2–4 megaohms (inside-out patches) or between 7–9 megaohms (cell-attached patches). All experiments were performed using EPC-10 patch-clamp amplifier (HEKA Electronics, Lambrecht/Pfalz, Germany) and PClamp software (Molecular Devices, Sunnyvale, CA, USA) at room temperature (21–24°C). Macroscopic records were filtered at 2.9 kHz and digitized at 50 kHz. Single channel records were filtered at 1 kHz and digitized at 10 kHz. The currents were allowed to stabilize for at least 5 min before drug treatment. The currents were recorded at 10 min after application of drugs, unless indicated otherwise. For cell-attached patches, the pipette solution contained (in mM): 140 KCl, 1.2 $MgCl_2$, 2.6 $CaCl_2$, 10 HEPES, pH 7.4 adjusted with NaOH. The bath solution contained (in mM): 110 KCl, 1.44 $MgCl_2$, 30 KOH, 10 EGTA, 10 HEPES, pH 7.2 adjusted with HCl. For inside-out patches, the pipette solution contained (in mM): 160 $MeSO_3K$, 10 HEPES, 2 $MgCl_2$, pH 7.0 adjusted with methanesulphonic acid ($MeSO_3$). The bath solution contained (in mM): 160 $MeSO_3K$, 10 HEPES, 5 EGTA, pH 7.0 adjusted with $MeSO_3$ at 0 μM Ca^{2+} bath solution. For 10 μM Ca^{2+} bath solution, 5 mM N-hydroxyethylenediaminetriacetic acid instead of 5 mM EGTA and Ca^{2+} were added to make 10 μM free Ca^{2+}, pH 7.0 adjusted with $MeSO_3$ [18].

Western blotting analysis

HEK 293 cells were grown to 40–60% confluency in 6-well culture plates and pre-incubated with different concentrations of LY294002 for 1 h. Before and after adding H_2O_2, the cells were harvested. Protein preparation and immunoblotting were performed as described [17]. The antibodies used were: anti-phospho-AKT (Ser 473) and anti-β-actin (Cell Signaling Technology, Danvers, MA, USA).

Tension measurement

Adult male Wistar rats (weighing 210~240 g; obtained from the Animal Center, Institute of Health and Epidemic Prevention, Hubei, China) were sacrificed by

decapitation under ether anaesthesia. The thoracic aortas were excised and rapidly immersed in ice-cold Krebs-Ringer buffer (in mM): 119 NaCl, 25 $NaHCO_3$, 11.1 glucose, 1.6 $CaCl_2$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, pH 7.4. The aortas were cleaned of adherent connective tissues and cut into ~ 3-mm rings. The rings were then quickly mounted on two stainless steel hooks in 5-ml organ baths filled with Krebs-Ringer buffer and continuously gassed with a mixture of 95% O_2/5% CO_2. The upper hook was connected to a force transducer (JZJ01H transducer, Chengdu Instrument Co., Chengdu, China). The rings were equilibrated at a resting tension of 1.0 g for 1 h at 37°C before measurement. During this period, the bathing solution was renewed every 20 min and the basal tone was maintained. After the equilibration, each ring physiological activity was confirmed by the administration of high-potassium buffer (80 mM). As rings were washed with Krebs-Ringer buffer until the baseline tone was re-maintained, the rings were contracted with 1 μM norepinephrine (NA) to the maximal contraction. Following washout of NA, cumulative concentration–response curves to stepwise cumulative application of H_2O_2 (100–800 μM) in the presence or absence of 50 nM Iberiotoxin and 50 μM LY294002 was established. The tension changes were measured by data acquisition and analysis system (BL-420F, Chengdu Taimeng Technology Co., Chengdu, China). Relaxation responses were expressed as a percentage of NA-induced contraction.

Data analysis

Data were analysed with SigmaPlot (SPSS Inc., Chicago, IL, USA), Clampfit 9.0 (Molecular Devices) and QUB (State University of New York, Buffalo, NY) softwares. All data were shown as means ± SEM. Statistical significance between two groups was tested using Student's *t*-test. Differences in the mean values were considered significant at a probability < 0.05. Dose–response relationships for H_2O_2 inhibiting BK_{Ca} channels were fitted to a Hill equation of the following form: $f = f_{max}/(1 + (IC_{50}/[H_2O_2])^n)$, where f is inhibition percentage of BK_{Ca} channel currents, f_{max} is the maximum values of f and $[H_2O_2]$ represents the concentration of H_2O_2. IC_{50} and n denote H_2O_2 concentration of half-maximal inhibition and the Hill coefficient, respectively. EC_{50}, counted as the same as IC_{50}, denotes H_2O_2 concentration of half-maximal activation. The *G–V* curves were fitted to a Boltzmann equation of the following form: $G/G_{max} = 1/(1 + \exp[(V - V_{1/2})/k])$, where G is the conductance of the channel, G_{max} is the maximum value of G, V is the holding potential, $V_{1/2}$ represents the voltage for half-maximal activation and k is the slope factor. Single channel amplitudes were measured by using all-points histograms of current records. The NP_O was calculated from the area under the curve of the Gaussian fit of all-points amplitude histograms as previously described [18].

Results

H_2O_2 inhibits BK_{Ca} channel currents in inside-out patches

HEK 293 cells do not express endogenous BK_{Ca} channels and are widely used as an expression system for ion channel studies. To determine the effects of H_2O_2 on the BK_{Ca} channel activity, HEK 293 cells were transiently transfected with pcDNA3.1-EGFP-mSlo plasmid and then treated with different concentrations of H_2O_2. BK_{Ca} channel currents were detected in mSlo-transfected HEK 293 cells in a Ca^{2+}-dependent manner and tetraethylammonium (TEA), a specific BK_{Ca} channels blocker, abolished the cloned BK_{Ca} channel activity (data not shown), suggesting that exogenous BK_{Ca} channels were expressed in HEK 293 cells. As shown in Figure 1, H_2O_2 (50–400 μM) causes a concentration-dependent generation of intracellular ROS. Consistent with previous data [7,10], BK_{Ca} channel currents were inhibited after exposure to 100 μM H_2O_2 in 0 μM Ca^{2+} bath solution in inside-out patches. H_2O_2 induced a 29 ± 5 mV rightward shift in the *G–V* curve. DTT reversed the inhibition and resulted in a 25 ± 4 mV leftward shift in the *G–V* curve (Figures 2A and B). However, H_2O_2-induced BK_{Ca} channel inhibition was abolished in 10 μM Ca^{2+} bath solution; 100 μM H_2O_2 only caused a 5 ± 3 mV rightward shift and DTT also only resulted in a 3 ± 2 mV leftward shift in the *G–V* curve, which is not significantly different in statistics ($p > 0.05$, $n = 5$) (Figures 2C and D). Similar to

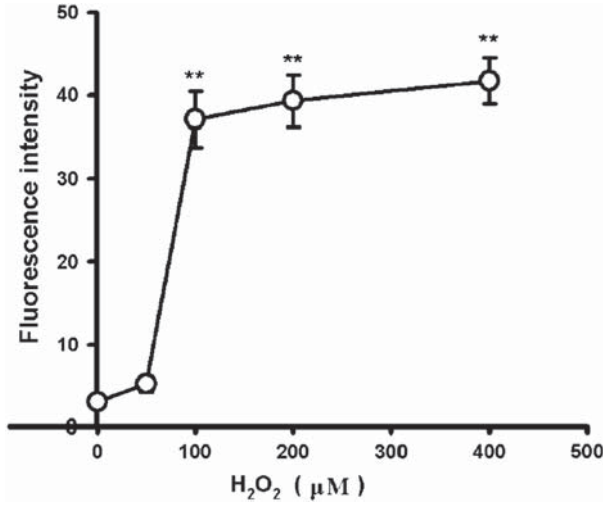

Figure 1. H_2O_2 increases intracellular ROS concentration. HEK 293 cells were stimulated with 50, 100, 200 and 400 μM H_2O_2 for 30 min. The intracellular ROS were determined by fluorescent spectrophotometry using DCFH-DA ($^{**}p < 0.05$ vs control group, $n = 6$).

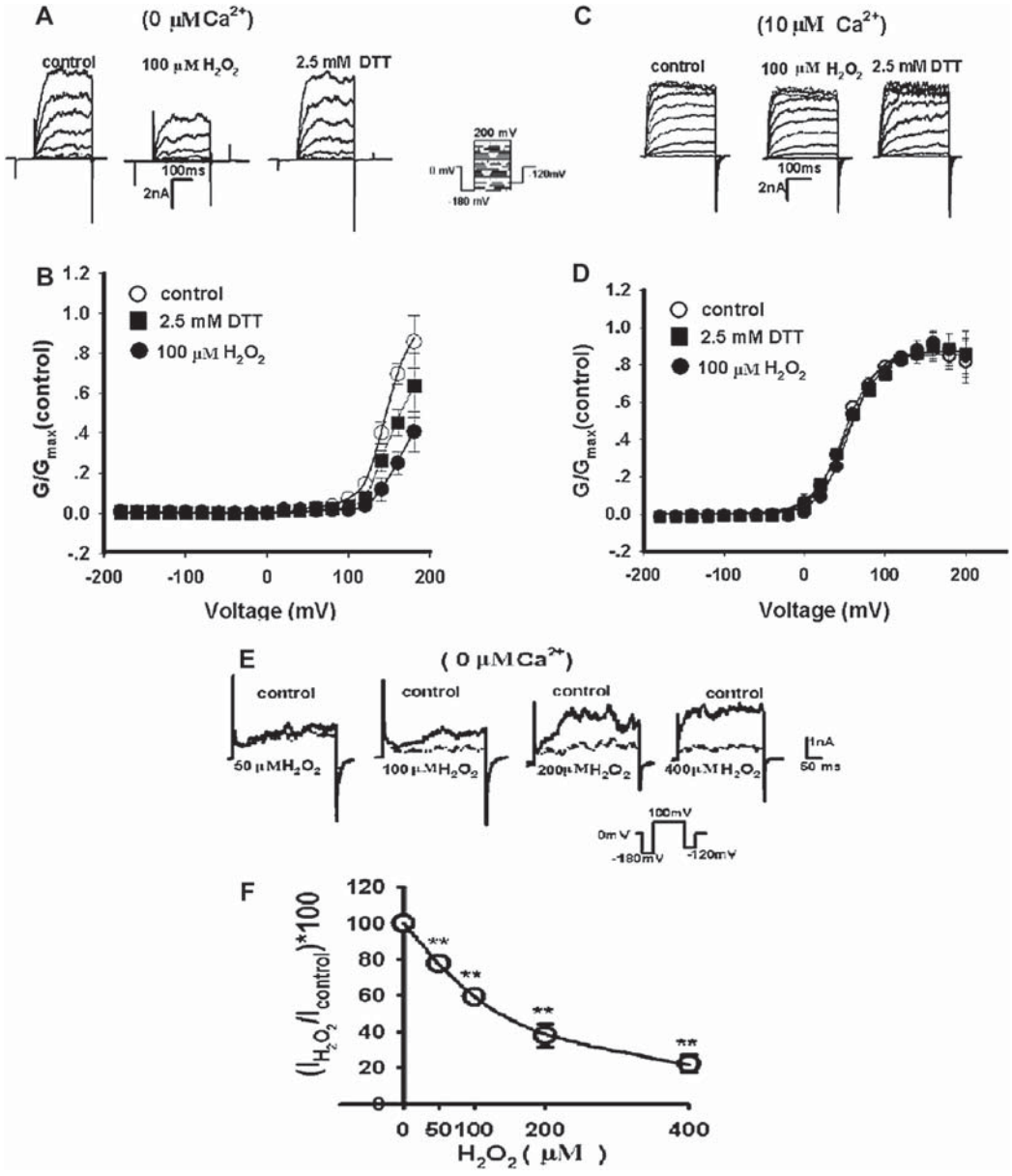

Figure 2. Inhibition of BK_{Ca} channels expressed in HEK 293 cells by H_2O_2 in inside-out patches. (A) and (C) Representative traces of BK_{Ca} channels obtained in control, 100 μM H_2O_2 and 2.5 mM DTT group as indicated. All currents were recorded in inside-out patches at 0 μM Ca^{2+} bath solution (A, B) and 10 μM Ca^{2+} bath solution (C, D), respectively. Currents were aroused by voltage steps as shown in the middle. (B) and (D) *G–V* curves from individual patches were normalized and then averaged. The fitted values for the $V_{1/2}$ of control, 100 μM H_2O_2 and 2.5 mM DTT are detected, respectively ($n = 7$). (E) and (F) The concentration-dependence of H_2O_2 on the inhibition of BK_{Ca} channel currents. The currents were recorded in inside-out patches at +100 mV and 0 μM Ca^{2+} bath solution. The solid line is control group and the dotted line is H_2O_2 exposure group (E). The dose–response curves of H_2O_2 on the inhibition of BK_{Ca} channel currents were determined. The solid line is a fit to the Hill equation (F) ($^{**}p < 0.01$ vs control group, $n = 5$).

macroscopic currents, NP_O of single BK_{Ca} channels was decreased after H_2O_2 treatment in inside-out patches (Figures 3A and B). To summarize all data($n = 8$), the NP_O of BK_{Ca} channels was reduced from 0.278 ± 0.0521 to 0.0754 ± 0.0291 after exposure to 100 μM H_2O_2 (Figure 3C).

To compare the effects of H_2O_2 on BK_{Ca} channel activity in inside-out patches, we detected the relationship between H_2O_2 dose and channel activity in 0 μM Ca^{2+} bath solution. The BK_{Ca} channel currents were recorded before and after 50, 100, 200 and 400 μM H_2O_2 treatment, respectively (Figure 2E). The dose–response curve was fitted to a Hill equation with IC_{50} values of 137 μM and a Hill coefficient of 1.21 (Figure 2F).

H_2O_2 enhances BK_{Ca} channel activity in cell-attached patches

The effects of H_2O_2 on BK_{Ca} channel activity were detected in cell-attached patches. The data showed that H_2O_2 significantly increased NP_O of BK_{Ca} channels (Figures 3E and F). To summarize all data ($n = 8$), the NP_O of BK_{Ca} channels increased from

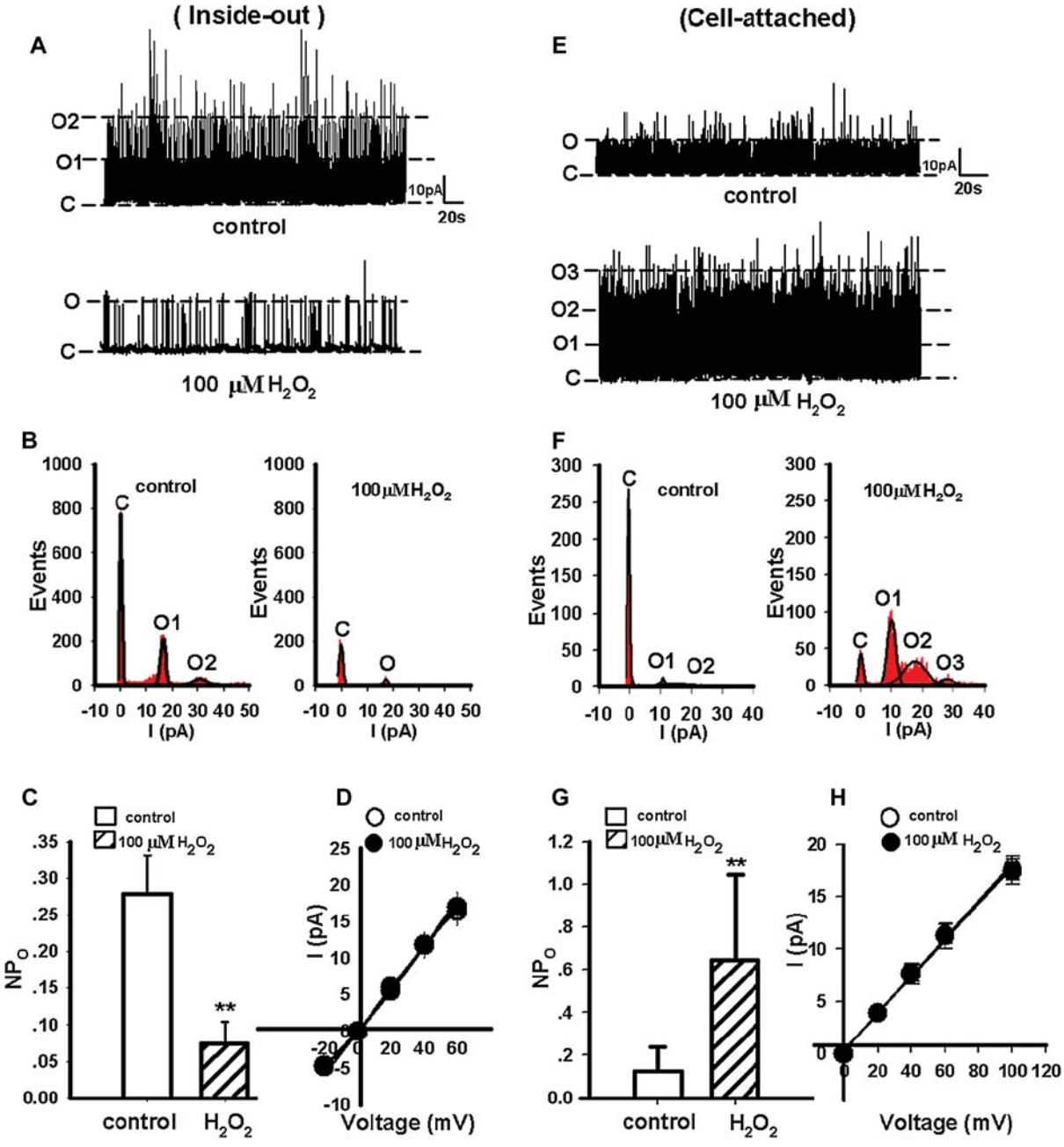

Figure 3. The biphasic effects of H_2O_2 on BK_{Ca} channels. (A) and (E) Single channel traces were obtained from inside-out (A–D) and cell-attached patches (E–H) before (control) and after application of 100 μM H_2O_2 to HEK 293 cells expressed with BK_{Ca} channels at +60 mV. The letters c, o1, o2, o3 indicate the closed, open 1, open 2, open 3 levels, respectively. (B) and (F) Each histogram illustrates all possible changes in open probabilities (NP_O). (C) and (G) Summary of the effects of 100 μM H_2O_2 on NP_O of BK_{Ca} channels ($^{**}p < 0.01$ vs control group, $n = 8$). (D) and (H) Channel current–voltage curves are plotted before and after application of 100 μM H_2O_2 ($n = 8$).

0.1247 ± 0.1151 to 0.6439 ± 0.4018 after exposure to 100 μM H_2O_2 (Figure 3G). The unitary conductance of BK_{Ca} channels was ~ 180 pS (Figure 3H). However, the unitary conductance of BK_{Ca} channels in inside-out patches was ~ 280 pS (Figure 3D).

H_2O_2 increases BK_{Ca} channel activity in a PI3K-dependent manner

To determine whether the PI3K/AKT signaling pathway was involved in H_2O_2-induced BK_{Ca} channel activation, two specific PI3K inhibitors LY294002 and Wortmannin were used. Pre-incubation with LY294002 inhibits p-AKT expression in the presence or absence of H_2O_2 (Figures 4E and F). When the cells were treated with LY294002 or Wortmannin in the absence of H_2O_2, the NP_O of BK_{Ca} channels was not changed (data not shown), suggesting that LY294002 and Wortmannin do not affect the BK_{Ca} channel basal activity. Consistent with the above data (Figures 3E and F), H_2O_2 significantly increased the NP_O of BK_{Ca} channels in cell-attached patches. How-

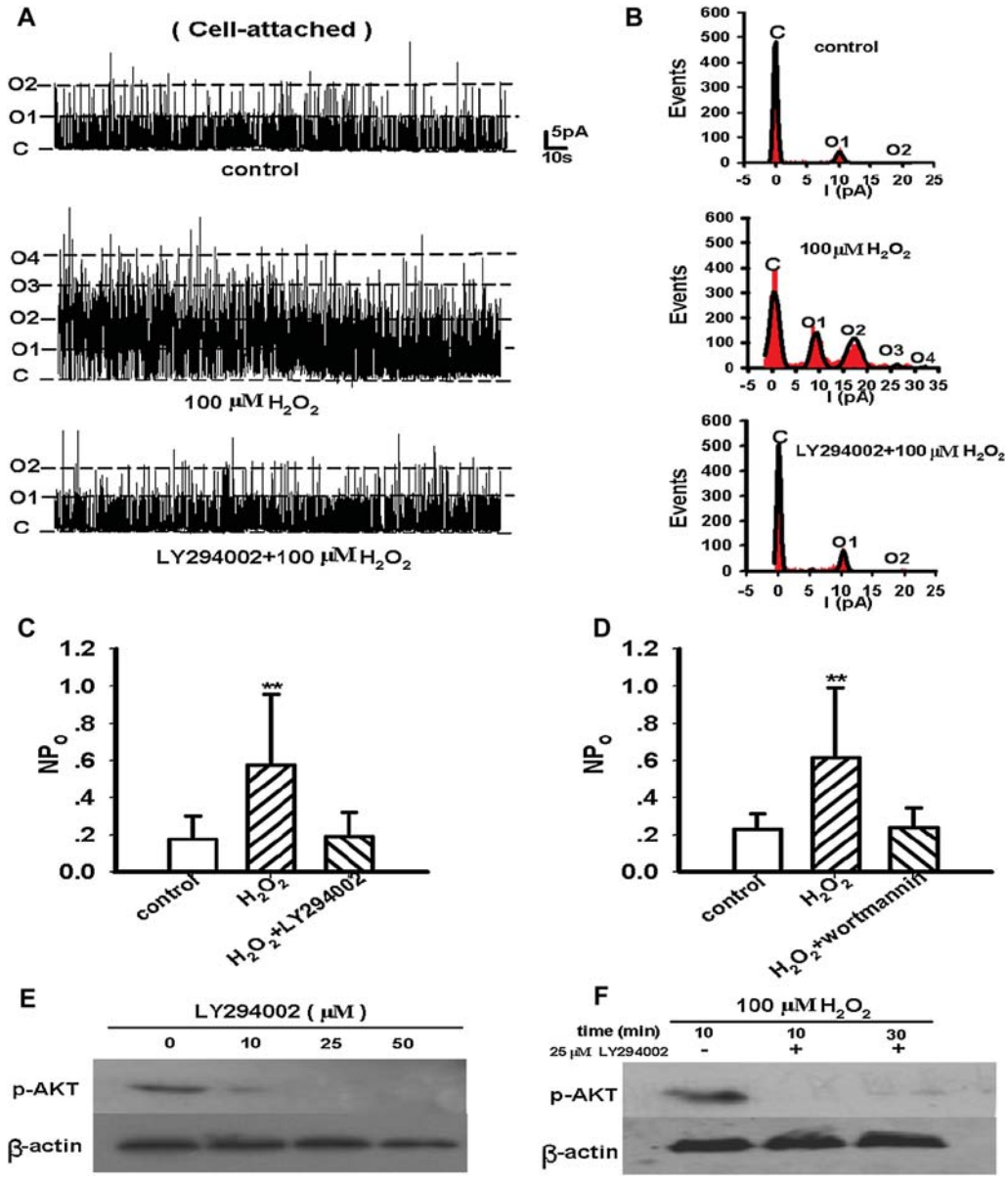

Figure 4. H_2O_2-induced BK_{Ca} channel activation in a PI3K-dependent manner. (A) Representative BK_{Ca} channel currents were recorded in cell-attached patches at +60 mV. HEK 293 cells expressed with BK_{Ca} channels were pre-incubated with or without 25 μM LY294002 or 500 nM Wortmannin for 1 h and then treated with H_2O_2 application for 10 min. (B) The histogram illustrates all possible changes in NP_O. (C) A summary of the effects of LY294002 on NP_O of BK_{Ca} channels induced by 100 μM H_2O_2 ($^{**}p < 0.05$, vs control group, $n=5$). (D) A summary of the effects of Wortmannin on NP_O of BK_{Ca} channels induced by ($^{**}p < 0.05$, vs control group, $n=6$). (E) and (F) The cells were pre-incubated with 10, 25, 50 μM LY294002 for 1 h (E) or pre-incubated with 25 μM LY294002 for 1 h and then exposed to 100 μM H_2O_2 for 10 or 30 min (F). The p-AKT expression was evaluated by Western blotting analysis.

ever, when the cells were pre-treated with LY294002 or Wortmannin, the H_2O_2-induced BK_{Ca} channel activation was abolished (Figures 4A–D).

BK_{Ca} channels are involved in H_2O_2-induced vasodilation through PI3K pathway

In accordance with previous data [19,20], H_2O_2 (100–800 μM) caused a concentration-dependent relaxations in rat thoracic aortic rings contracted by NA (1 μM) and the EC_{50} was calculated to be 247.98 μM (Figure 5A). To determine the mechanism of H_2O_2 relaxing rat thoracic aortic rings, we pre-treated rat thoracic aortic rings with the specific BK_{Ca} channel blocker Iberiotoxin or PI3K inhibitor LY294002. The results showed that Iberiotoxin and LY294002 significantly inhibited H_2O_2-induced relaxations (Figure 5B), suggesting that BK_{Ca} channels and PI3K signaling pathway may be involved in H_2O_2-induced vasodilation. However, when the rat thoracic aortic rings were pre-treated with both Iberiotoxin and LY294002, the inhibitory effect on H_2O_2-induced

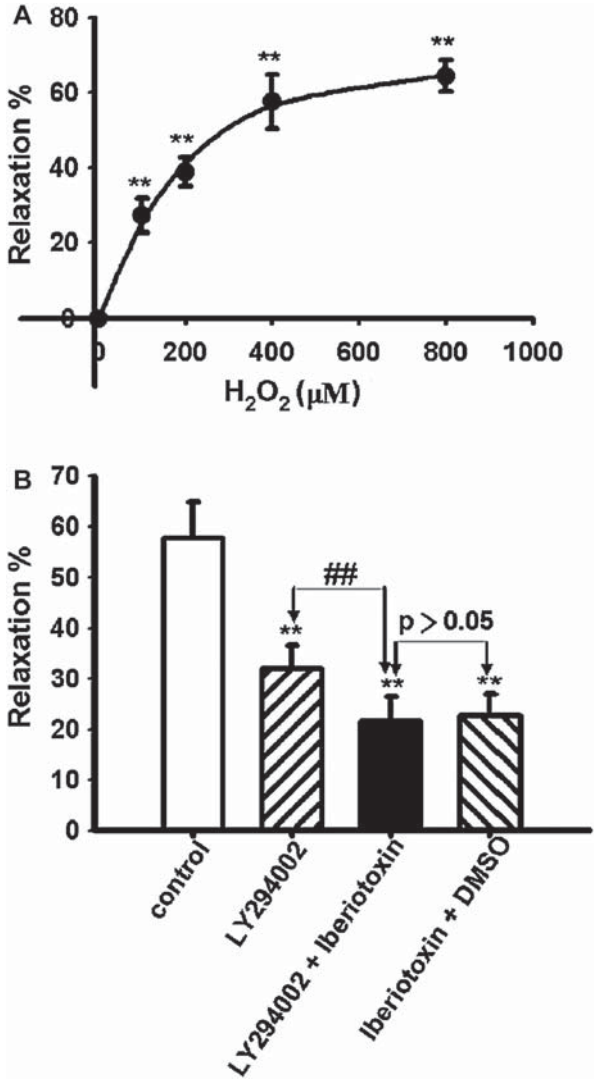

Figure 5. BK_{Ca} channels and PI3K signaling are involved in H_2O_2-induced vasodilation in isolated rat aorta. Rat aortic rings were contracted with NA (1 μM) before H_2O_2 was added. (A) Vascular relaxation responded to H_2O_2 in a concentration-dependent manner (**, $p < 0.05$ vs control group, $n = 4$). (B) H_2O_2-induced relaxation in the presence or absence of 50 μM LY294002 or 50 μM LY294002 before 400 μM H_2O_2 was added (**, $p < 0.05$ vs control group, $n = 5$; ##, $p < 0.05$, LY294002 + Iberiotoxin vs LY294002, $n = 5$).

relaxations was similar to that in the group pre-treated with Iberiotoxin only. The results indicated that the inhibitory effect on H_2O_2-induced vasodilation by LY294002 was mainly due to modulation of BK_{Ca} channel activity.

Discussion

BK_{Ca} channels play a pivotal and specific role in many pathophysiological conditions. The native BK_{Ca} channels consist of a pore-forming α sub-unit and β sub-unit. The α sub-unit encoded by a single gene *Slo* is a key part of BK_{Ca} channel function and contains six transmembrane segments S1–S6. Two regulators of the potassium conductance domains and 'calcium bowl' have been identified in segments S7–S10, where a majority of cysteine residues in the COOH terminus could be modified directly by oxidative stress [21–23]. Pantopoulos et al. [24] demonstrated that some proteins regulated by H_2O_2 respond differentially to alterations of extra- and intracellular H_2O_2 levels. In this study, we performed patch-clamp experiments in inside-out and cell-attached configurations to study the effects of H_2O_2 on BK_{Ca} channel activity. We found that H_2O_2 induced intracellular ROS. Application of H_2O_2 to the cytosolic face in inside-out patches inhibited BK_{Ca} channel activity. However, BK_{Ca} channel activity was increased when intact cells were treated with H_2O_2 in cell-attached patches. The results suggested that two distinct effects of H_2O_2 on BK_{Ca} channels would relate to the mode of action.

Our studies demonstrated that H_2O_2 significantly inhibited the activity of BK_{Ca} channels in inside-out patches, shifting the *G–V* relationship to the right in 0 μM Ca^{2+} bath solution. DTT reversed the inhibition induced by H_2O_2. These results suggested that direct oxidation of cysteine residues, such as cysteine 911 near the 'calcium bowl' in the pore-forming α sub-unit [9,10] may inhibit the BK_{Ca} channel activity directly. However, it was possible that the oxidation of other membrane proteins that regulate BK_{Ca} channel function indirectly inhibited the BK_{Ca} channel activity. We further found that the inhibition induced by H_2O_2 was abolished in 10 μM Ca^{2+} bath solution, indicating that the inhibitory effects of H_2O_2 on BK_{Ca} channels was in a Ca^{2+}-dependent manner. High internal [Ca^{2+}] would act as a protective Ca^{2+} agent to modulate and potentially protect groups that can be oxidized and mediate the closing of the BK_{Ca} channels, which is consistent with the previous finding [7].

Previous studies showed that H_2O_2 opened BK_{Ca} channel currents via a PLA_2-AA signaling pathway [6]. It was known that PLA_2 and PI3K signaling function similarly in some aspects [25]. In this study, we found that PI3K inhibitors LY294002 and Wortmannin, which inhibited p-AKT expression, abolished the activating effect of H_2O_2 on BK_{Ca} channel activity during 10 min in cell-attached patches, suggesting that the PI3K/AKT signaling pathway may be involved in BK_{Ca} channel activation by H_2O_2. Some evidences indicated that BK_{Ca} channels play a crucial role in H_2O_2-induced vascular diseases [26]. To explore the potential physiological significance of H_2O_2 activating BK_{Ca} channels, the mechanism on H_2O_2-induced vasodilation was investigated. The results showed that the specific BK_{Ca} channel inhibitor Iberiotoxin and PI3K inhibitor LY294002 significantly inhibited H_2O_2-induced relaxations, suggesting that BK_{Ca} channels and the PI3K signaling pathway may be involved in H_2O_2-induced vasodilation. We further found that pre-treatment with Iberiotoxin and

LY294002 together inhibited H_2O_2-induced vasodilation to a similar extent to that in the group pre-treated with Iberiotoxin only. The results indicated that the H_2O_2-induced vasodilation by PI3K pathway was mainly due to modulation of BK_{Ca} channel activity, which is consistent with the finding that H_2O_2 activates BK_{Ca} channel activity in a PI3K-dependent manner.

H_2O_2 has been reported as a vasoconstrictor [27] and vasodilator [28]. The differences may depend on the concentration of H_2O_2 used or whether studies are performed *in vivo* or *in vitro*. The intracellular H_2O_2 concentration was controlled by some antioxidant enzymes such as catalase in physiological condition and it only reaches 1~15% of the applied extracellular concentration [29]. Our results have shown that H_2O_2 inhibited BK_{Ca} channel activity in inside-out patches and activated BK_{Ca} channel activity in cell-attached patches. Considering the physiological significance of BK_{Ca} channels in H_2O_2-regulated vascular reactivity, we speculate that an amount of H_2O_2 activated BK_{Ca} channels and caused vasodilation. If higher concentrations of H_2O_2 were used, some antioxidant enzymes were inactivated and BK_{Ca} channels may be closed to contract vessels. Whether BK_{Ca} channels are involved in H_2O_2-induced vasoconstriction needs to be further elucidated. In this paper, to exclude the presence of other channels that could be contributing to the effects of H_2O_2 on BK_{Ca} channels, we used an artificial expression system in which BK_{Ca} α sub-unit (mslo) was expressed in BK_{Ca} channels-null HEK 293 cells to mimic native BK_{Ca} channels in the vascular smooth muscle cells. Further studies on the effects of H_2O_2 on the BK_{Ca} channel activity in isolated vascular smooth muscle cells needs to be confirmed.

In conclusion, the inhibitory and activating effects of H_2O_2 on BK_{Ca} channels were detected. The biphasic effects of H_2O_2 on BK_{Ca} channels depended on the mode of action. Oxidative modification of cysteine residues in BK_{Ca} channels or other membrane proteins that regulate BK_{Ca} channel function account for the inhibition by H_2O_2 in inside-out patches. PI3K/AKT signaling modulates the H_2O_2-induced BK_{Ca} channel activation in cell-attached patches. BK_{Ca} channels and the PI3K signaling pathway were involved in H_2O_2-induced vasodilation and H_2O_2-induced vasodilation by the PI3K pathway was mainly due to modulation of BK_{Ca} channel activity. BK_{Ca} channels may present a potential therapeutic target for the prevention and treatment of impaired vascular relaxation.

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