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Sulfhydryl Modification Affects Coronary Artery Tension by Changing Activity of Delayed Rectifier K^+ Current

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Abstract

It has been reported that a change in the cellular redox state may be involved in the regulation of vascular tone, but the underlying mechanism is not fully understood. The present study was designed to investigate the cellular effect of sulfhydryl modifying agents in the coronary artery of rabbit using the tension measurement and whole cell clamping method. The application of diamide, a sulfhydryl oxidizing agent, relaxed the endothelium denuded coronary arteries in a dose dependent manner. The fact that this diamide-induced relaxation was significantly attenuated by a pretreatment of 4-AP, and the coronary arteries precontracted with 100 mM K^+ instead of histamine, suggests the involvement of 4-AP sensitive K^+ channels in the diamide-induced relaxation of coronary arteries. Whole cell patch clamp studies revealed that the 4-AP sensitive I_{dK} was significantly enhanced by the membrane permeant oxidizing agents, diamide and DTDP, and were reversed by subsequent exposure to the reducing agent, DTT. Neither the membrane impermeant oxidizing or reducing agents, GSSG or GSH, had any effect on the activity of I_{dK}, indicating that intracellular sulfhydryl modification is critical for modulating I_{dK} activity. The Diamide failed to significantly alter the voltage dependence of the activation and inactivation parameters, and did not change the inactivation process, suggesting that diamide increases the number of functional channels without altering their gating properties. Since I_{dK} has been believed to play an important role in regulating membrane potential and arterial tone, our results about the effect of sulfhydryl modifying agents on coronary arterial tone and I_{dK} activity should help understand the pathophysiology of the diseases, where oxidative damage has been implicated.

Key Words: Coronary artery, delayed rectifier K^+ current, diamide, dithiothreitol, glutathione, redox

INTRODUCTION

One of the major biological targets of free radical oxidation is the cardiovascular system. In particular, the coronary smooth muscle cells are directly exposed to reactive oxygen species during ischemia-reperfusion, atherosclerosis, and hypertension.[1-3] It has been reported that reactive oxidant species markedly affect the vascular tone by changing the transsarcolemmal Ca^{2+} influx or Ca^{2+} release from intracellular stores.[4-6] However, the mechanism for the oxidant induced change of vascular contractility has not been fully characterized.

There is increasing evidence that many cellular effects of reactive oxygen species are mediated by changes in the membrane ionic conductance. Oxidation of sulfhydryl groups on or in an ion channel protein might directly affect the ion channel activity or a change in cellular redox status might modulate the ion channel function.[7,8] There is substantial evidence that the K^+ current activity is regulated by an oxidation/reduction reaction, and the changes in the K^+ current activity can regulate the vascular tone by changing the membrane potential of the vascular smooth muscle cells.[9-11]

At least four different types of macroscopic K^+ currents have been identified in the coronary smooth muscle cells, including the delayed rectifier K^+ current (I_{dK}), Ca-activated K^+ current ($I_{K\text{-}Ca}$), inward rectifier K^+ current (I_{Kir}), and ATP-sensitive K current ($I_{K\text{-}ATP}$).[12-15] Among these currents, I_{dK} is a major determinant of the resting membrane potential and has been shown to be inhibited during atherosclerosis.[16,17] These studies, therefore, suggest that I_{dK} might be the principal target that is affected by reac-

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tive oxygen species. However, there have been no reports on the oxidant-induced change of I_{dK} in the coronary smooth muscle cells, or whether this change in I_{dK} is responsible for the change in coronary tone during oxidative stress.

In light of these uncertainties, the objective of the present study was to determine whether I_{dK} is responsible for oxidant-induced change in the coronary vascular tone using sulfhydryl oxidizing and reducing agents, and to examine the mechanism of how sulfhydryl modifying agents affect I_{dK} using the whole cell patch clamp technique in coronary smooth muscle cells. We present direct evidence that sulfhydryl oxidant increases the activity of I_{dK} in coronary smooth muscle cells, and this enhancement of I_{dK} is responsible for the oxidant-induced relaxation of the coronary artery.

MATERIALS AND METHODS

Measurement of tension

Albino rabbits (2.0−2.5 Kg) of either sex were anaesthetized by injecting 60 mg/Kg sodium pentobarbital into the ear vein, and they were exsanguinated by cutting a femoral artery. The left anterior descending coronary artery was excised and placed into Krebs-Henseleit (KH) solution with the following composition: 136 mM NaCl, 5.4 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 24 mM $NaHCO_3$, 0.01 mM EDTA and 5.5 mM Glucose. The pH of the solution was maintained at 7.4 after bubbling with 95% O_2 and 5% CO_2 gas mixture. Arterial rings with a width of 2−3 mm were prepared for isometric contractile force recordings as described previously.[13] To eliminate any possible involvement of endothelium-derived vasoactive factors, the endothelium was removed by gently rubbing the intimal surface, and its functional integrity was checked by the method of Furchgott and Zawadzki.[18]

The coronary rings were mounted using two triangular stainless wires, with one fixed to the bottom of the tissue bath and the other attached to a force transducer, and the force was continuously recorded using a chart recorder. The KH solution was used as a tissue bath solution, which was continuously oxygenated (95% O_2 and 5% CO_2) and heated to 37°C. The coronary rings were equilibrated for 120 minutes under a 0.5 g of resting tension before each experiment. During equilibration, rings were exposed to 100 mM K^+ solution, which maximally contracted the arteries and ensured the stabilization of the muscle. The 100 mM K^+ solution was prepared by equimolar replacement of NaCl with KCl in a normal KH solution to maintain normal osmolarity and Cl^- concentration. The effect of the sulfhydryl modifying agent was measured after the coronary rings reached a stable maximum contraction with agonist (histamine 10^{-5} M) or 100 mM K^+.

Measurement of I_{dK}

Preparation of the coronary smooth muscle cells: After removing the surrounding connective tissues, the coronary artery was exposed longitudinally and bathed in a Ca^{2+}/Mg^{2+} free Tyrode solution for five The coronary artery was incubated for an additional 60 minutes in a temperature controlled (37°C) water bath after changing with a solution containing 0.1% collagenase. After this incubation, the coronary strips was transferred to a Ca^{2+}/Mg^{2+} free Tyrode solution, and agitated with fine glass rod. Single coronary smooth muscle cells were stocked in the Tyrode solution at 4°C until the experiment.

Patch clamp method: An aliquot of single coronary smooth muscle cells in suspension was added to a recording chamber mounted on a Nikon diaphot inverted microscope. The solutions were superfused at a rate of 1 ml/min, and the experiments were performed at room temperature (23−25°C). The standard whole cell patch clamp technique was used to voltage-clamp the single coronary smooth muscle cells.[19] Borosillicate patch pipettes were pulled on a two-stage horizontal puller and heat polished using a microforge. The tip diameter of each patch pipette was 1−2 μm and the pipette resistance ranged from 2−3 MΩ when the pipette was filled with internal pipette solution.

Voltage command pulses were generated and the resulting membrane currents were measured with an Axopatch 1-D patch clamp amplifier using pClamp software (Version 6.0, Axon Instruments, Foster City, CA, USA). The membrane currents and voltage commands were monitored on a storage oscilloscope and simultaneously stored on a computer hard disk for later analysis. The data were low pass filtered at 5 kHz with an 8-pole Bessel filter and digitized at 25 kHz using a 12 bit A/D converter.

Solutions and chemicals

The tyrode solution for cell preparation contains (mM); NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1.0; HEPES, 10; Glucose 5.5, and the pH was adjusted with Tris to 7.4. The standard micropipette solution for the whole-cell experiments contained 100 mM potassium gluconate, 30 mM KCl, 5.7 mM $MgSO_4$, 5 mM K_2ATP, 1 mM Na_2GTP, 10 mM BAPTA, and 10 mM HEPES, and the pH was adjusted with Tris to 7.2. The standard external solution for the whole-cell experiments contained 135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.5 mM $MnCl_2$, 10 mM glucose, and 10 mM HEPES, and pH was adjusted with Tris to 7.4. All chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

RESULTS

Characterization of vasorelaxation to sulfhydryl oxidizing agent

As shown in Fig. 1, diamide (sulfhydryl oxidizing agent) produced concentration dependent relaxation of the endothelium-denuded coronary ring preparations precontracted with histamine (10^{-5} M). On average, 0.3 mM diamide induced a $78.3 \pm 4.6\%$ relaxation (n=10). The relationship between the concentration response and diamide-induced relaxation was determined, and the EC_{50} was calculated to be $62.3 \pm 0.2\ \mu M$ (n=7). This diamide induced relaxation was completely reversed by applying DL-dithiothreitol (DTT, 1 mM), a sulfhydryl reducing agent (n=10).

To elucidate the underlying mechanism of the diamide-induced relaxation, we first tried to compare the relaxing effect of diamide under different external K^+ concentration conditions. The coronary rings exhibited a contraction after exposure to 100 mM K^+ solution due to an increased Ca^{2+} influx via depolarization of the membrane, and this depolarization reduced the driving force for the K^+ efflux. In the coronary rings precontracted with 100 mM K^+, the relaxation responses of diamide were significantly attenuated relative to the case of a normal external K^+ concentration (5.6 mM). The relaxing effect of 0.3 mM diamide in 80 mM K^+-induced contraction was $23.1 \pm 10.2\%$, which was significantly attenuated compared to the effects of diamide in the histamine-induced contraction ($p < 0.01$, n=10, Fig. 2A and C).

These results suggest the involvement of the K^+ channel in the diamide-induced relaxation response. In rabbit coronary smooth muscle cells, I_{dK} plays an important role in determining the resting membrane potential and vascular tension[16], so we measured the effect of pretreating with 4-aminopyridine (4-AP, a blocker of I_{dK}[20]) on the diamide-induced relaxation response. The 4-AP pretreatment significantly attenuated relaxation response induced by 0.3 mM diamide ($78.3 \pm 4.6\%$ vs. $24.1 \pm 3.2\%$ in the control vs. in the 4-AP, n=10, $p < 0.01$, Fig. 2B and C).

Sulfhydryl oxidizing agents increase I_{dK}

The result obtained from the tension measurement

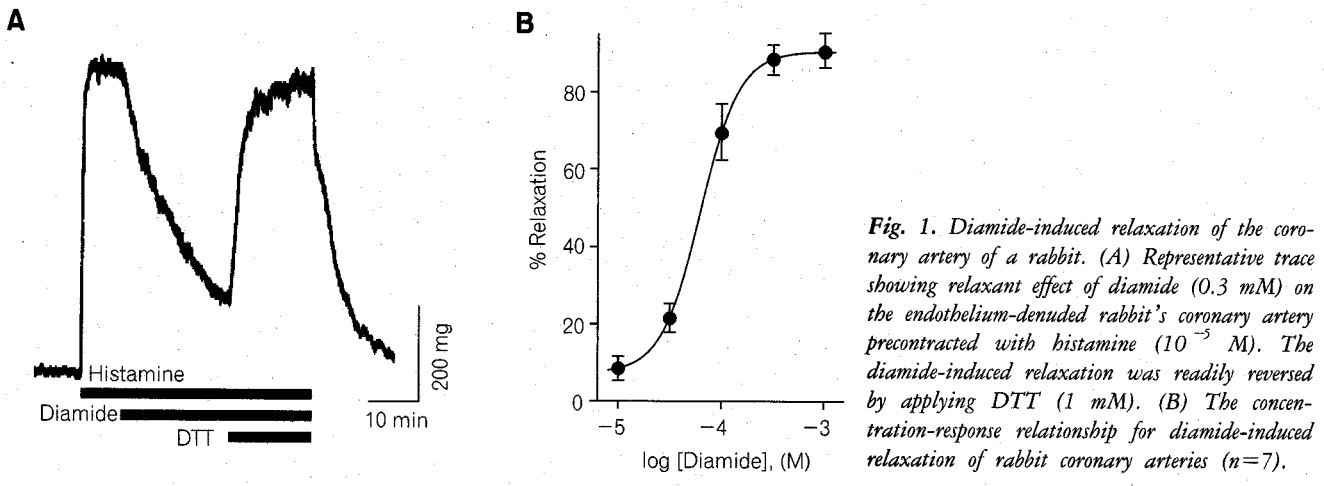

Fig. 1. Diamide-induced relaxation of the coronary artery of a rabbit. (A) Representative trace showing relaxant effect of diamide (0.3 mM) on the endothelium-denuded rabbit's coronary artery precontracted with histamine (10^{-5} M). The diamide-induced relaxation was readily reversed by applying DTT (1 mM). (B) The concentration-response relationship for diamide-induced relaxation of rabbit coronary arteries (n=7).

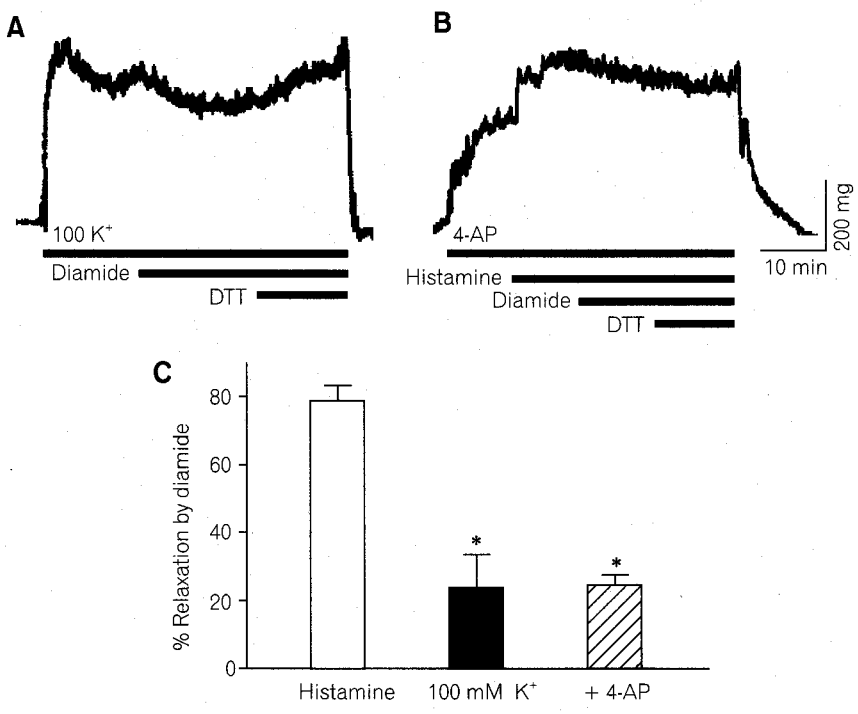

*Fig. 2. Inhibition of the diamide-induced relaxation by high external K^+ and 4-AP. Typical traces showing the suppression of diamide-induced relaxation in a coronary artery precontracted with high external K^+ (A) and by pretreatment of 10 mM 4-AP in a histamine-induced contraction (B). (C) Comparison of the diamide-induced relaxation responses in coronary arteries precontracted with histamine, 4-AP pretreatment, and in arteries precontrated with 100 mM KCl. * represents statistically significant data (n=10).*

strongly suggest the involvement of I_{dK} in the diamide-induced relaxation responses in the coronary rings, so a whole cell patch clamp was applied to determine whether I_{dK} increased due to sulfhydryl oxidizing agent. For the whole-cell experiments, I_{dK} was isolated from $I_{K\text{-}Ca}$ with 10 mM BAPTA in the pipette solution to buffer the intracellular Ca^{2+} and also by replacing the external Ca^{2+} with Mn^{2+}. Under these experimental conditions, the contribution of $I_{K\text{-}Ca}$ to the total outward current was minimal or absent, especially at potentials less than +30 mV, as assessed using iberiotoxin, a specific blocker of $I_{K\text{-}Ca}$ channels.[21] The contribution of $I_{K\text{-}ATP}$ to the macroscopic outward currents was minimized by adding 5 mM ATP in the internal solution.[22] These experimental conditions were used for all whole-cell experiments reported in this study

Fig. 3A shows the representative whole-cell I_{dK} currents elicited by a series of depolarizing voltage-clamp steps over the range −50 to +25 mV from a holding potential of −80 mV during the control, and 10 minutes after superfusion with the membrane permeant oxidizing agent, 1 mM diamide[23]. During exposure to diamide, the amplitudes of I_{dK} were higher over the entire range of potentials. In most of the cells that we examined, the diamide increased I_{dK} at the concentration greater than 10 μM, and reached to a maximum effect at 1 mM. When we used 1 mM diamide, the I_{dK} typically started to increase within one minute of its application, and washing the bath with a diamide free solution for up to ten minutes did not reverse the effect of diamide on enhancing the I_{dK} amplitude. This diamide-induced enhancement of I_{dK} was completely reversed by applying DTT (Fig. 3B). It is interesting to note that DTT completely reversed the effects of diamide with the I_{dK} currents amplitudes returning to nearly the same as in the control, suggesting that these effects can be attributed to alterations in redox state of I_{dK} channel. Fig. 3C shows the relationship between the mean current and applied voltage of I_{dK} obtained from six different cells under control conditions, after ten minutes of superfusion with diamide, followed by superfusion with DTT (n=6).

We also tested the effect of another sulfhydryl modifying agent on the amplitude of I_{dK}. When we used a 2,2-dithiodipyridine (DTDP), a membrane permeable oxidizing agent,[24] produced a similar enhancement of I_{dK} as with the diamide treatment. However, applying the oxidized form of glutathione (GSSG), a membrane impermeable oxidizing agent or the reduced form of glutathione (GSH), a membrane impermeable reducing agent showed no apparent effect on I_{dK}. These results suggest that a modifica-

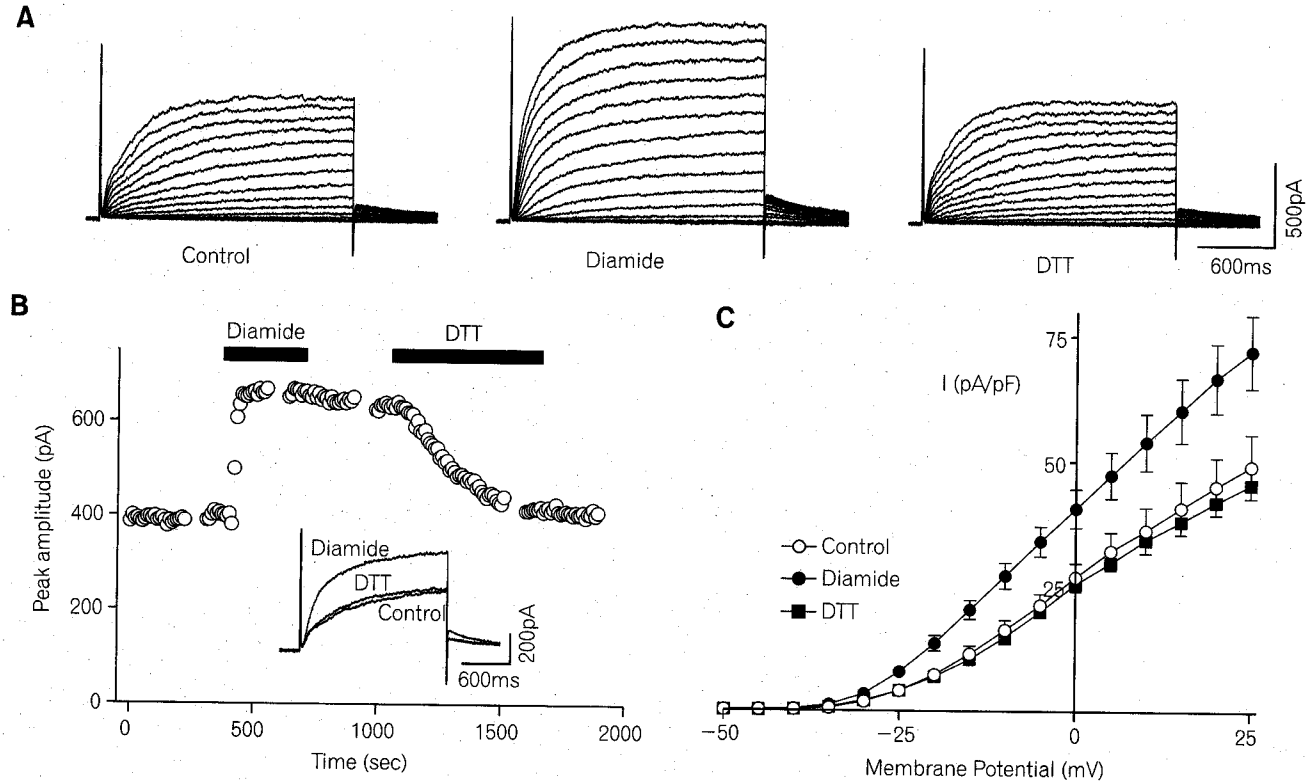

Fig. 3. The Effect of diamide on I_{dK} in rabbit coronary arterial smooth muscle cells. K^+ currents were evoked by stepping the membrane potential from a holding potential of −80 mV to potentials ranging from −50 to +25 mV in 5 mV increments. (A) A family of K^+ currents was recorded under control, 1 mM diamide, and after 5 mM DTT. (B) A plot of K^+ current amplitude, evoked by repetitive step depolarizations from −80 mV to 0 mV versus time. The horizontal bars show the time during which the cells were exposed to diamide (1 mM) or DTT (5 mM). The inset shows the representative current traces during control, 1 mM diamide, and 5 mM DTT. (C) A summary of diamide and DTT effects on the current-voltage relations of I_{dK} (n=6). The current amplitudes were normalized with cell capacitance.

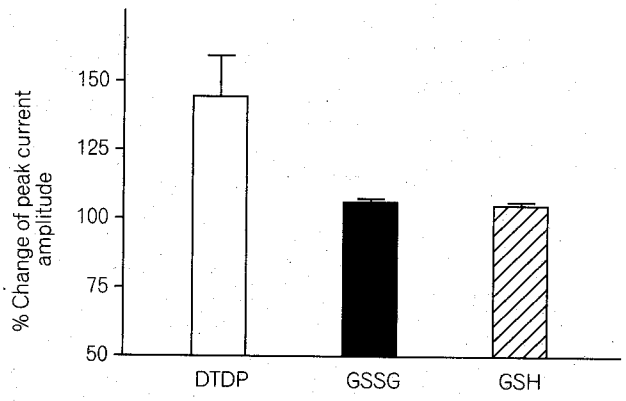

Fig. 4. Comparison of membrane permeable and impermeable sulfhydryl modifying agents on the activity of I_{dK}. The relative changes in I_{dK} in response to 0 mV voltage steps after treatment with 50 μM DTDP (n=5), 5 mM GSSG (n=3), and 5 mM GSH (n=3).

tion of sulfhydryl in I_{dK} from the intracellular side is critical for modulating I_{dK} activity (Fig. 4).

Effects of oxidizing agents on gating properties of I_{dK}

Several possible mechanisms exist that explain the diamide-induced enhancement of I_{dK}. It is possible that diamide enhances the I_{dK} by altering the voltage dependence of the steady state activation or inactivation so that more channels are open at a lesser negative voltage or more channels are available to open on depolarization.[25] To test these possibilities, we examined the effects of diamide on the voltage-dependence of the steady-state activation and inactivation of I_{dK}. The midpoint of the activation curve was not affected statistically by diamide (-16.2 ± 0.5 mV in the control group and -18.1 ± 0.5 mV in the diamide groups; $p>0.05$, $n=6$). The midpoint of the inactivation curve before and after oxidation was also statistically insignificant (-32.8 ± 0.4 and -31.8 ± 0.4 mV in the control and diamide groups, respectively; $p>0.05$, $n=7$). These results suggest that

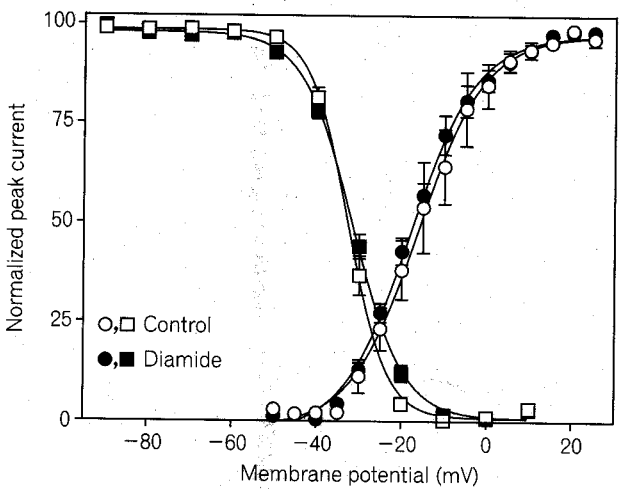

Fig. 5. The effects of diamide on the steady-state activation and inactivation of I_{dK}. The voltage-dependence of the steady-state activation was determined using a standard double-pulse protocol as described below. The K^+ currents were elicited by 280 ms depolarizing steps from −50 to 25 mV in 5 mV increments from a holding potential of −80 mV, and the tail currents that were evoked upon repolarization to −50 mV for 400 ms were measured with control and after bath application of 1 mM diamide. The normalized tail current amplitudes were fit to a Boltzman equation, $Y=\{1+\exp\{(V_{1/2}-V)/k\}\}^{-1}$, where $V_{1/2}$ represents half-maximal activation potential and k is the slope factor. Under control conditions (○), $V_{1/2}=-16.2\pm0.5$ mV and $k=7.8\pm0.5$, and after 1 mM diamide treatment (●), $V_{1/2}=-18.1\pm0.5$ mV and $k=7.8\pm0.5$ ($n=6$). In order to measure the steady state inactivation parameters, the K^+ currents were elicited by 20 s preconditioning pulses from −90 to 10 mV in 10 mV increments from a holding potential of −80 mV and a 1 s test pulse to 20 mV was applied with control and after bath application of 1 mM diamide. The normalized peak amplitudes of the test pulses were fit to a Boltzman equation, $Y=\{1+\exp\{(V-V_{1/2})/k\}\}^{-1}$, where $V_{1/2}$ represents half-maximal inactivation potential and k is the slope factor. Under control conditions (□), $V_{1/2}=-32.8\pm0.4$ mV, $k=-4.4\pm0.3$ and after diamide treatment (■), $V_{1/2}=-31.8\pm0.4$ mV and $k=-5.8\pm0.4$ ($n=7$). Diamide treatment did not affect the voltage dependence of the activation and inactivation parameters of I_{dK} ($p>0.05$).

diamide enhanced the I_{dK} without altering the steady-state voltage dependency (Fig. 5).

It has been reported that a slowed inactivation may enhance the peak current amplitude,[26,27] so we examined the kinetics of inactivation before and after applying the diamide. Fig. 6A shows typical current traces obtained in the control and after the diamide. These currents were evoked by 20 s steps to +20 mV from a holding potential of −80 mV. The inactivation time course was well described by the sum of 2 exponentials, and the inactivation time constants at 20 mV from eight different cells are plotted in Fig.

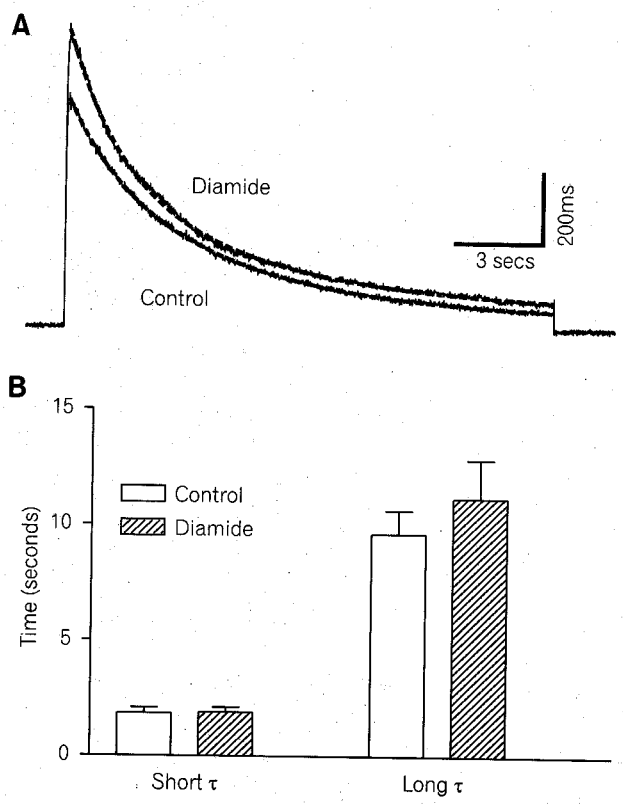

Fig. 6. Diamide did not alter the inactivation time course of I_{dK}. (A) Representative I_{dK} currents recorded in response to 20 s pulses to 20 mV before and after the diamide treatment. The current traces were fitted well with double exponential functions and showed as dotted lines through the original current traces. (B) A summary of the inactivation times constant of I_{dK} was obtained for 11 cells. After applying the diamide, the fast inactivation time constant was changed from 1856.8±239.4 to 1907.8±215.1 ms ($p>0.05$), and the slow inactivation time constant was slightly increased from 9611.6±1026.4 to 11176.4±1669.9 ms, but it was statistically insignificant ($p>0.05$).

6B. The diamide did not affect either the fast time constants (1856.8±239.4 in the control group and 1907.8±215.1 ms in the diamide group; $p>0.05$, $n=8$) or the slow time constant (9611.6±1026.4 in the control group and 11176.4±1669.9 ms in the diamide group; $p>0.05$, $n=8$) of I_{dK} recorded at 20 mV depolarizing step.

DISCUSSION

Our findings clearly indicate that I_{dK} in rabbit coronary smooth muscle cells can be regulated by the cellular redox status, and this change in I_{dK} activity might be responsible for the oxidant-induced change

of coronary arterial tone. The redox regulation of K^+ channels in vascular smooth muscle cells has previously been demonstrated and is suggested to play an important potential role in the modulation of K^+ channels and resting membrane potential during oxidative stress.[10,28] However, such effects have not been demonstrated for I_{dK} in coronary smooth muscle cell, even though this current is known to be an important determinants of the resting membrane potential and tension in the coronary artery of rabbits.

Involvement of K^+ channel activation in diamide-induced relaxation of coronary artery

In the present study, diamide relaxed a coronary artery precontracted with agonist, a histamine. In contrast, diamide-induced relaxation was significantly attenuated in the coronary arteries precontracted by high extracellular K^+. Raising the extracellular K^+ decreases the driving force for K^+ efflux because the electromotive force for K^+ flux ($E_m - E_K$) becomes smaller in high external K^+ than in normal external K^+. In this case, Activating or opening the K^+ channels had a lesser effect on the membrane potential and tension.[29] The decreased ability of diamide to relax arteries precontracted with high external K^+ suggested that the diamide-induced relaxation involved the activation of the K^+ channels.

At least four different types of macroscopic K^+ currents have been identified in cthe oronary smooth muscle cells.[12-15] Among these currents, I_{dK} is a major determinant of the resting membrane potential and important effector systems that mediate changes in the agonist induced vascular tone by altering the membrane potential in the coronary artery of rabbits.[16] As shown in Fig. 2B, a pretreatment of 4-AP (a effective blocker of I_{dK}) significantly attenuated the diamide induced coronary relaxation. Moreover, pretreatment with a low dose of TEA (a sensitive blocker of $I_{K\text{-}Ca}$[30]) had only a small effect on diamide-induced coronary relaxation (data not shown). These results best fit the hypothesis that diamide relaxed the coronary artery by activating the I_{dK} in the coronary smooth muscle cells.

Mechanism of diamide-induced activation of I_{dK}

Several precautions were taken in the present study to insure effective isolation of I_{dK} from contamination by $I_{K\text{-}Ca}$, $I_{Cl\text{-}Ca}$, or $I_{K\text{-}ATP}$. We included 5 mM ATP in the pipette solution to inhibit $I_{K\text{-}ATP}$ and the activity of $I_{K\text{-}Ca}$, $I_{Cl\text{-}Ca}$ was minimized by using 10 mM BAPTA to buffer the intracellular Ca^{2+} and replacing the external Ca^{2+} with Mn^{2+}. Under these conditions, we were able to record a time and voltage dependent outward current that represented the properties of I_{dK}. Furthermore, this current was markedly inhibited by 4-AP, a effective blocker of I_{dK}. The contribution of I_{K1} on the outward current also seemed negligible, since the density of I_{K1} in the coronary artery was very low compared to the I_{dK} and the voltage we used to record the I_{dK} was much more positive from E_K, where the activity of I_{K1} was minimal[15].

The I_{dK} was enhanced by the membrane permeable oxidizing agents, diamide and DTDP, and this enhancement was completely reversed by the reducing agent, DTT. These effects are consistent with the results of earlier studies, which demonstrated that a variety of voltage dependent K^+ channels have a critical cysteine residue, and that the changing of the redox status of these cysteine residues affect the channel function.[26,27] We assumed in our experiments the intracellular cysteine residues were responsible for the diamide or DTDP-induced increase in I_{dK}, since GSSG and GSH (two membrane impermeable oxidizing and reducing agents) had a very small effect on the I_{dK} activity.

It is still uncertain whether diamide or DTDP directly interact with the cysteine residues of a channel protein. Previous studies have proposed a variety of molecular pathways that can be stimulated by oxidizing agents. For example, applying H_2O_2 can activate guanylate cycylase, protein kinase C, and phospholipase A_2 in the vascular smooth muscle cells, which might be related to the oxidant-induced change of vascular tone.[28,31] However, the involvement of these intracellular messenger systems in our diamide-induced coronary relaxation or activation of I_{dK} seems unlikely, since pretreatment of indomethacin or eicosatrinoic acid did not inhibit the diamide-induced relaxation in our preparation (unpresented results). Furthermore, in the canine pulmonary artery, diamide could activate I_{dK} in the cell-free, inside-out patches, even in the presence of protein kinase C or protein kinase G inhibitors.[32]

In many voltage dependent channels, oxidizing agents alter the gating properties by changing the redox status of the cysteine residue of the channel

protein, which may be related to the change in channel activity. For example, oxidizing the methionine or cysteine residue of the transient outward K^+ current enhanced the current amplitude with concomitant prolongation of inactivation.[26,27] It is possible that the diamide-induced enhancement of I_{dK} in our preparation was due to prolonged inactivation process or negative shift of the steady state voltage dependency. However, the diamide failed to significantly alter the voltage dependence of activation or inactivation parameters, and it did not change the inactivation process.

There are some possibilities that explain these discrepancies between the enhancement of I_{dK} and failure of alteration of gating properties. Firstly, The macroscopic I_{dK} current in the smooth muscle cells have been attributed to multiple types of delayed rectifier K^+ channels,[33] and it is conceivable that the possible differential effects on these multiple channels might prevent the detection of clear-cut alterations in the composite channel gating properties at the macroscopic current level. Secondly, oxidation of the cysteine residues by diamide might increase the number of functional channels in the cell without altering the gating properties. However, the precise biophysical mechanism responsible for the redox alteration of I_{dK} requires substantially more information than is currently available on the molecular identity of the delayed rectifier K^+ channels in coronary smooth muscle cells.

In summary, these data suggest that the delayed rectifier K channels in coronary smooth muscle cells contain at least one cysteine residue on the cytoplasmic side of the membrane, which is readily available for redox modulation. Oxidation of this site(s) is capable of increasing the magnitude of I_{dK}, and inducing the vasorelaxation by hyperpolarizing the membrane potential in rabbit coronary smooth muscle cells. Understanding the mechanism of oxidant-induced relaxation might help understand the pathophysiologic progress of those diseases in which oxidative damage has been implicated.

REFERENCES

1. Rubanyi GM. Vascular effects of oxygen-derived free radicals. Free Radic Biol Med 1988;4:107-20.
2. Prasad K, Kalra J, Chan WP. Effects of oxygen free radicals on cardiovascular function at organ and cellular levels. Am Heart J 1989;117:1196-202.
3. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-9.
4. Krippeit-Drews P, Harberland C, Fingerle J, Drews G, Lang F. Effects of H_2O_2 on membrane potential and $Ca^{2+}{}_i$ of cultured rat arterial smooth muscle cells. 1995;209: 139-45.
5. Bielefeldt K, Whiteis CA, Sharma RV, Abbound FM, Conklin JL. Reactive oxygen species and calcium homeostasis in cultured human intestinal smooth muscle cells. Am J Physiol 1997;272:G1439-50.
6. Suzuki YJ, Cleemann L, Abernethy DR, Morad M. Glutathione is a cofactor for H_2O_2-mediated stimulation of Ca^{2+}-induced Ca^{2+} release in cardiac myocytes. Free Radic Biol Med 1998;24:318-25.
7. Lacampagne A, Duittoz A, Bolanos P, Peineau N, Argibay JA. Effect of sulfhydryl oxidation on ionic and gating currents associated with L-type calcium channels in isolated guinea pig ventricular myocytes. Cardiovasc Res 1995;30:799-806.
8. Fearon M, Palmer ACV, Balmforth AJ, Ball SG, Varadi G, Peers C. Modulation of recombinant human cardiac L-type Ca channel α_{1C} subunits by redox agents and hypoxia. J Physiol 1999;514:629-37.
9. Duprat F, Guillemare E, Romey G, Fink M, Lesage F, Lazdunski M, et al. Susceptibility of cloned K^+ channels to reactive oxygen species. Proc Natl Acad Sci USA 1995;92:11796-800.
10. Post JM, Weir EK, Archer SL, Hume JR. Redox regulation of K+ channels and hypoxic pulmonary vasoconstriction. In: Weir EK, Hume JR, Reeves JT, editors. Ion Flux in Pulmonary Vascular Control. New York, NY: Plenumn Publishing Corporation; 1993. p.189-204.
11. Wang ZW, Nara M, Wang YX, Kotlikoff MI. Redox regulation of large conductance Ca^{2+}-activated K^+ channels in smooth muscle cells. J Gen Physiol 1997;110:35-44.
12. Volk KA, Shibata EF. Single delayed rectifier potassium channels from rabbit coronary artery myocytes. Am J Physiol 1993;264:H1146-53.
13. Ahn DS, Jeong YK, Lee YH, Kang BS. Activation of Ca^{2+}-activated K^+ channels by beta agonist in rabbit coronary smooth muscle cells. Yonsei Med J 1995;36:232-42.
14. Gollasch M, Ried C, Bychkov R, Luft FC, Haller H. K^+ currents in human coronary artery vascular smooth muscle cells. Circ Res 1996;78:676-88.
15. Quayle JM, Dart C, Standen NB. The properties and distribution of inward rectifier potassium currents in pig coronary arterial smooth muscle. J Physiol 1996;494:715-26.
16. Leblanc N, Wan X, Leung PM. Physiological role of Ca^{2+}-activated and voltage-dependent K^+ currents in rabbit coronary myocytes. Am J Physiol 1994; 266:C1523- 37.
17. Ahn DS, Ha My, Lee CH, Kang BS. Effect of lysophosphatidylcholine on whole cell K^+ current in rabbit coronary smooth muscle cells. FASEB J 1999;13:A514.
18. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980;288:373-6.
19. Hamil OP, Marty A, Neher El, Sakmann B, Sigworth FJ.

Improved patch clamp techniques for high resolutuion current recording from cells and cell-free membrane patches. Pflugers Arch 1981;391:85-100.
20. Robertson BE, Nelson MT. Aminopyridine inhibition and voltage dependence of K^+ currents in smooth muscle cells from cerebral arteries. Am J Physiol 1994; 267:C1589-97.
21. Ahn DS, Hume JR. pH regulation of voltage-dependent K^+ channels in canine pulmonary arterial smooth muscle cells. Pflugers Arch 1997;433:758-65.
22. Clapp LH, Gurney AM. ATP sensitive K^+ channels regulate resting potential of pulmonary arterial smooth muscle cells. Am J Physiol 1992;262:H916-20.
23. Kosower NS, Kosower ED. Diamide: An oxidant probe for thiols. Methods Enzymol 1995;251:123-33.
24. Chiamvimonvat N, O'Rourke B, Kamp TJ, Kallen RG, Hofman F, Flockerzi V, et al. Functional consquences of sulfhydryl modification in the pore forming subunits of cardiovascular Ca^{2+} and Na^+ channels. Circ Res 1995;76: 325-34.
25. Li A, Segui J, Heinemann SH, Hoshi T. Oxidation regulates cloned neuronal voltage dependent Ca^{2+} channels expressed in xenopus oocytes. J Neurosci 1988;18:6740-7.
26. Ruppersberg JP, Stocker M, Pongs O, Heinemann SH, Frank R, Koenen M. Regulation of fast inactivation of cloned mammalian $I_{K(A)}$ channels by cysteine oxidation. Nature 1991;352:711-4.
27. Ciorba MA, Heienmann SH, Weissbach H, Brot N, Hoshi T. Regulation of voltage dependent K^+ channels by methionine oxidation: effect of nitric oxide and vitamine C. FEBS Lett 1999;442:48-52.
28. Barlow RS, White RE. Hydrogen peroxide relaxes porcine coronary arteries by stimulating BK_{Ca} channel activity. Am J Physiol 1998;275:H1283-9.
29. Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. Am J Physiol 1995;268:C799-C822.
30. Nelson MT, Patlak JB, Worley JF, Standen NB. Calcium channels, potassium channels and voltage dependence of arterial smooth muscle tone. Am J Physiol 1990;259:C3-C18.
31. Yang ZW, Zheng T, Zhang A, Altura B, Altura B. Mechanisms of hydrogen peroxide-induced contraction of rat aorta. Eur J Pharmacol 1998;344:169-81.
32. Ahn DS, Hume JR. Sulfhydryl modification of delayed rectifier K^+ currents in canine pulmonary smooth muscle cells. Biophys J 1997;72:A263.
33. Carl A. Multiple component of delayed rectifier K^+ current in canine colonic smooth. J Physiol 1995;484:339-53.