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Hydrogen peroxide selectively increases TREK-2 currents via myosin light chain kinases

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1. ABSTRACT

Two-pore domain K^+ (K_{2P}) channels play a critical role in cellular responses to various stimuli, such as stretch or changes in pH and are considered to be important in pathological responses such as apoptosis and tumorigenesis. We investigated effects of H_2O_2 on various K_{2P} channels expressed in CHO cells. Application of H_2O_2 did not affect TASK-1, TASK-3, TRAAK currents, but specifically increased TREK-2 currents recorded using a nystatin perforated whole cell technique. The H_2O_2-induced activation of TREK-2 currents was also observed at single channel levels in cell-attached patches, and the effect was reversed by the reducing agent, dithiothreitol. In contrast, TREK-2 currents recorded using ruptured whole cell technique or single channel recording in inside-out patches were not affected by H_2O_2. Furthermore, direct application of 5,5'-dithiobis-(2-nitrobenzoic acid) inhibited TREK-2, suggesting that the H_2O_2-induced activation does not result from direct oxidation of TREK-2 proteins. Among the cell signaling agents, myosin light chain kinase (MLCK) inhibitors significantly inhibited the H_2O_2-induced activation of TREK-2 currents. These results suggest that TREK-2 channels have a potential to play a specific role in cellular responses to reactive oxygen species and that MLCK activation is involved in this process.

2. INTRODUCTION

Oxidative stress may alter membrane structure and function, membrane fluidity and permeability, enzymes activity, channels, transport proteins and receptors. These effects of oxidative stress are mainly initiated by reactive oxygen species (ROS), such as superoxide(O_2^-), hydroxyl radicals (HO·), lipid hydroperoxides and H_2O_2. ROS have been viewed traditionally as damaging to the cell, but the role of ROS in cell signaling networks has been recently appreciated (1). ROS were shown to modulate various biological processes, including cell growth, apoptosis and cell adhesion (2-4). In these actions, increased intracellular Ca^{2+} concentration (5), activation of cytosolic phospholipase A_2 ($cPLA_2$) (6), rearrangement or disruption of the cytoskeleton (7), and increased ion permeability (8) were suggested to be involved.

Recently, two-pore domain K^+ (K_{2P}) channels are cloned and regarded to function as leak channels or background channels. Because they are open at the physiological range of membrane potentials and exhibit little or no voltage- or time-dependence, they are expected to play a major role in setting the resting membrane potential in many cell types (9, 10). Roles of K_{2P} channels in cell apoptosis and tumorigenesis have recently been

recognized to be important (11). Another important feature of K_{2P} channels is the diversity of their regulatory mechanisms. The weak inward rectifiers TWIK-1 and TWIK-2 are stimulated by activators of protein kinase C and decreased by internal acidification, the baseline TWIK-related acid-sensitive K^+ (TASK)-1 and TASK-2 channels are sensitive to external pH changes in a narrow range near physiological pH (12). The TWIK-related (TREK)-1 and TWIK-related arachidonic acid-stimulated K^+ (TRAAK) channels are the first cloned polyunsaturated fatty acids-activated and mechanogated K^+ channels (13, 14). However, the effect of ROS on K_{2P} channels has not been investigated.

In this study, we tested effects of H_2O_2 on four K_{2P} channels; TASK-1, TASK-3, TRAAK, and TREK-2. We found that H_2O_2 selectively increases TREK-2 activity and this effect is reversed by DTT. Unlike the direct activation of two-pore domain K^+ channels by pH or arachidonic acid, H_2O_2-induced activation of TREK-2 was abolished by making inside-out patch or whole-cell configurations. Furthermore, direct application of chemical oxidizing agent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), to inside-out patches induced the decrease in TREK-2 activity. These results indicate that H_2O_2-induced activation of TREK-2 is not attributable to the direct redox modulation of TREK-2 channels, but occurs via, intracellular signaling pathways. We demonstrated that myosin light chain kinase (MLCK) is involved, at least in part, in the H_2O_2-induced activation of TREK-2.

3. MATERIALS AND METHODS

3. 1.Cell culture and transfection

CHO cells were plated on 35 mm culture dishes 24 h before transfection. TREK-2 and other clones subcloned in pcDNA3.1 were co-transfected with green fluorescent protein (GFP) into CHO cells using the LipoFectamine reagent (Invitrogen, Calsbad, CA). Green fluorescence from cells expressing GFP was detected with the aid of a Nikon microscope equipped with a mercury lamp light source. Cells were used for 2–3 days after transfection

3.2. Solutions

Normal Tyrode (NT) bath solution contained (mM) 143 NaCl, 5.4 KCl, 0.5 NaH_2PO_4, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES and 10 glucose, and was adjusted to pH 7.4 with NaOH. When whole cell configuration was performed, the high-K^+ pipette solution contained (mM) 140 K-aspartate, 10 NaCl, 0.01 GTP, 1 $MgCl_2$, 5 Mg-ATP and 2 EGTA, and was titrated to pH 7.3 with KOH. For single channel recording, the pipette and bath solution was composed of (mM) 140 KCl, 1 $MgCl_2$, 10 HEPES and 5 EGTA titrated to pH 7.2 with KOH. MLCK inhibitors were from Biomol, and other chemicals were from Sigma. Drugs were prepared as concentrated stock solutions in distilled water and dimethyl sulfoxide. To ensure a rapid turnover solution, the rate of perfusion was kept above 5ml min^{-1}, which corresponded to 50 times the bath volume (100 microliter) per minute. The reference electrode was a 3M KCl-agar bridge electrode.

3.3. Electrophysiological studies

3.3.1. Voltage clamp recording and analysis

Membrane currents were recorded from single CHO cells in a perforated patch configuration using nystatin (ICN, 200 microgram/ml), ruptured whole cell configuration, cell-attached or inside-out patches configuration. Voltage clamp was performed using an Axopatch-1D (Axon Instruments, Union city, CA). The patch pipettes were pulled from borosilicate capillaries (Harvard Apparatus) using a Narishige puller (PP-83, Tokyo). The patch pipettes used for whole cell configuration had a resistance of 2-3 megaohm when filled with the pipette solutions. Pipette resistance for single channel recording ranged between 3 and 5 megaohm, and pipettes were coated with Sylgard. Electrical signals were displayed during experiments using an oscilloscope (Tektronix, TDS 210). Voltage clamp and data acquisition was performed using a digital interface (BNC-2110, National Instruments) coupled to an IBM-compatible computer at a sampling rate of 5 kHz. Data were filtered at 1 kHz (cut-off frequency at -3 dB points). With single-channel analysis, the threshold of opening transition was set at one-half of the unitary current using Igor Pro (Version 4.1; WaveMetrics, Lake Oswego, OR). Current tracings shown in figures were filtered at 1 kHz.

3.3.2 Statistics

Data are given as means ± SEM, *n* represents the number of cells tested. The significance of differences between means was established using Student's t- test. $P < 0.05$ was regarded as significant. All statistical analyses were conducted with standard software (Origin version 6.0, Microcal, Northampton, Mass.). All experiments were done at room temperature (23 ± 1°C).

4. RESULTS

4.1. TREK-2 is activated by H_2O_2

Effects of H_2O_2 on two-pore domain K^+ channels were tested for TREK-2, TRAAK, TASK-1 and TASK-3 expressed in CHO cells (Figure 1). Whole cell currents were recorded using the nystatin perforated patch clamp technique. In response to the voltage ramp from –120 mV to +60 mV, characteristic current responses corresponding to each channel were observed. The reversal potentials of two-pore K^+ channels expressed in CHO cells were near K^+ equilibrium potential [TASK-1 : –63 ± 5 mV (n = 3), TASK-3 : –60 ± 4 mV (n = 4), TRAAK: –65 ± 5 mV (n = 5), TREK-2: –68 ± 7 mV (n = 276)]. When H_2O_2 (5 mM) was added to the bath solution, current magnitude increased in the case of TREK-2 (Figure 1B). The increase in TREK-2 currents persisted even after the wash-out of H_2O_2, but was gradually reversed by the application of the reducing agent, DTT (5 mM). Neither H_2O_2 nor DTT had significant effects on TRAAK (n = 3), TASK-1 (n = 4) or TASK-3 (n = 3) (Figure 1A). These results suggest that, of the two-pore domain K^+ channels tested in this study, TREK-2 is sensitive to H_2O_2. We investigated I-V relationships after exposure to H_2O_2 in TREK-2 expressed cells. As shown in Figure 1C, the current response to the voltage ramp displayed an outward-rectifying I-V relation in the presence of physiological solution, which is characteristic of TREK-

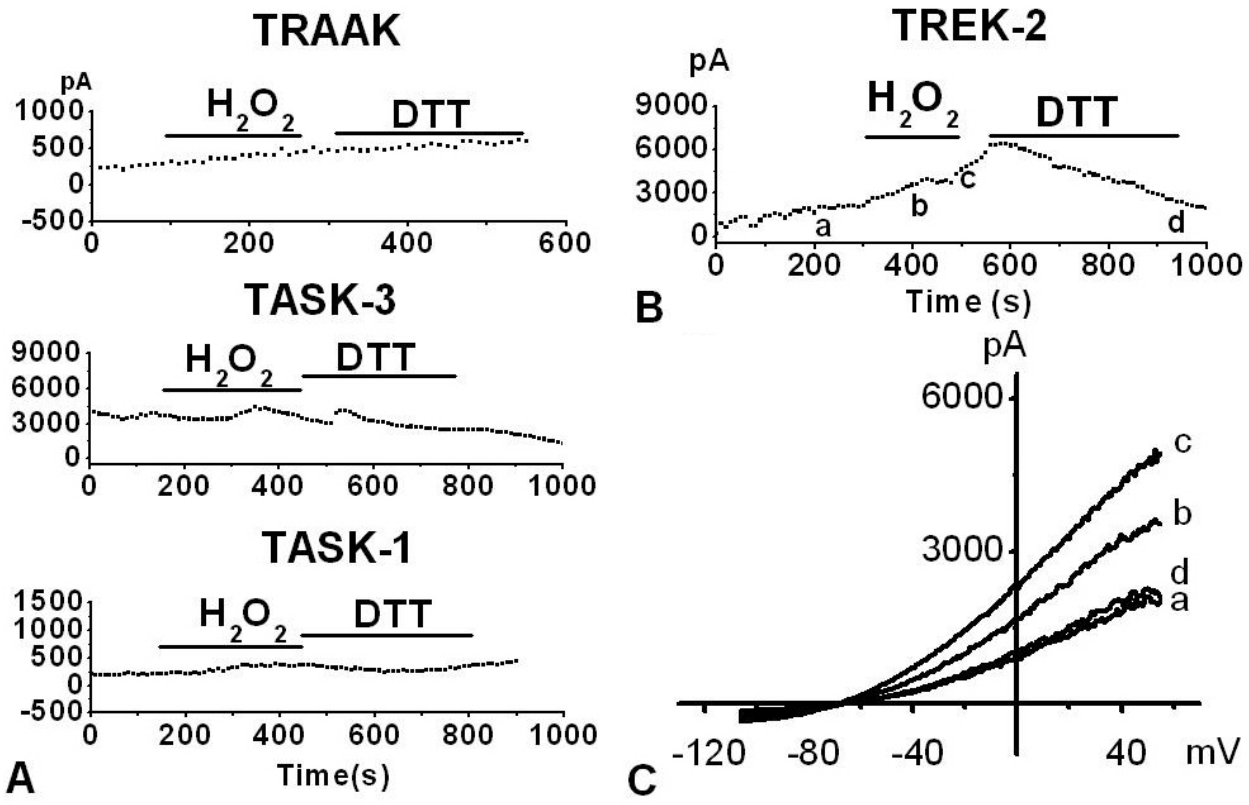

Figure 1. The effect of H_2O_2 and DTT on two-pore domain K^+ channels. Current were recorded at 50 mV in a nystatin (0.2 mg/ml) perforated patch configuration. A; Representative results obtained from cells expressing TRAAK (*upper panel*), TASK-3 (*middle panel*) and TASK-1 (*lower panel*). Changes in current amplitude were plotted along time. Bars above current traces indicate periods of time when H_2O_2 and DTT were applied. Of three two-pore domain K^+ channels, none of them were affected by H_2O_2 and DTT. B; A representative result obtained from a cell expressing TREK-2. It is noticed that the magnitude of current increased upon application of H_2O_2, returning to the original level by DTT. C; Current-voltage relationships obtained by applying voltage ramps from –120 to 60 mV for 300 ms. Alphabets indicate the time points when the voltage ramps were applied as indicated under the current trace of B.

2. The time course of the H_2O_2 effect on TREK-2 channels is shown in Figures 1B and 1C. The outward-rectifying current increased gradually, and reached a maximum steady state within 10 min of application of 5 mM H_2O_2 to the extracellular solution. It is much slower than the time course of the current increase by other conditions that induce a direct modulation of channel protein, such as acid or arachidonic acid (9, 15, 16). This slow time course may suggest that the H_2O_2 effect does not result from the redox-modulation of channel protein itself, but is mediated via the intracellular signaling process involved in channel activation. The reversal potential did not change with time during channel activation by H_2O_2 (Figure 1C).

Since the H_2O_2 effect developed gradually and the time course seemed to depend on concentrations, it was not practical to obtain dose-response relationship for the H_2O_2 effect on TREK-2 currents. We could observe the H_2O_2 effect at concentrations from 100 micromole to 500 micromole, but it was not easy to determine the lowest concentration for the effect, because variation of TREK-2 currents could be caused by other factors when the recording time was prolonged. In the subsequent experiments to investigate the mechanism of H_2O_2-induced TREK-2 activation, we used 5 mM of H_2O_2 to get clear responses in short time periods

To confirm that the H_2O_2-induced current increase was due to TREK-2, we applied the typical K^+ channel blocker, tetraethylammonium chloride (TEA), which is known not to affect background channels such as TREK-2 or other two-pore domain K^+ channels. The current was not blocked by 1 mM TEA in presence of H_2O_2 as we expected (data not shown). We also tested effects of H_2O_2 in CHO cells that did not have TREK-2 channel expression. In these cells, the current magnitude measured at 50 mV was less than 50 ± 20 pA (n = 10), and the current was not affected by H_2O_2. We did not observe any channel opening in non-transfected CHO cells or CHO cells transfected with GFP alone using cell-attached patch or inside-out patch configurations (n = 11). These results suggest that the selective increase in membrane current induced by H_2O_2 is mainly due to an increase in activity of TREK-2 channels.

In Figure 2A, the effect of H_2O_2 on TREK-2

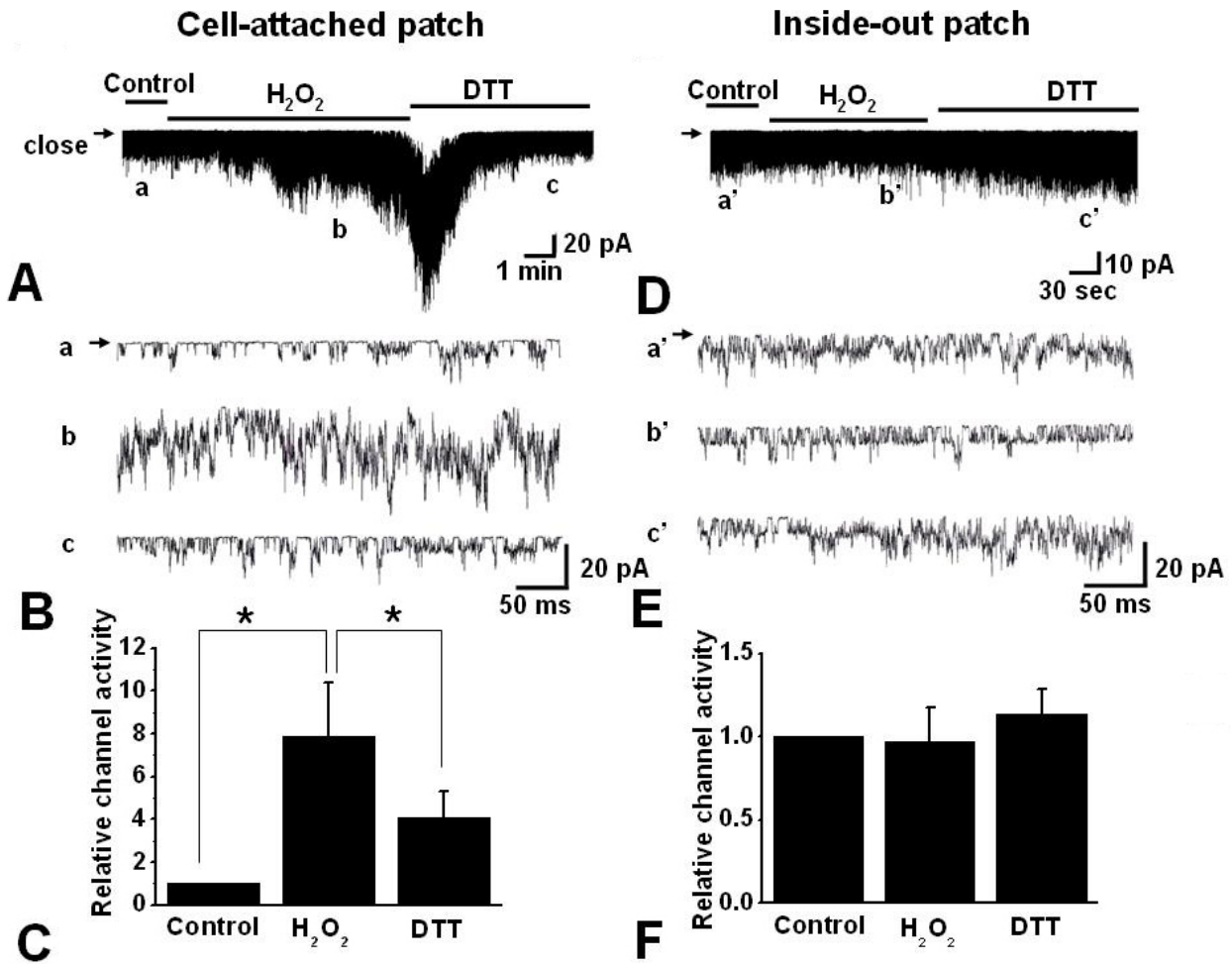

Figure 2. The effects of H_2O_2 and DTT on TREK-2 in cell-attached (A, B, and C) and inside-out patch (D, E, and F) configurations. Current recordings obtained at –40 mV. A and D; Bars above current traces indicate periods of time when H_2O_2 and DTT were applied. Arrows on the left of current traces indicate zero current level representing channel closure. Alphabets under the current traces indicate the time points when current traces shown in an expanded scale (B and E) are obtained. C and F; Summary of the effects of H_2O_2 and DTT on TREK-2 currents in cell-attached and inside-out patch configurations. Channel activity (expressed as number of active channels in a patch x open probability, NP_o) in the presence of H_2O_2 and DTT are normalized to the control NP_o to obtain relative channel activity. Data obtained from six (C) and twenty (F) cells were averaged. Bars represent the mean ± S.E.M. The *asterisks* indicate a significant difference from the respective control (P<0.05).

channels was observed in cell-attached configurations (Figure 2A). The pipette and bath solutions were composed of 140 mM KCl, 5 mM EGTA, 10 mM HEPES, and 1 mM $MgCl_2$. The holding potential was –40 mV. The amplitude of TREK-2 at –40 mV was –4.4 ± 0.3 pA (110 ± 7.5 pS), which was similar to previously reported results, and the channel kinetics showed typical TREK-2 channel kinetics (Figure 2B). Effects of H_2O_2 and DTT on the channel activation did not differ between the perforated patch configuration and the cell-attached patch configuration. In cell-attached patch configuration, addition of H_2O_2 (5 mM) increased the channel activity by 7.8 ± 2.5 folds in symmetrical 140 mM KCl solution, and it was recovered to the control level by DTT (Figure 2C).

To test whether H_2O_2 effect is due to direct redox modulation of TREK-2 channel, we studied effects of H_2O_2 on TREK-2 in excised inside-out patch configurations (Figure 2D). The solutions used for these recordings were the same as those used for the cell-attached patches. In contrast to the effect of H_2O_2 on TREK-2 channels recorded in cell-attached configurations, H_2O_2 had no effect on TREK-2 channels recorded in inside-out configurations (n = 20, Figure 2D, E, and F). We also confirmed that the H_2O_2-induced increase in TREK-2 current was abolished in ruptured whole-cell patches, and even the decrease was observed in some cases.

Furthermore, application of chemical oxidizing agent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 1 mM), to the bath solution in the inside-out patch configuration induced inhibition of TREK-2 activity, which was opposite

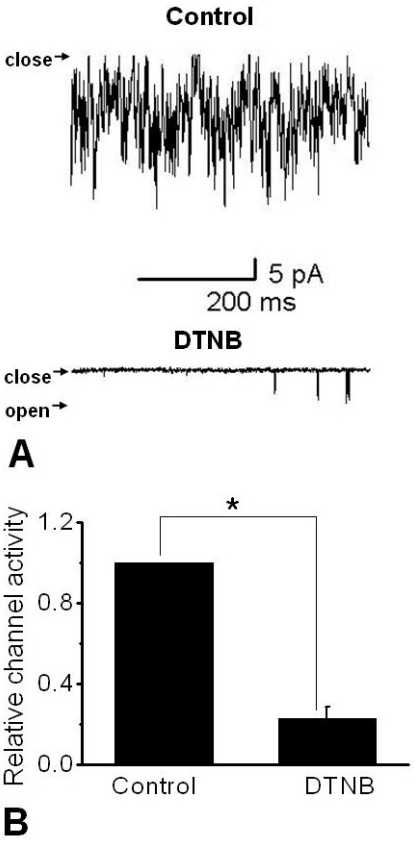

Figure 3. The effects of the chemical oxidizing agent, DTNB on TREK-2 in inside-out patch configurations. A; Current recordings at –40 mV before (*upper panel*) and after (*lower panel*) applying DTNB (1 mM) to the bath. The channel activity of TREK-2 was dramatically reduced by DTNB. B; Summary of the effect of DTNB on TREK-2. The relative channel activity in the presence of DTNB (n= 25). The *asterisk* indicates a significant difference from the respective control (P<0.05).

to the effect of H_2O_2 (Figure 3). This inhibition was reversed by DTT. These results suggest that H_2O_2-induced TREK-2 channel activation is not attributable to direct redox modulation of the TREK-2 channel protein, but involves an activation of signaling pathway that may be disrupted in inside-out patch or in ruptured whole cell patch configurations.

4.2. Myosine light chain kinase (MLCK) is involved in H_2O_2-induced TREK-2 channel activation

To investigate the mechanism of the channel activation by H_2O_2, we tested the involvement of several transduction pathways that may possibly be activated by H_2O_2. We assumed that the activation of TREK-2 by H_2O_2 would result from the increase of intracellular Ca^{2+} or an increase of AA via phospholipase A_2 (PLA_2), because the TREK-2 channel is known to be sensitive to AA, and AA would have arisen from the activation of PLA_2 by intracellular Ca^{2+}. We first tested whether intracellular Ca^{2+} would influence TREK-2 in inside-out patch mode, and found that TREK-2 channel activity was not affected by the application of various intracellular Ca^{2+} concentrations (0, 100 nanomole, 500 nanomole, 1 micromole, and 100 micromole, n = 4). This result indicates that TREK-2 channels are not directly affected by intracellular [Ca^{2+}] increase. To test the possibility that the H_2O_2 effect occurs in a Ca^{2+}-dependent manner, we pretreated cells with BAPTA-AM (20 micromole), a Ca^{2+}-chelator, for 30 min – 1 hr and tested effects of H_2O_2 in Ca^{2+}-free solution, in which possible Ca^{2+} increase was completely prevented. In cell-attached configuration, channel activation by H_2O_2 was still observed (n=6, Figure 4). Next, we examined effects of the cyotsolic PLA_2 inhibitor, $AACOCF_3$, (20 micromole, n = 4) in cell-attached patch configurations, and it did not inhibit TREK-2 activation by H_2O_2. These results indicate that intracellular Ca^{2+} rise or PLA_2/arachidonic acid pathway is not involved in TREK-2 activation by H_2O_2.

Previous reports suggested that H_2O_2 provokes an increase in ion permeability because of rearrangement of the cytoskeleton via Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK) activation in endothelial cells (7, 17). To investigate the possibility that MLCK might be involved in H_2O_2-induced TREK-2 activation, we tested effects of MLCK antagonists, 1-(5-iodonaphthalene-1-sulphonyl)-1H-hexahydrol-1,4-dazepine HCl (ML-7) and 1-(5-chloronaphthalenesulfonyl)-1H-hexahydro-1,4-diazepine HCl (ML-9) on H_2O_2-induced TREK-2 current. For this test, we used the cell-attached patch configuration at holding potential of 0 mV in the presence of a Na-rich solution in the bath and a K-rich solution in the pipette (Figure 5). ML-7 and ML-9 are known to act by binding to or competing with ATP for binding to kinase (18). Basal TREK-2 currents before H_2O_2 application were little affected by the MLCK inhibitors in the cell attached patch mode (Figure 5A). However, H_2O_2-induced TREK-2 activation was inhibited by 82 ± 15% by 2 micromole ML-7 (n = 4) and 85 ± 25% (n = 5) by 10 micromole ML-9, respectively (Figures 5B and 5C). This shows that H_2O_2-induced TREK-2 channel activation may involve MLCK.

To obtain a dose-dependency curve for the effect ML-7 on H_2O_2-induced TREK-2, we measured the channel activity at various concentrations of ML-7. The current trace was obtained from a holding potential of 0 mV and the channel activity was normalized to the fully activated level. The H_2O_2-induced TREK-2 channel activity was reduced by application of 2, 5, and 10 micromole of ML-7 by 50 ± 12%, 70 ± 3%, 90 ± 0.01%, respectively (n = 3) (Figure 6A). Fitting these values to the Hill equation showed that the concentration of ML-7 for half-maximal inhibition was 2.6 micromole (Figure 6B).

5. DISCUSSION

5. 1. TREK-2 as a specific target for H_2O_2-induced signaling

In the present study, we showed that, of four K_{2P} channels (TASK-1, TASK-3, TRAAK, and TREK-2), only TREK-2 can be activated by H_2O_2, and that this effect is mediated by intracellular signal pathways involving myosin light chain kinase (MLCK). We also confirmed that application of the chemical oxidizing agent to K_{2P} channels recorded in inside-out patch configuration induces the

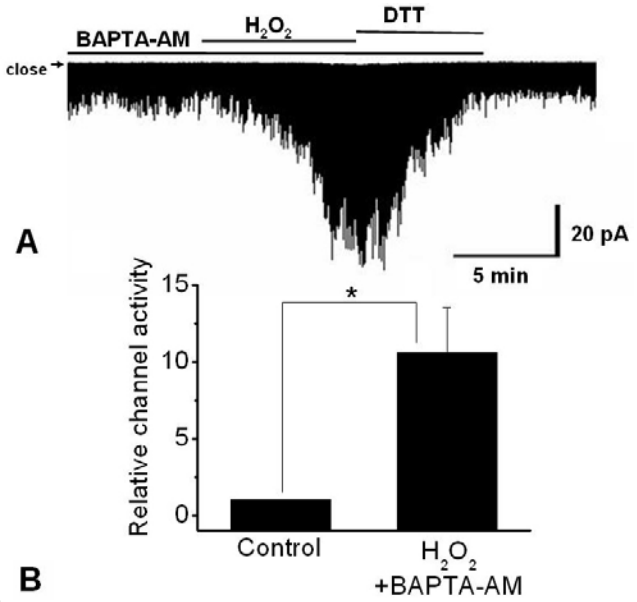

Figure 4. The effect of Ca^{2+} chelator on H_2O_2 –induced TREK-2 current in CHO cells using the cell-attached patch configuration. A; Ca^{2+} -chelator, BAPTA-AM (20 micromole) was applied to the bath 40 min prior to recording. The pipette solution consisted of 140 mM KCl, 5 mM EGTA, 10 mM HEPES, 1 mM $MgCl_2$; the bath solution consisted of 143 mM NaCl, 5.4 mM KCl, 0.5 mM NaH_2PO_4, 0.5 mM $MgCl_2$, 0 mM $CaCl_2$, 1 mM EGTA, 5 mM HEPES, 10 mM glucose. The recording potential was 0 mV. B; Summary of the effect of Ca^{2+} chelator on H_2O_2 –induced TREK-2 current. Data were obtained from estimates of channel activity. Bars represent the mean ± S.E.M. The *asterisk* indicates a significant difference from the respective control (P<0.05)

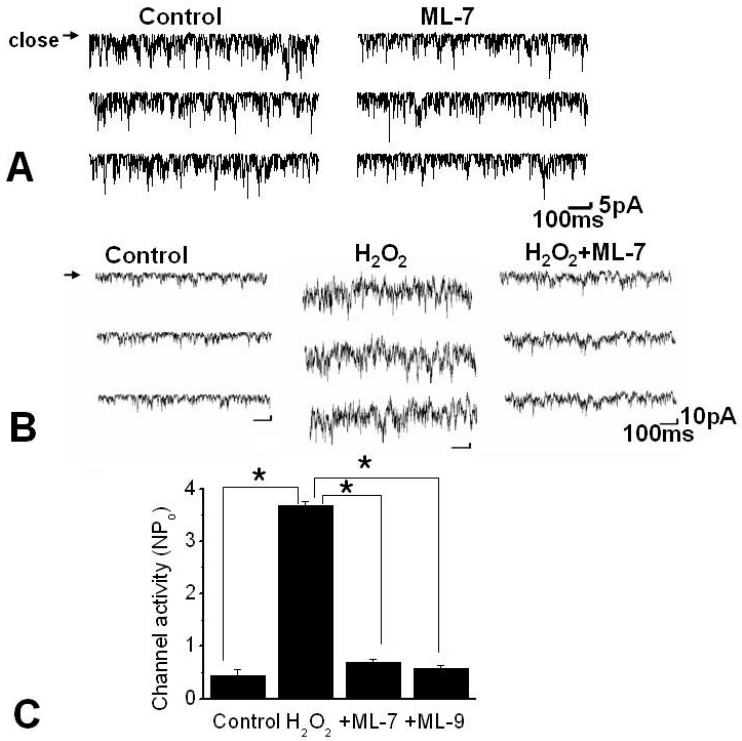

Figure 5. The effect of MLCK inhibitors on TREK-2 current in the absence and presence of H_2O_2. Currents were recorded at 0 mV using the cell-attached patch configuration. A; Tracings show the effect of ML-7 on TREK-2 in the absence of H_2O_2. B; Tracings show the effect of ML-7 on TREK-2 in the presence of H_2O_2. C; Bar graphs show channel activity after treatment of TREK-2 that had been activated by H_2O_2 with ML-7 (2 micromole) and ML-9 (10 micromole). Bar graphs represent mean ± S.E.M. The *asterisks* indicate a significant difference from the respective control (P<0.05).

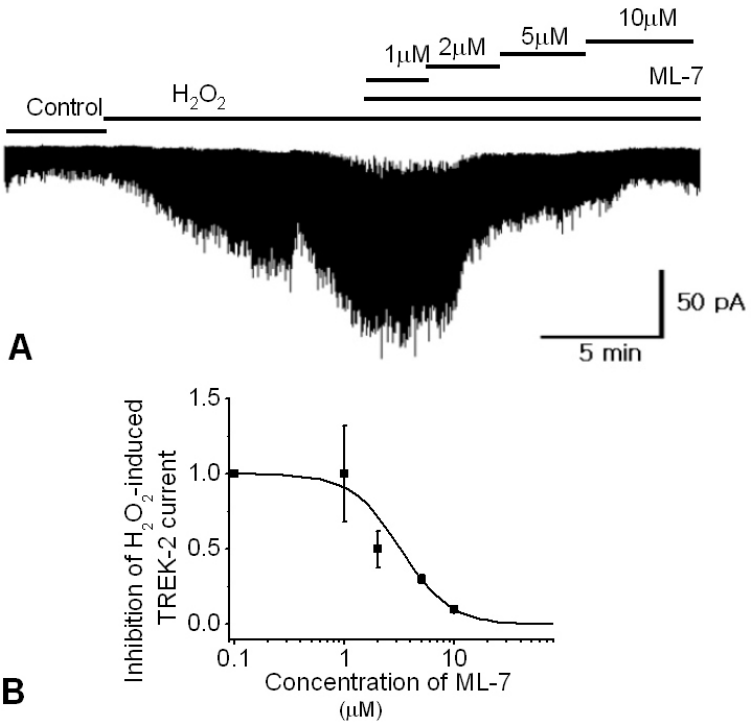

Figure 6. Dose dependence of ML-7 in the inhibition of H_2O_2 –induced TREK-2 current. The pipette solution consisted of 140 mM KCl, 5 mM EGTA, 10 mM HEPES, 1 mM $MgCl_2$; the bath solution consisted of 143 mM NaCl, 5.4 mM KCl, 0.5 mM NaH_2PO_4, 0.5 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, 10 mM glucose. The current trace was obtained from a holding potential of 0 mV and the channel activity was normalized to the fully activated level measured before ML-7 was applied (B).

opposite effect, resulting in the decreased activity, and that this effect is also specific to TREK-2 (data not shown). The target site for this differential effect remains to be investigated. Considering that TASK-1 is known to be oxygen-sensing channels (19, 20), it is therefore rather surprising that TREK-2 channel is the specific target for H_2O_2-induced activation via MLCK and direct inhibition by oxidizing agent.

As far as it is known, regulatory properties of TREK-1 are similar to TREK-2 channel ; both are activated by unsaturated free fatty acid (including arachidonic acids), volatile anesthetics and mechanical stretch (9). In the present study, we did not investigate the effect of H_2O_2 on TREK-1 channels in details, but could observe that H_2O_2 increased TREK-1 activity similarly. In contrast, the effect of chemical oxidizing agent (DTNB) was not observed in TREK-1 (data not shown), but only observed in TREK-2 channel. Considering that TREK-1 has been recognized as a oxygen-sensing channel (21, 22), it will be interesting in future studies to compare the molecular mechanism of TREK-1 activation by hypoxia with that of H_2O_2.

5.2. Involvement of MLCK in H_2O_2-induced signal pathways

Oxidant-mediated signaling mechanisms involve an increase in intracellular Ca^{2+}, protein kinase C (PKC), mitogen-activated protein kinase (MAPK) [extracellular signal-regulated kinase (ERK), c-Jun amino terminal kinase (JNK), p39 MAP kinase (p39 MAPK)], tyrosine kinases, Rho GTPases (Rho A-E, G and H), redox sensitive transcription factors [AP-1 (Jun Fos) and NF-kappaB] and cytokines (TNF-alpha, IL-1alpha). Here, we found that MLCK might be involved in the regulation of H_2O_2-induced activation of TREK-2. The involvement of MLCK in ROS-induced signaling has been suggested in cultured pulmonary endothelial cells (7). Modulation of ion channels by MLCK-dependent phosphorylation was reported in noradrenalin-evoked cation current studies of rabbit portal vein myocytes (23). Direct regulation of channels by MLCK has been demonstrated in guinea pig gastric myocytes (24) and bullfrog sympathetic neurons (25). In addition to these studies, our present study proposes that ion channels are important target for MLCK.

Since it was reported that MLCK contributes to H_2O_2-induced myosin light chain phosphorylation and actin rearrangement and that the rearrangement of the cytoskeleton may affect the activity of ion channels and exchangers (26), we tested the possibility of the involvement of cytoskeletons using cells pretreated with cytochalasin D, an actin disrupting agent, or phalloidin, an actin stabilizing agent (data not shown). The result showed that H_2O_2-induced activation of TREK-2 channels appeared to be affected but was not abolished in these cells. Since the statistical test did not show a significant difference due to a wide variation in basal channels activity, we could not conclude that signaling pathway for H_2O_2-induced activation of TREK-2 channels involves cytoskeletal rearrangement.

5.3. Functional implications

The result of the present study suggests the possibility that ROS can induce K^+ efflux through TREK-2 activation. Increased ion permeability to potassium ions was shown to be involved in neuronal differentiation (27), apoptotic cell death (3), or protection from ischemia and excitotoxicity (28) .

The activation of K^+ channels by insults such as H_2O_2 was suggested to be an initiator of the apoptotic process and K^+-efflux-mediated apoptosis has been reported to exist in various cell types (3). Increase in delayed rectifier K^+ current (29) and HERG channel (30) were reported to be involved in ROS-induced apoptosis. The application of K^+ channel blockers inhibited apoptosis in porcine granulosa cells (31).

Recently, the involvement of K_{2P} channels in apoptotic cell death was suggested. Trimarchi et al (2002) suggested that TASK might underlie the K^+ efflux related to apoptotic shrinkage in mouse embryos (32). The involvement of K_{2P} channels in neuronal development has been suggested in Fioretti et al. (2004) who showed that histamine, an important modulator of developing neurons, activated a background, arachidonic acid-sensitive K^+ channels in chick dorsal root ganglion cells (33). Here we suggest that TREK-2 participates in K^+ efflux after exposure to ROS. Considering that TREK-2 is highly expressed in granule cell layers of cerebellum and olfactory bulb, as well as particular brainstem nuclei, e.g. pontine nucleus, trapezoid body, locus coeruleus, spinal trigeminal nucleus and gigantocellular neurons throughout the reticular formation (34), the role of H_2O_2-induced TREK-2 activation should be investigated in neurons in future study.

6. ACKNOWLEDGMENTS

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Abbreviations: K_{2P} : Two-pore domain K^+, TWIK: weakly inward rectifying two-pore K^+ channel, TASK: TWIK-related acid-sensitive K^+ channel, TREK: TWIK-related K^+ channel, TRAKK: TWIK-related arachidonic acid-stimulated K^+ channel, DTT: dithiothreitol, DTNB: 5,5'-dithiobis-(2-nitrobenzoic acid), hydrogen peroxide (H_2O_2), MLCK : myosin light chain kinase, ML-7: 1-(5-iodonaphthalene-1-sulphonyl)-1H-hexahydrol-1,4-dazepine HCl , ML-9: 1-(5-chloronaphthalenesulfonyl)-1H-hexahydro-1,4-diazepine HCl

Key Words: Two-pore K^+ channel, TREK-2, H_2O_2, DTT, CHO cells, DTNB, MLCK, ML-7

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