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Thiol-dependent Redox Mechanisms in the Modification of ATP-Sensitive Potassium Channels in Rabbit Ventricular Myocytes

Jin Han, Nari Kim, Dang Van Cuong, Chunghui Kim, and Euiyong Kim

Department of Physiology and Biophysics, College of Medicine, Inje University, Busan 614–735, Korea

Cellular redox state is known to be perturbed during ischemia and that Ca^{2+} and K^+ channels have been shown to have functional thiol groups. In this study, the properties of thiol redox modulation of the ATP-sensitive K^+ (K_{ATP}) channel were examined in rabbit ventricular myocytes. Rabbit ventricular myocytes were isolated using a Langendorff column for coronary perfusion and collagenase. Single-channel currents were measured in excised membrane patch configuration of patch-clamp technique. The thiol oxidizing agent 5,5'-dithio-bis-(2-nitro-benzoic acid) (DTNB) inhibited the channel activity, and the inhibitory effect of DTNB was reversed by dithiothreitol (disulfide reducing agent; DTT). DTT itself did not have any effect on the channel activity. However, in the patches excised from the metabolically compromised cells, DTT increased the channel activity. DTT had no effect on the inhibitory action by ATP, showing that thiol oxidation was not involved in the blocking mechanism of ATP. There were no statistical difference in the single channel conductance for the oxidized and reduced states of the channel. Analysis of the open and closed time distributions showed that DTNB had no effect on open and closed time distributions shorter than 4 ms. On the other hand, DTNB decreased the life time of bursts and increased the interburst interval. N-ethylmaleimide (NEM), a substance that reacts with thiol groups of cystein residues in proteins, induced irreversible closure of the channel. The thiol oxidizing agents (DTNB, NEM) inhibited of the K_{ATP} channel only, when added to the cytoplasmic side. The results suggested that metabolism-induced changes in the thiol redox can also modulate K_{ATP} channel activity and that a modulatory site of thiol redox may be located on the cytoplasmic side of the K_{ATP} channel in rabbit ventricular myocytes.

Key Words: K_{ATP} channel, Patch-clamp technique, Rabbit ventricular myocytes, Thiol redox

INTRODUCTION

A number of potassium channels are present in cardiac myocytes, where they serve various roles critical for the electrophysiological function of the heart, ranging from the maintenance of the membrane potential to changes in action potential duration and pacemaker activity. One of these, called the ATP-sensitive K^+ (K_{ATP}) channels, is thought to activate when intracellular ATP levels drop during conditions of metabolic impairment, such as under hypoxia or myocardial ischemia. Discovered in cardiac myocytes (Noma, 1983), the channels have also been identified in many other cell types including pancreatic β-cells in relation with glucose-induced insulin secretion (Ashcroft et al, 1984; Fosset et al, 1988; Ashcroft & Ashcroft, 1990), skeletal (Spruce et al, 1985; Burton et al, 1988; Davies, 1990) and smooth muscle cells (Standen et al, 1989; Clapp & Gurney, 1992; Beech et al, 1993; Bonev & Nelson, 1993), central and peripheral neurons (Jonas et al, 1991; Ohno-Shosaku & Yamamoto, 1992), as well as epithelial and follicular cells (Honore & Lazdunski, 1993). The properties of K_{ATP} channels vary between cell types, leading to the premise that this K^+ channel family may be composed of heterogenous K^+ channel proteins.

The functional role of K_{ATP} channels has been well established in pancreatic β-cells, where these channels mediate glucose-induced insulin secretion (Ashcroft & Rorsman, 1989; Dune & Petersen, 1991). K_{ATP} channels participate in diverse cellular functions in the body, such as secretion of growth hormone (Bernardi et al, 1991), smooth muscle relaxation and vasodilation (Standen et al, 1989; Daut et al, 1990; Nelson et al, 1990; Jackson et al, 1993; Nelson, 1993; Dart et al, 1994; Daut et al, 1994; Quayle & Standen, 1994), regulation of skeletal muscle excitability (Weik & Neumcke, 1989), neurotransmitter release (Amoroso et al, 1990), excitability of neuronal tissues (Mourre et al, 1989), appetite control in the

Corresponding to: Jin Han, Department of Physiology and Biophysics, College of Medicine, Inje University, Kaegeum-Dong, Busanjin-Gu, Busan 614-735, Korea. (Tel) +82-51-890-6714, (Fax) +82-51-894-4500, (E-mail) phyhanj@ijnc.inje.ac.kr

ABBREVIATIONS: K_{ATP} channels, ATP-sensitive K^+ channels; DTNB, 5,5'-dithio-bis-(2-nitro-benzoic acid); DTT, dithiothreitol; NEM, N-ethylmaleimide.

hypothalamus (Ashford et al, 1990), K^+ recycling in renal epithelia (Tsuchiya et al, 1992), and oocyte maturation (Wibrand et al, 1992).

When activated pharmacologically (Fosset et al, 1988), these channels drastically reduce action potential duration, and it has been suggested that they may be responsible for action potential shortening and the cellular loss of K^+ that occurs during various forms of metabolic stress, including ischemia, hypoxia, and inhibition of glycolysis and/or oxidative phosphorylation (Fosset et al, 1988; Elliott et al, 1989; Findlay et al, 1989; Faivre & Findlay, 1990; Nichols & Lederer, 1991; Nichols et al, 1991; Escande & Henry, 1993; Findlay, 1994). During early ischemia, opening of K_{ATP} channels and the electrophysiological effect of extracellular K^+ accumulation may lead to conditions that promote the induction of cardiac arrhythmias (Gasser & Vaughan-Jones, 1990; Wilde et al, 1990; Wilde, 1993; Wilde & Janse, 1994). Opening of K_{ATP} channels has also been implicated as cardioprotective mechanism underlying ischemia-related preconditioning (Downey, 1992; Gross & Auchampach, 1992; Cole, 1993; Grover et al, 1993; Yao et al, 1993; Grover, 1994; Parratt & Kane, 1994; Han et al, 2002a, 2002b). In ischemia-reperfusion models, pretreatment of the myocardium with K^+ channel-opening drugs, that open K_{ATP} channels, delays the time of ischemic contracture and the occurrence of irreversible cell injury (Quast, 1992; Edwards & Weston, 1993; Gopalakrishnan et al, 1993). Furthermore, sulfonylureas, which are specific blockers of channels, abolish the cardioprotective effect of ischemic preconditioning (Lazdunski et al, 1992; Han et al, 2002a, 2002b).

The regulation of the channel appears to be more complex than a change in the intracellular concentration of ATP or ATP/ADP ratio, and may modulate various metabolic changes, including inorganic phosphate, lactate, and the breakdown products of ATP and lipids (Lederer & Nichols, 1989; Kim & Duff, 1990; Kirsch et al, 1990). Therefore, some channels may open during ischemia at least in part. Considering that only a few channels need to be open to have considerable electrophysiological effects, this is especially important (Faivre & Findlay, 1990).

Many biologically active proteins, including enzymes, membrane ionic pumps, and exchangers, contain critical cystein residues, and assessable free thiol groups are present on the channel in various tissues (Weik & Neumcke, 1989; Islam et al, 1993). The function of these proteins often depends on the oxidation state of thiol (SH) groups (Ziegler, 1985). The tissue content of reduced glutathione (GSH) has been known to decrease during ischemia and reperfusion (Singh et al, 1989), whereas that of oxidized glutathione (GSSG) to increase, suggesting that cellular thiol redox may be perturbed. Therefore, their function may be altered during ischemia (Means et al, 1971). Ca^{2+} and K^+ channels have been shown to have functional groups (Krippeitdrew et al, 1994; Post et al, 1994; Ruppersberg et al, 1991). The regulation of the K_{ATP} channel also appears to be modulated by alteration of the structure of the channel protein.

It is of particular important to know whether there is any interaction between modification of thiol group and cardiac K_{ATP} channel activity. In the present study, we have investigated the effect of thiol modification on the K_{ATP} channels in rabbit ventricular myocytes using single channel recordings.

METHODS

Cell isolation

Single ventricular myocytes were isolated from rabbit hearts by enzymatic dissociation, as discribed previously (Han et al, 1993, 2001, 2002c). Initially, hearts were perfused retrogradely for 5 min with Tyrode solution until all signs of blood were removed, when gently squeezed the heart. The hearts were then perfused with a normally Ca^{2+}-free Tyrode's solution for 5 min, followed by perfusion with Ca^{2+}-free Tyrode's solution containing 0.01% collagenase (5 mg/50 cc, Yakult, Japan). After 15~25 min of enzymatic treatment, the hearts were perfused with Krafts Bürhe (KB) solution. After 5 min of perfusion with KB solution, the hearts were removed from the cannula, the atria were discarded, and the ventricular walls and septa were cut vertically into four to six pieces. They were gently agitated in a small beaker with KB solution to obtain single cells. Isolated ventricular cells were stored in a KB solution at 4°C and used within 12 hours. Langendorff column was kept at 37°C during all the steps described above.

Electrophysiological methods

Single-channel currents were measured in inside-out and outside-out patch configurations of the gigaohm seal patch-clamp technique (Hamill et al, 1981). Channel activity was measured using a patch-clamp amplifier (EPC-7, LIST, Darmstadt, Germany; Axopatch-1D, Axon Instruments, Union City, CA, USA). Pipettes of 5~10 MΩ resistance were pulled from borosilicate glass capillaries (Clark Electrochemical, Pangbourne, England) using a vertical puller (Narishige PP-83, Tokyo, Japan). Their tips were coated with Sylgard and fire polished. Membrane currents were digitized at a sampling rate of 48 kHz and stored in digitized format on digital audio tapes using a Biologic DTR-1200 recorder (Grenoble, France). For the analysis of single channel activity, the data were transferred to a computer (IBM-PC, 80486 DX2-66, Busan, Korea) with pCLAMP v 6.0 software (Axon Instruments, Union City, CA, USA) through an analogue-to-digital converter interface (Digidata-1200, Axon Instruments, Union City, CA, USA).

Data analysis and quantification of channel activity

The threshold for judging the open state was set at half of the single-channel amplitude (6). The open time histogram was formed from continuous recordings of more than 60 sec. The open probability (P_O) was calculated using the formula:

$$P_O = (\sum_{j=1}^{N} t_j)/(T_d N)$$

where t_j is the time spent at current levels corresponding to j=0, 1, 2, ... N channels in the open state, T_d is the duration of the recording and N is the number of channels active in the patch. The number of channels in a patch was estimated by dividing the maximum current observed during an extended period at zero ATP, with the mean unitary current amplitude. P_O was calculated over 30 sec records.

Rundown of K_{ATP} channels

The activity of K_{ATP} channels in rabbit ventricular myocytes decreases slowly with time after patches are excised into ATP-free solution. This phenomenon is known as "rundown". Upon excision, patches were continuously exposed to ATP (0.5 or 1 mM), except for a brief exposure to zero ATP at the beginning and end of experiments to estimate the number of channels in a patch and the degree of rundown. Data from patches exhibiting more than 50% rundown were discarded. This concentration of ATP was chosen to represent a near-physiological level of ATP, while giving a P_O to allow single-channel events to be observed.

Solutions and drugs

Normal Tyrode solution contained (in mM): 143 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 5.5 glucose, 5 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) and adjusted the pH to 7.4 with NaOH. The solutions facing the outside of the cell membrane in the excised patch recordings contained (in mM): 140 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES and adjusted the pH to 7.4 with KOH. The solutions facing the inside of the cell membrane in the excised patch recordings contained (in mM): 127 KCl, 13 KOH, 1 $MgCl_2$, 5 ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), 10 glucose, 10 HEPES and adjusted the pH to 7.4 with KOH. The modified KB solution had the following composition (in mM): 25 KCl, 10 KH_2PO_4, 16 KOH, 80 glutamic acid, 10 taurine, 14 oxalic acid, 10 HEPES, and 11 glucose at pH 7.4, adjusted by KOH.

ATP (500 μM or 1 mM) and glibenclamide (10 μM) were added to either the extracellular or intracellular solutions according to the experimental protocols described in the text. After addition of drugs to the test solution, the pH was re-adjusted to 7.4 with KOH. Unless otherwise noted, these agents were obtained from Sigma (St. Louis, MO, USA). Experiments were done at 25±2°C.

Metabolically compromised cells

Rabbit ventricular myocytes were incubated with an ischemic solution for more than 60 min. The ischemic solution and normal Tyrode solution were similar, except for the following changes: 8 mM KCl, no glucose, pH 6.0, and bubbled with 100% nitrogen gas for more than 3 h before the experiment was started. The P_{O2} of ischemic solution was reduced by 82%: 661±25 and 128±23 mmHg in the normal Tyrode (n=4) and ischemic (n=3) solutions, respectively. In some cases, we included 10 mM deoxyglucose in the ischemic solution to induce absolute depletion of energy source.

Statistical analysis

The data were statistically analyzed using either the Student's unpaired *t* test when two treatment groups were compared, or one-way analysis of variance (ANOVA) followed by a *post hoc* Student-Newman-Keuls test when all pairwise comparisons among the different treatment groups were made. Tests were considered significant when $P<0.05$. All data are presented as means±S.E.

RESULTS

ATP-sensitive K^+ (K_{ATP}) channels in rabbit ventricular myocytes have distinctive single-channel characteristics. The unitary current amplitude increases with hyperpolarization and decreases with depolarization. At around 0 mV (E_K; the equilibrium potential for K^+), outward currents through the channel are observed in the more positive potentials. The openings of the channel appear in bursts at various potentials, and the flickerings within bursts decreases with depolarizing the membrane. The hyperpolarlizing potentials, ranging from 20 to 80 mV negative to E_K, generated a linear current-voltage relationship with a slope conductance of 71.7 pS. (data not shown). Fig. 1 shows typical recordings of K_{ATP} channels in an excised inside-out membrane patches from rabbit ventricular myocytes under control conditions, with both sides of the patch bathed in isotonic KCl solution. During the recording of the K_{ATP} channel at −70 mV, the application of 0.5 mM ATP (Fig. 1A) and 10 μM glibenclamide (Fig. 1B) to the internal solution markedly depressed the channel opening in a reversible manner.

To test the hypothesis that thiol modification is involved in the activation of K_{ATP} channels, the effects of thiol modulating reagents were investigated with an inside-out patch configuration. Dithiothreitol (DTT, 2 mM), a thiol reducing agent, itself did not have any effect on the channel activity in inside-out patch at −60 mV (Fig. 2A). Fig.2B

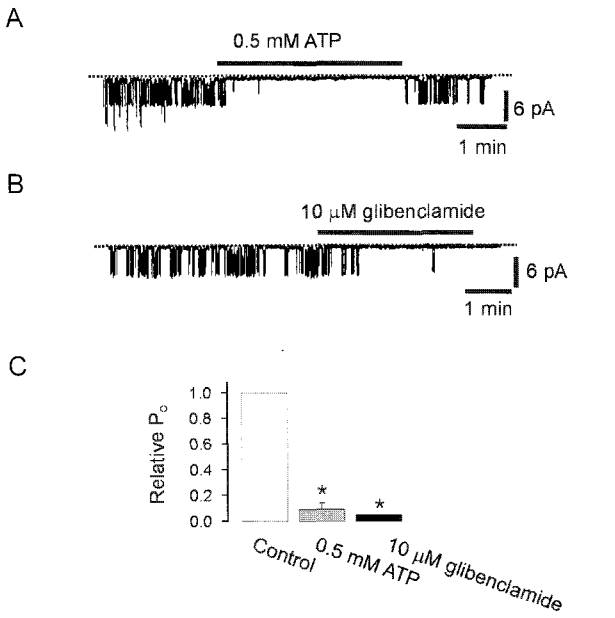

Fig. 1. Single channel currents recorded from the K_{ATP} channel. A. Inhibition of the single K_{ATP} channel currents by either ATP (A) or glibenclamide (B). On formation of the inside-out patches, the K_{ATP} channel was activated. ATP (0.5 mM) and glibenclamide (10 μM) were added as indicated by the horizontal line. The channel activity was almost completely inhibited by ATP and glibenclamide. The bath was perfused with the internal solution. The holding potential of the patch was −70 mV. Dashed line indicates the closed level. C. Change of channel activity in response to ATP and glibenclamide. Histogram showing the pooled data (mean±SE) for Po for the following conditions: control, ATP and glibenclamide. *P< 0.05 relative to control.

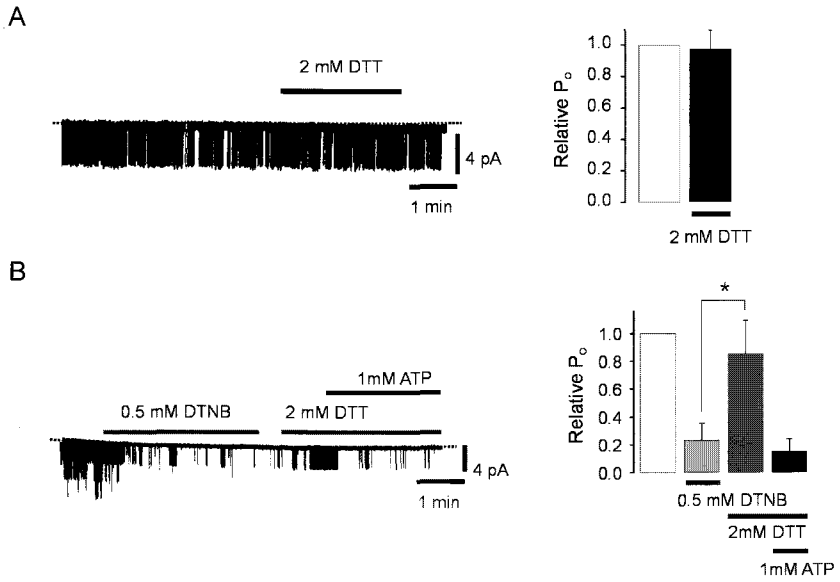

Fig. 2. Effect of thiol modification on the K_{ATP} channel activity in rabbit ventricular myocytes. Single-channel recordings from excised inside-out membrane patches. The pipette contained extracellular solution and the cell was perfused with intracellular-like solution. A. left panel, currents at −60 mV before (control), 2 min after application of 2 mM DTT. DTT did not affect the K_{ATP} channel activity under control condition. Data were sampled at 40 kHz and filtered at 3 kHz. Dashed line represents the closed level. Right panel, change of channel activity in response to 2 mM DTT in an inside-out patch configuration. Histogram showing the pooled data (mean±SE) for Po for the following conditions: control and intracellular DTT (2 mM). B. left panel, 2 mM DTT restored the K_{ATP} channel activity after addition of thiol oxidizing agent, DTNB. Right panel, change of channel activity in response to 2 mM DTT in an inside-out patch configuration. Histogram showing the pooled data (mean±SE) for Po for the following conditions: control, 0.5 mM DTNB, 2 mM DTT and additional application of 1 mM ATP. Note that the amplitude of the channel was not affected by thiol modification. *$P<0.05$.

shows the inhibitory effect of representative reactive disulfide, 5,5'-dithio-bis-(2-nitro-benzoic acid) (DTNB), on the K_{ATP} channel at −60 mV. The control currents in Fig. 2B originate from the openings and closings of at least three K_{ATP} channels in the patch, and the current through a single channel was 3.9±0.3 pA (n=8 patches). As shown by the currents in Fig. 2B, the thiol oxidizing agent (DTNB, 0.5 mM) in the internal solution induced an inhibited the channel activity, and DTT (2 mM) substantially reversed the inhibitory effect of DTNB, however, did not block the inhibitory action of ATP on the K_{ATP} channel. The current through an open channel was not affected by DTNB or DTT. The current-voltage relationships were linear in the negative membrane potential range, with slope conductance of 70.5±0.5 pS (n=8 patches) in the presence of DTNB and 71.3±1.4 pS (n=8 patches) in the presence of DTT (data not shown). There were no statistical difference between DTNB and DTT-treated series, suggesting that the oxidized and reduced state of the channel did not affect the conductance of single-channel currents of the channel. Interestingly, on the other hand, 2 mM DTT itself was able to increase the channel activity in the patches excised from the metabolically compromised cells (Fig. 3).

We obtained single channel recordings from three experiments and successfully analyzed the open- and closed-time of K_{ATP} channel in both control and the presence of DTNB. Fig. 4 shows representative results of the analysis in the

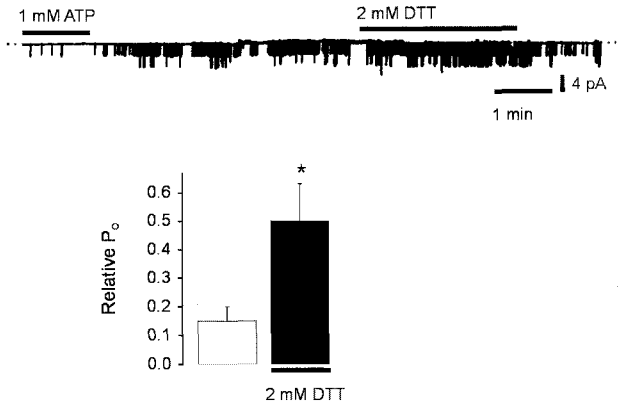

Fig. 3. Effect of cellular metabolic conditions on the action of DTT on the K_{ATP} channels. Single-channel recordings from excised inside-out membrane patches held at −50 mV. Upper panel, 2 mM DTT restored the K_{ATP} channel activity in the patch excised from metabolically compromised cell. Data were sampled at 40 kHz and filtered at 3 kHz. Lower panel, change of channel activity in response to 2 mM DTT. Histogram showing the pooled data (mean ±SE) for Po for the following conditions: control and intracellular DTT (2 mM). *$P<0.05$ relative to control.

presence of 0.5 mM DTNB. As it has been described above, the channel opening appeared in a burst. The fast open and closed kinetics within the bursts were analyzed from the records filtered at 10 kHz. In the control, the open time distribution was described by a single exponential with a time constant (τ_o) of 2.3±0.1 ms (n=3 patches). The closed time distribution was best fitted by two exponentials. The time constant of the fast exponential component (τ_{c1}) was 0.3±0.1 ms (n=3 patches) and that of the slow component (τ_{c2}) was 9.1±2.0 ms (n=3 patches). Both open and closed time distributions were not affected by DTNB: In DTNB solution, the exponential time constants of the distribution of open and closed times were 2.2±0.2 (τ_o, n=3 patches), 0.3±0.1 (τ_{c1}, n=3 patches) and 9.0±3.0 ms (τ_{c2}, n=3 patches), respectively.

The lifetime of each bursting of opening was measured from the recordings filtered at a cutoff frequency of 0.1 kHz. It seems that the open time histogram was well fitted to a single exponential function. In these histograms, the time constant of burst duration (τ_b) was decreased from 40.5± 3.8 (n=3 patches) to 14.3±4.1 ms (n=3 patches) by 0.5 mM DTNB. The interburst time histograms were fitted with two exponential functions. The time constant of the fast exponential component (τ_{c3}) was not affected by DTNB (from 9.0±3.4 to 12.0±2.1 ms, n=3 patches), and the time constant of the slow exponential component (τ_{c4}) was increased from 58.0±9.3 to 311.3±25.4 ms by DTNB (n=3 patches). Thus, DTNB must have increased the long closed times between bursts of openings and/or reduced the number of functional channels.

The property of N-ethylmaleimide (NEM), an alkylating agent, is to modify thiol residues in proteins. As seen in Fig. 5A, an irreversible inhibition of the channel was observed after application of NEM (2 mM), and the channel activity was blocked rapidly after the addition of ATP (1 mM) and reappeared when NEM was first washed out from the internal solution, followed by ATP (Fig. 5B). This result suggests that the presence of internal ATP prevents the irreversible inhibition of K_{ATP} channels by NEM. DTT at 2 mM could not reverse the inhibitory effect of NEM on the channel activity (Fig. 6).

When performed on outside-out patches excised from cells, the and adjusted the pH to 7.4 addition of thiol oxidizing agents (DTNB and NEM) and reducing agent (DTT) to the perfusion solution no longer blocked the channel activity. Fig. 7 shows a representative experiment using outside-out patch configuration.

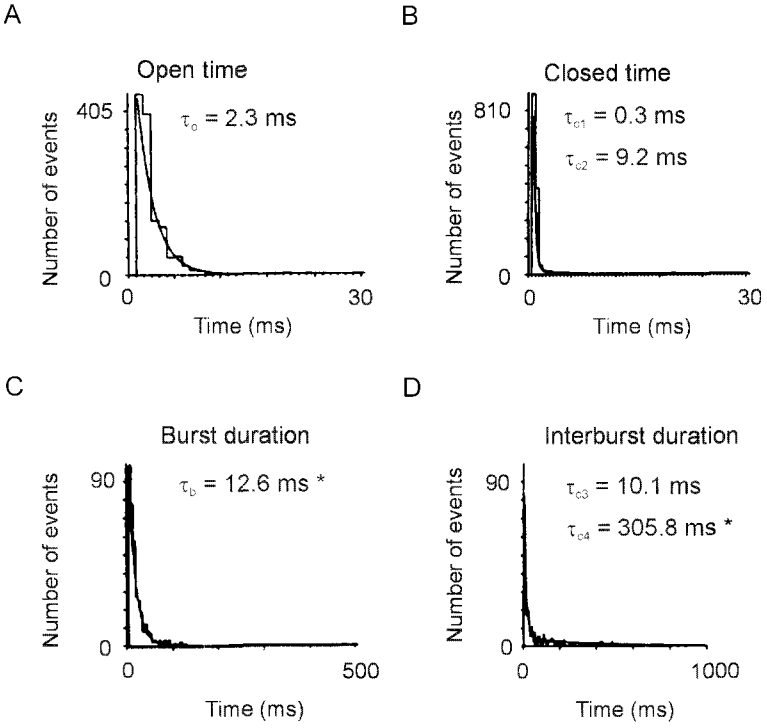

Fig. 4. Effect of thiol oxidation on the distribution of open-time, closed-time, life time of burst duration and interburst duration of the K_{ATP} channel current from an inside-out patch. The histograms of the open time (A) and closed time (B) within bursts were analyzed from the current records at 10 kHz. The histograms of the burst (C) and interburst duration (D) were analyzed from the current records at 0.1 kHz. The membrane potential was held at −50 mV. Time constants of closed time and interburst duration histograms were fitted to two exponentials (fast and slow), and the others were fitted to single exponentials. Note that the thiol oxidizing agent (DTNB) decreased the burst duration and increased the interburst duration. *$P < 0.05$ relative to the control.

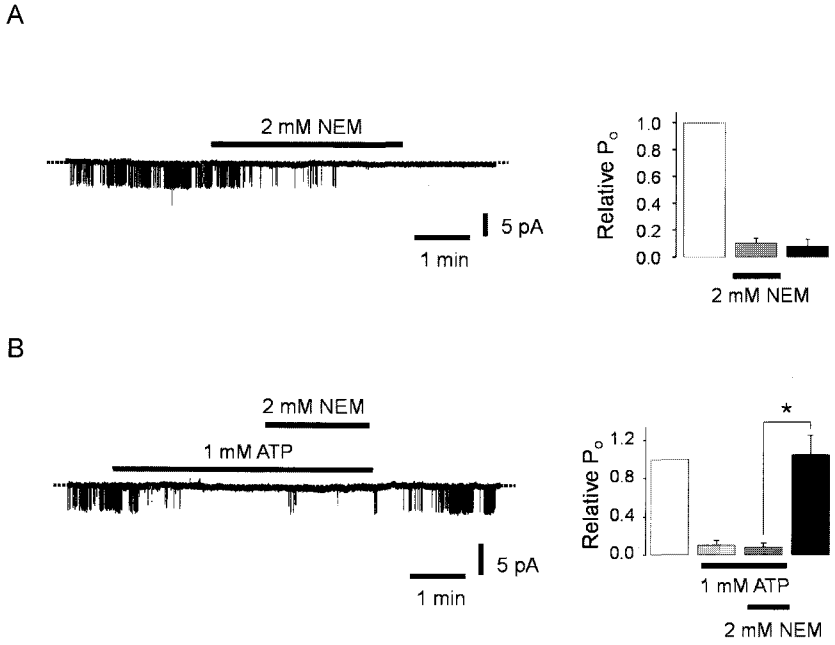

Fig. 5. Effects of an alkylating agent on the K_{ATP} channel activity. A. Single-channel recordings from excised inside-out membrane patches held at −50 mV. The K_{ATP} channels were inhibited irreversibly by 2 mM NEM. Data were sampled at 40 kHz and filtered at 3 kHz. Dashed line represents the closed level. Change of channel activity in response to 2 mM NEM in an inside-out patch configuration (right panel). Histogram showing the pooled data (mean±SE) for Po for the following conditions: control, NEM (2 mM) and wash-out of NEM. *$P<0.05$. B. Effects of NEM on the K_{ATP} channel activity in the presence of ATP. Single-channel recordings from excised inside-out membrane patches held at −50 mV. The K_{ATP} channel currents were rapidly blocked after the addition of ATP (1 mM). The channel activity reappeared after NEM was first washed out from the internal solution, followed by ATP. Data were sampled at 40 kHz and filtered at 3 kHz. Dashed line represents the closed level. Histogram showing the pooled data (mean±SE) for Po for the following conditions: control, ATP (1 mM) alone, ATP (1 mM) and NEM (2 mM) and washing-out of ATP and NEM. *$P<0.05$.

DISCUSSION

In the present study, we demonstrated the effects of thiol group-modifying substances on K_{ATP} channel activity in rabbit ventricular myocytes. The K_{ATP} channels were inhibited by the presence of 0.5 mM DTNB and 2 mM NEM in the internal solution. The result suggests that the inhibition of the channel activity was caused by thiol redox modulation, because a common property among DTNB and NEM is to modify thiol groups in proteins. Furthermore, the effect of DTNB was reversed by the addition of excess amount of the disulfide reducing agent, DTT (2 mM). This also indicates that the inhibition of the channel activity was caused by thiol oxidation, but not due to a nonspecific effect on the channel. These data are in agreement with those of Coetzee et al (1995), who reported that the membrane-impermeable compound, p-chloromercuri-phenylsulfonic acid (pCMPS) and thimerosal induced a quick and irreversible inhibition of K_{ATP} channel activity in guinea pig ventricular myocytes, and that DTT (3 mM) was able to reverse this inhibition. Interestingly, oxidized glutathione (3 mM) did not block K_{ATP} channel activity.

There is a controversy regarding the relationship between activation of K_{ATP} channels and thiol modification. The data in our study showed that thiol oxidizing agent blocked K_{ATP} channel activity without affecting the blocking mechanism of ATP. This result is in contrasts with the data of Tokube et al (1996; 1998) in guinea pig ventricular myocytes, which showed that oxygen free radicals significantly increased the open probability of the channel at a relatively narrow range of ATP concentrations (0.2~2 mM), and this effect was enhanced in the presence of ADP (0.1 mM) and abolished in the presence of either free radical scavengers or glibenclamide. This observation indicates that oxygen free radicals activate K_{ATP} channels by modulating ATP binding sites of the K_{ATP} channels, without affecting ADP binding or glibenclamide binding sites. The difference between ours and the result of Tokube et al (1996; 1998) might be due to the differences in animal species used and thiol redox modulating system, since they used guinea pig ventricular myocytes and oxygen free radicals produced by xanthine oxidase reaction.

Ischemic preconditioning is a phenomenon whereby brief periods of ischemia are paradoxically protective against subsequent ischemic injury (Murry et al, 1986). Although the molecular basis of this endogenous protective mechanism remains elusive, several key components have been identified. Among these, the K_{ATP} channel has been demon-

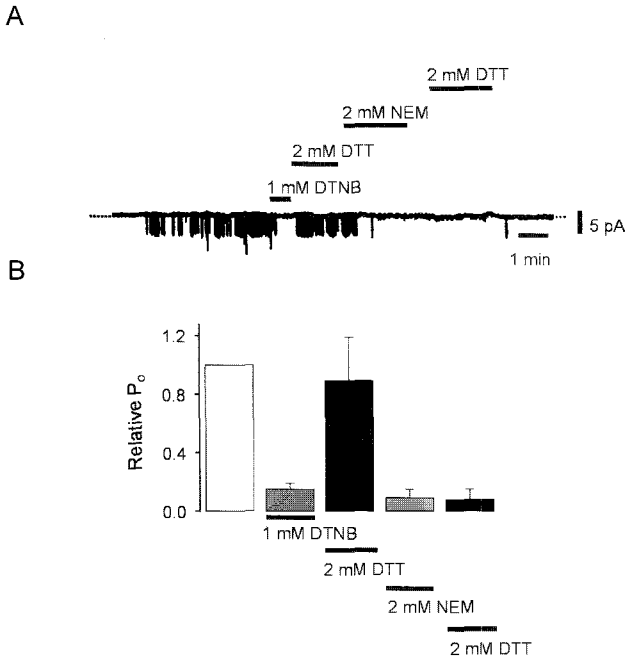

Fig. 6. Effects of an alkylating agent on the action of thiol reducing agent on K_{ATP} channel activity. A. Single-channel recordings from excised inside-out membrane patches held at −50 mV. DTT (2 mM) did not affect the K_{ATP} channel activity after pretreatment of 2 mM NEM. Data were sampled at 40 kHz and filtered at 3 kHz. Dashed line represents the closed level. B. Change of channel activity in response to 2 mM DTT in an inside-out patch configuration after pretreatment of NEM. Histogram showing the pooled data (mean ± SE) for Po for the following conditions: control, 1 mM DTNB, 2 mM DTT, 2 mM NEM and subsequent application of 2 mM DTT.

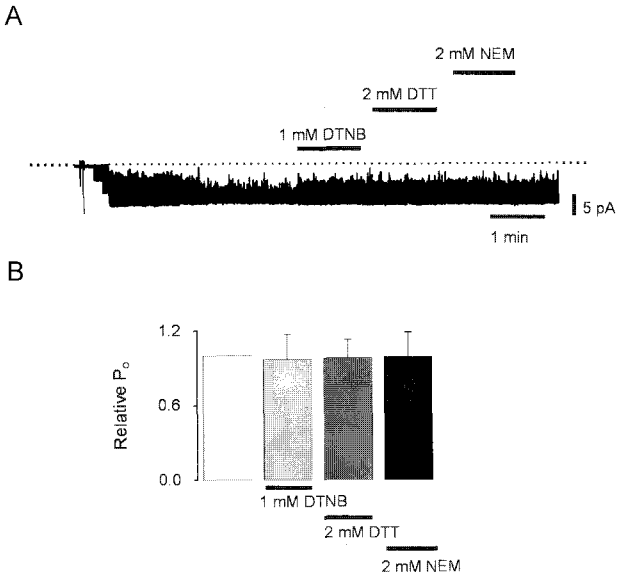

Fig. 7. Effect of thiol group modulators on the single-channel currents from an outside-out patch configuration. A. Single-channel recordings from excised outside-out membrane patches held at −50 mV. The bars indicate the application of the three agents. The current spikes at the beginning of recording are not channel currents but artifacts from excised patch formation. Data were sampled at 40 kHz and filtered at 3 kHz. Dashed line represents the closed level. B. Change of channel activity in response to the three agents in an outside-out patch configuration. Histogram showing the pooled data (mean ± SE) for Po for the following conditions: control, 1 mM DTNB, 2 mM DTT and 2 mM NEM.

strated to be an important component (Garlid et al, 1997; Liu et al, 1998). There is some controversy regarding the relationship between the cardioprotection and change in redox state. It is demonstrated that the thiol groups are essential for the cardioprotection in ischemic hearts (Sargent et al, 1993). On the contrary, however, although high levels of reactive oxygen species are known to be detrimental (Jeroudi et al, 1994), moderate levels of H_2O_2 and $^{\cdot}O_2^-$ have been shown to elicit a cardioprotective effect similar to that observed with ischemic preconditioning (Baines et al, 1997; Tritto et al, 1997). Consequently, further experiments are necessary to determine the relationships between K_{ATP} channel opening, the mode of action of thiol modulating agents, and cardioprotection against ischemic injury.

The fact that DTT itself did not have any effect on the channel activity, suggests that thiol groups were in the reduced form in the intact channels. This hypothesis was supported by our observation that DTT increased the channel activity in the patches excised from the metabolically compromised cells. DTNB and NEM were effective only, when added to the intracellular surface of the channel in the excised membrane patches. The result indicates that a functionally important thiol group is located on the cytoplasmic side of the channel, and this was confirmed in experiment using the outside-out patch configuration. There are evidences to suggest that the K_{ATP} channel of mouse skeletal muscle (Weik & Neumcke, 1989; Tricarico & Camerino, 1994) and pancreatic β-cells (Islam et al, 1993; Lee et al, 1994; Song et al, 1997; Trapp et al, 1998) contain functionally important thiol groups.

It has been shown that thiol groups play an important role in the activity of many membrane proteins and enzymes (Whisler et al, 1995; Meldrum et al, 1998; Takeishi et al, 1999; Chung et al, 2002; Tupling & Green, 2002). This may occur by the formation of intramolecular disulfide bridges between adjacent thiol groups by thiol group oxidation, resulting in the formation of intermolecular mixed disulfides (Means & Feeney, 1971) and thus causing changes in tertiary structure and function. Change in redox state is proposed as a cellular signaling, influencing cell death as well as survival (Finkel, 2000; Irani, 2000). There are a plethora of articles on alterations of the function of different ion channels caused by modification of thiol or disulfide groups on the channel proteins, such as Ca^{2+}, K^+, Na^+ channels (Islam et al, 1993; Egorova et al, 1997; Yao et al, 1997; Gong et al, 2002; Kerst et al, 2002) as well as Na^+-Ca^{2+} exchanger (Reeves et al, 1986). Interestingly, thiol oxidation leads to opening of the intracellular calcium channels, whereas in the case of the K_{ATP} channel a similar chemical modification gives rise to an opposite result, indicating that various signaling targets for thiol modification are involved in cardioprotective mechanisms against ischemia.

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